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Morphological, Physiological and Biochemical Responses to High Temperatures and Drought Stress in Lentil (*Lens culinaris* Medikus)

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Dedication

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Abstract

This thesis explores a critical challenge facing lentil production globally: climate change, focusing particularly on the impact of heat and drought stresses. The aim was to identify new sources of tolerance to these stresses in lentil using the Focused Germplasm Identification Strategy. To achieve this, a set of 162 accessions was screened for heat and combined heat-drought stress tolerance under field conditions at two diverse locations (Marchouch and Tessaout) in Morocco. Novel potential sources of heat tolerance, such as ILL 7833, ILL 6338, and ILL 6104 at Marchouch, and ILL 7814 and ILL 8029 at Tessaout, were identified based on the heat tolerance index. Similarly, using the stress tolerance index, ILL 7835, ILL 6075, and ILL 6362 were found as the most tolerant accessions at Marchouch, and ILL 7814, ILL 7835, and ILL 7804 at Tessaout, under combined heat-drought stress conditions. Subsequently, 20 lentil accessions were selected for further evaluation under field conditions to explore the impact of both stresses on various traits related to phenology, grain yield, nutritional quality, and canopy temperature. These accessions were also assessed for genotypic variability in the limited transpiration (TRlim) trait in response to increased vapor pressure deficit (VPD) under controlled conditions. The study validated previous field screening results, showing that all tolerant genotypes exhibited high adaptation to high temperatures and combined temperature-drought stress under field conditions, along with clear TRlim at high VPD under controlled conditions. Two tolerant accessions (ILL 7833 and ILL 7835) demonstrated the lowest breakpoint observed in cultivated lentil. Additionally, accessions like ILL 7835, ILL 7814, and Bakria (ILL 4605) demonstrated a good nutritional quality alongside moderate to high yields under both heat and combined heat-drought stress. Afterward, twelve accessions were selected to elucidate the mechanisms underlying heat and drought tolerance in lentil accessions. Results revealed that tolerant lentil accessions exhibited higher antioxidant activity, osmolytes, and secondary metabolites under high-temperature and drought-stress conditions compared to sensitive accessions. This work contributes to a deeper understanding of lentil mechanism responses to drought and heat stresses, serving as a valuable resource for lentil stress tolerance breeding efforts.

Keywords: lentil, heat tolerance, drought tolerance, vapor pressure deficit, metabolite mechanisms, physiological responses, grain yield, nutritional quality

Résumé

Cette thèse explore un défi critique auquel est confrontée la production mondiale de lentille : le changement climatique, en mettant particulièrement l'accent sur l'impact des contraintes de chaleur et de sécheresse. L'objectif était d'identifier de nouvelles sources de tolérance à ces contraintes chez la lentille en utilisant la Stratégie d'Identification de Germplasm Ciblé. Pour ce faire, un ensemble de 162 accessions a été examiné pour la tolérance à la chaleur et à la combinaison chaleur-sécheresse dans des conditions de terrain à deux sites (Marchouch et Tessaout) au Maroc. De nouvelles sources potentielles de tolérance à la chaleur, telles que ILL 7833, ILL 6338 et ILL 6104 à Marchouch, et ILL 7814 et ILL 8029 à Tessaout, ont été identifiées sur la base de l'indice de tolérance à la chaleur. De même, en utilisant l'indice de tolérance au stress, ILL 7835, ILL 6075 et ILL 6362 ont été identifiés comme les accessions les plus tolérantes à Marchouch, et ILL 7814, ILL 7835 et ILL 7804 à Tessaout, sous des conditions de stress combiné chaleur-sécheresse. Par la suite, 20 accessions ont été sélectionnées pour une évaluation plus approfondie en conditions de terrain afin d'explorer l'impact des deux contraintes sur divers traits liés à la phénologie, au rendement en grain, à la qualité nutritionnelle et à la température du couvert végétal. Ces accessions ont également été évaluées pour la variabilité génotypique dans le trait de transpiration limitée (TRlim) en réponse à une augmentation du déficit de pression de vapeur (DPV) dans des conditions contrôlées. L'étude a validé les résultats précédents de criblage sur le terrain, montrant que tous les accessions tolérants ont présenté une forte adaptation aux températures élevées et au stress combiné chaleur-sécheresse sur le terrain, ainsi qu'une TRlim claire à des DPV élevés dans des conditions contrôlées. Deux accessions tolérantes (ILL 7833 et ILL 7835) ont démontré le plus bas point de rupture observé chez les lentilles cultivées. De plus, des accessions comme ILL 7835, ILL 7814 et Bakria (ILL 4605) ont présenté une bonne qualité nutritionnelle ainsi que des rendements modérés à élevés sous des contraintes de chaleur et de sécheresse combinées. Ensuite, douze accessions ont été sélectionnées pour élucider les mécanismes sous-jacents de tolérance à la chaleur et à la sécheresse. Les résultats ont révélé que les accessions tolérantes de lentilles ont présenté des réponses plus élevée pour l'activité antioxydante, les osmolytes et les métabolite secondaires sous des conditions de température et de sécheresse par rapport aux accessions sensibles. Cette étude contribue à une compréhension plus approfondie des réponses métaboliques des lentilles au stress hydrique et thermique, servant de ressource précieuse pour les efforts de sélection de la tolérance au stress des lentilles.

Mots-clés: lentille, tolérance à la chaleur, tolérance à la sécheresse, déficit de pression de vapeur, mécanismes métaboliques, réponses physiologiques, rendement en grains, qualité nutritionnelle.

Scientific productions

a. List of publications

1. **El Haddad, N.**, En-Nahli, Y., Choukri, H., Aloui, K., Mentag, R., El-Baouchi, A., Hejjaoui, K., Rajendran, K., Smouni, A., Maalouf, F. and Kumar, S., **2023**. Metabolic Mechanisms Underlying Heat and Drought Tolerance in Lentil Accessions: Implications for Stress Tolerance Breeding. Plants, 12(23), p.3962. https://doi.org/10.3390/plants12233962.
2. Aloui, K., Choukri, H., **El Haddad, N.,** Gupta, P., El Bouhmadi, K., Emmrich, P.M., Singh, A., Edwards, A., Maalouf, F., Bouhlal, O. and Staples, J., **2023**. Impact of Heat and Drought Stress on Grasspea and Its Wild Relatives. Plants, 12(19), p.3501. https://doi.org/10.3390/plants12193501.
3. Benali, A., **El Haddad, N.**, Patil, S.B., Goyal, A., Hejjaoui, K., El Baouchi, A., Gaboun, F., Taghouti, M., Ouhssine, M. and Kumar, S., **2023**. Impact of Terminal Heat and Combined Heat-Drought Stress on Plant Growth, Yield, Grain Size, and Nutritional Quality in Chickpea (*Cicer arietinum* L.). Plants, 12(21), p.3726. https://doi.org/10.3390/plants12213726.
4. Choukri, H., **El Haddad, N.,** Aloui, K., Hejjaoui, K., El-Baouchi, A., Smouni, A., Thavarajah, D., Maalouf, F. and Kumar, S., **2022**. Effect of High Temperature Stress During the Reproductive Stage on Grain Yield and Nutritional Quality of Lentil (*Lens culinaris* Medikus). Frontiers in Nutrition. 9:857469. https://doi.org/10.3389/fnut.2022.857469.
5. Elango, D., Rajendran, K., Van der Laan, L., Sebastiar, S., Raigne, J., Thaiparambil, N.A., **El Haddad, N**., Raja, B., Wang, W., Ferela, A. and Chiteri, K.O., **2022**. Raffinose Family Oligosaccharides: Friend or Foe for Human and Plant Health?. *Frontiers in Plant Science*, *13*, pp.Art-829118. https://doi.org/10.3389/fpls.2022.829118.
6. **El Haddad, N.,** Choukri, H., Ghanem, M.E., Smouni, A., Mentag, R., Rajendran, K., Hejjaoui, K., Maalouf, F. and Kumar, S., **2021**. High-temperature and drought stress effects on growth, yield and nutritional quality with transpiration response to vapor pressure deficit in lentil. *Plants*, *11*(1), p.95. https://doi.org/10.3390/plants11010095.
7. **El Haddad, N.**, Rajendran, K., Smouni, A., Es-Safi, N.E., Benbrahim, N., Mentag, R., Nayyar, H., Maalouf, F. and Kumar, S., **2020**. Screening the FIGS set of lentil (*Lens culinaris* Medikus) germplasm for tolerance to terminal heat and combined drought-heat stress. *Agronomy*, *10*(7), p.1036. doi :10.3390/agronomy10071036. https://doi.org/10.3390/agronomy10071036.
8. Choukri, H., Hejjaoui, K., El-Baouchi, A., **El Haddad, N.**, Smouni, A., Maalouf, F., Thavarajah, D. and Kumar, S., **2020**. Heat and drought stress impact on phenology, grain yield, and nutritional quality of lentil (*Lens culinaris* Medikus). *Frontiers in Nutrition*, *7*, p.596307. https://doi.org/10.3389/fnut.2020.596307.

b. Oral presentations

1. **N. El Haddad**, H. Choukri, M. E. Ghanem, A. Smouni, R. Mentag, K. Rajendran, K. Hejjaoui, F. Maalouf and S. Kumar. High-Temperature and Drought Stress Effects on Growth, Yield and Nutritional Quality with Transpiration Response to Vapor Pressure Deficit in Lentil, doctoriale faculté des sciences Rabat du 18 au 22 juillet **2022**.
2. **N. El Haddad**, K. Hejjaoui, H. Choukri, R. Mentag, M. Ghanem, A. Smouni and S. Kumar Genotypic variation in Lentil (*Lens culinaris* Medikus) for transpiration response to Vapor Pressure Deficit during the Third International Legume Society Conference ILS3 2019 Legume for human and planet health in Poznań, Poland, 21 to 25 May **2019**.
3. **N. El Haddad**, A. Smouni, R. Mentag, M. Ghanem and S. Kumar. Physiological, genetic and molecular characterization of heat and drought stress tolerance in lentil (*Lens culinaris* Medikus). In International Students Symposium at ICARDA, Morocco, January 20, **2017**.
4. **N. El Haddad**, K. Rajendran, R. Mentag, N. Benbrahim, N. Es-Safi and S. Kumar. Screening in field lentil (*Lens culinaris* Medikus) FIGS subset for tolerance to terminal heat and drought stress during reproductive stage. In the second edition of the international conference on natural substances and sustainable development, 2ème Edition du congrès International 'substance naturelles et développement durable', Faculté des sciences de Rabat, 19-21 mai **2016**.
5. **N. El Haddad**, K. Rajendran, R. Mentag, N. Benbrahim, N. Es-Safi and S. Kumar. Screening in field lentil (*Lens culinaris* Medikus) FIGS subset for tolerance to terminal heat and drought stress during reproductive stage. In the second edition of the international conference on natural substances and sustainable development, 5ème édition des Doctoriales FSR 2016 'Recherche scientifique Eco-Innovatrice: acquisition du savoir et perfectionnement des compétences' 09, 10 et 11 Mars **2016**.

c. List of poster presentations

1. **N. El Haddad**, K. Rajendran, S. Kumar, R. Mentag, A. Smouni, F. Maalouf, and S. Kumar. Screening the FIGS set of lentil (*Lens culinaris* Medikus) germplasm for tolerance to terminal heat and drought stress during the Third International Legume Society Conference ILS3 2019 "Legume for human and planet health" in Poznań from 21 to 25 May 2019, Poland.
2. **N. El Haddad**, K. Hejjaoui, H. Choukri, R. Mentag, M. Ghanem, A. Smouni and S. Kumar. Evaluating heat and drought stress impact on growth and yield of lentil under controlled environments. In The IFLRC-VII, Marrakesh, Morocco, May 6-8, **2018** ap 142.
3. H. Choukri, K. Hejjaoui, **N. El Haddad**, A. El-Baouchi, A. Smouni, M. Amri, and S. Kumar. Mainstreaming biofortification in global lentil improvement program. In The IFLRC-VII, Marrakesh, Morocco, May 6-8, **2018** ap 028.
4. **N. El Haddad**, K. Hejjaoui, H. Choukri, R. Mentag, M. Ghanem, A. Smouni and S. Kumar. Evaluating heat and drought stress impact on growth and yield of lentil under controlled environments. Doctoriales du centre organisées à la faculté des sciences, 1ere édition des doctoriales du centre Biotechnologies Végétales et Microbienne, Biodiversité et Environnement. Université Mohammed V de Rabat, 25 et 26 Décembre **2018**.
5. **N. El Haddad**, K. Rajendran, S. Kumar, R. Mentag, N. Benbrahim, N. Es-Safi, A. Smouni F. Maalouf and S. Kumar. Screening FIGS set of lentil (*Lens culinaris* Medikus) germplasm for tolerance to terminal heat and drought stresses. In The ICP_2016, Marrakesh, Morocco, April 18-20, **2016** pp 106.
6. **N. El Haddad**, K. Rajendran, R. Mentag, N. Benbrahim, N. Es-Safi, A. Smouni F. Maalouf and S. Kumar. Screening in field lentil (*Lens culinaris* Medikus) FIGS subset for tolerance to terminal heat and drought stress during reproductive stage. In the 7th scientific days of CEDoc SVS and the 4th scientific days of AMADOC SVS, Faculty of Medicine and Pharmacy, Rabat, Morocco. Mars 16-19, **2016**.
7. **N. El Haddad**, K. Rajendran, R. Mentag, N. Benbrahim, N. Es-Safi, A. Smouni, F. Maalouf and S. Kumar. Screening lentil (*Lens culinaris* Medikus) FIGS subset for tolerance to terminal heat and drought stress. In the 5th International Conference Environment and Sustainable Development 'Climate Change: causes, Impacts, Mitigation and Adaptation, Rabat-Kénitra, Morocco. October 10-15, **2016**.
8. **N. El Haddad**, K. Rajendran, R. Mentag, N. Benbrahim, N. Es-Safi. A. Smouni, F. Maalouf and S. Kumar (2016). Screening lentil (*Lens culinaris* Medikus) FIGS subset for tolerance to terminal heat and drought stress. In the International Symposium of natural substances: Stress and cellular metabolisms, Settat, Morocco. December 15-16, **2016**.
9. **N. El Haddad**, K. Rajendran, R. Mentag, N. Benbrahim, N. Es-Safi. A. Smouni, F. Maalouf and S. Kumar (2016). Screening lentil (*Lens culinaris* Medikus) FIGS subset for tolerance to terminal heat and drought stress. In the 5th edition of the FSR doctoral **2016** Innovative scientific research, Rabat, Morocco. Mars 09-11, 2016.

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List of abbreviations

ABA: Abscisic acid
ANFs: Antinutritional factors
ANOVA: Analysis of variance
APX: Ascorbate peroxidase
BP: Breakpoint
BR: Brassinosteroid
CAT: Catalase
CP: Crude protein content
CV: Coefficient of variation
CWANA: Central and West Asia and North Africa
CwINVs: Cell wall invertases
CWR: Crop wild relatives
D50F: Days to 50% flowering
D50P: Days to 50% podding
DFF: Days to first flowering
DM: Days to maturity
DPPH: 1,1-diphenyl-2-picrylhydrazyl
DS: Drought sensitive
DT: Drought tolerant
EGV: Early growth vigor
ET: Ethylene
FAOSTAT: Food and agriculture organization of the United Nations
Fe: Iron content
FIGS: Focused identification of germplasm strategy
GABA: Gibberellic acid
GLM: General linear model
GMP: Geometric mean productivity
GPS: Global positioning system
GPX: Guaiacol peroxidase
GR: Glutathione reductase
GYP: Grain yield per plant
H_2O_2: Hydrogen peroxidase
HARM: Harmonic mean
HPr: Heat priming
HS: Heat sensitive
HSW: Hundred seed weight
HTI: Heat tolerance index
HT: Heat tolerant
HXKs: Hexokinases
ICARDA: International center for agriculture in the dry areas
ICP-OES: Inductivity coupled plasma-optical emission spectrometry
IPCC: Intergovernmental panel on climate change
JA: Jasmonic acid
LSD: Least significant difference
MAS: Marker assisted selection

MDT: Moderately drought tolerant
MHT: Moderately heat tolerant
MP: Mean productivity
NFPP: Number filled pods per plant
NTPP: Number total pods per plant
NUPP: Number unfilled pods per plant
$^1O_2$: Singlet oxygen
O^{2-}: Superoxide anion
OH: Hydroxyl
PBPP: Number of primary branches per plant
PC: Proline content
PCA: Principal component analysis
PLH: Plant height
POX: Peroxidase
QTL: Quantitative trait loci
RH: Relative humidity
RO: Alkoxy radicals
ROS: Reactive oxygen species
RS: Reducing sugars
SBPP: Number of secondary branches per plant
SOD: Superoxide dismutase
STI: Stress tolerance index
STPs: Sugar transporters
SUSs: Sucrose synthases
TAA: Total antioxidant activity
TBPP: Number of tertiary branches per plant
TC: Tannin content
TFC: Total flavonoids content
TOL: Stress tolerance
TPC: Total phenolic content
TR: Transpiration rate
TRlim: Limited transpiration rate
TSS: Total soluble sugars
VPD: Vapor pressure deficit
Yp: Yield potential
Ys: Yield under stress
Zn: Zinc content

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Summary

General Introduction

Lentil (*Lens culinaris* Medikus) is the third-most important cool season grain legume in the world after chickpea and pea (Sehgal et al., 2021), and accounted 6% of the total dry pulse production with an average yield of 854 kg ha^{-1} between 2015 and 2020. The current global production of lentil is estimated at 6.5 million tons in 2020. Canada, India, Australia, United states of America, Nepal and Syria are biggest producer countries of lentil producing around 88% of the global lentil production (FAOSTAT, 2021). Morocco is ranked seventeen worldwide and second in Africa after Ethiopia in term of lentil production, with a total production of 9044 tons in 2020. In Morocco, lentil is grown often as rainfed crop in rotation with cereals, occupying 14% of the area yearly dedicated to food legumes (Idrissi et al., 2020).

Due to its great nutritional content, particularly protein and micronutrients such as iron and zinc, lentil is a vital dietary component in many Mediterranean and Asian countries, contributing to reduce malnutrition and enhance health for low-income people in developing regions. Lentil is known as poor man's meat, since it is the cheap source of protein (20-36%) (Choukri et al., 2020; Saricaoglu, 2020). It provides also a range of important dietary compounds such as carbohydrates, prebiotics, crude fiber, vitamins (folic acid, pantothenic acid and vitamin B6), amino acids and antioxidants. In addition, the crop provides a series of additional agronomic, environmental and socioeconomic benefits. As a leguminous crop, lentil is able to enhance soil fertility and thus contributes to farming sustainability by fixing atmospheric nitrogen in soils due to the symbiotic association of its roots with the bacterium *Rhizobium* species (Sijilmassi et al., 2021).

Lentil requires low temperature during the vegetative stage, while warmer temperatures are essential at the period of maturity (Choudhury et al., 2012). The optimum temperature for its best growth has been reported to be 18-30 °C (Dikshit et al., 2022). Of the abiotic stresses experienced by lentil worldwide, numerous studies reported that high-temperatures and drought stress are the most severe constraints for global productivity in lentil (Choukri et al., 2020; Sehgal et al. 2017, Sehgal et al., 2019b) and many other crops (Cohen et al., 2021b; Dreccer et al., 2018; Sehgal et al., 2018; Ullah et al., 2019). Global climate change is an added concern, increasing overall temperatures, altering the distribution of precipitation and aggravating drought conditions in semiarid and arid areas, which further exacerbates food security and malnutrition (Li et al., 2021). Food production has been significantly affected by climate change in central and south America,

Africa and Australia. In the mid and late 21st century, global mean surface temperature is projected to rise by 2 °C which will lead to an extreme variation in precipitation events, more heat waves and fewer cold temperature extremes (NASA/GISS), aggravating more the production of major crops.

Temperatures over 32 °C cause several morpho-anatomical, physiological, reproductive and biochemical changes in lentil, which can affect plant growth and development and ultimately lead to a reduction in economic yield (Bhandari et al., 2016; Sita et al., 2018). The effects of heat stress, mainly, at the time of the reproductive stage and seed development of plants, are gaining attention as they are a serious threat to the productivity of leguminous crops by reducing pollen viability, fertilization and pod set (Coyne et al., 2020). Grain legumes are highly sensitive to high-temperature stress and lead to high yield losses because of impaired seed filling and seed development (Kumar et al., 2020; Liu et al., 2019). In addition to heat stress, lentil is also influenced by moisture stress under low precipitation conditions, principally in arid and semi-arid areas, leading to forced maturity and lower yield (Kumar et al., 2012). Previous studies reported numerous devastating effects of drought stress on various activities such as decreased turgor, increased oxidative damage, and changes in leaf gas exchange, thereby leading to yield reduction in lentil (Mishra et al., 2016; Priya et al., 2021; Singh et al., 2017b).

However, the combined effects of the heat and drought on lentil growth and productivity are more severe than those of the individual effects, and the reproductive stages are more susceptible than the vegetative stage (Sehgal et al., 2017). The prevalence of drought accompanied by high temperatures, is expected to increase in the near future (ICPP, 2022). In Morocco, the rainfall has been fluctuating year to year and thus significantly impacted lentil production in the country, resulting in high yield losses. For example, the successive drought stress during the cropping seasons 2006-2007 and 2007-2008 that hit Morocco decreased the production of lentil by 25% compared to 2005-2006 (FAOSTAT, 2022). However, the year 2016 was the most severe drought stress year in Morocco with a total rainfall less than 250 mm, decreasing lentil production from 50,004 tons in 2015 to 2004 tons, and its productivity has also declined from 10,991 to 2,092 kg ha^{-1}. Likewise, the low rainfall of 2020 (less 250 mm) in Morocco decreased the production (9044 tons) and the productivity (2230 kg ha^{-1}) of lentil by 24 %, compared to the year 2019. In fact, as lentil is often cultivated under in rainfed conditions of Morocco, its productivity is very influenced by irregular rainfall. In addition, local populations and traditional varieties being used by farmers

have long cycles in which flowering and pod onset are delayed toward late spring with low soil water availability and high temperatures causing significant increase of flower and pod abortion and thus reducing grain yield (Idrissi et al., 2020). For that, Morocco still relies on imports of lentil grains, mainly from Canada, United states, France and Spain to cover the high local demand.

Breeding towards high yielding, drought and heat tolerant and adapted varieties that meet farmers requirements are at utmost importance to enhance and sustain crop productivity. To overcome these challenges and increase the production, the International Center for Agricultural Research in the Dry Areas (ICARDA) is one of the biggest centers that collect and test genetic sources of various crops under specific nurseries including drought and heat stresses. Large numbers of lentil varieties were released from germplasm produced by ICARDA for North Africa, Middle East, and Central Asia. In May 2022, ICARDA has established a new genebank in Morocco, which is among the largest in the world containing about 14,512 accessions of lentil, thus offer an exciting opportunity for screening and characterizing promising accessions with high adaptation to high-temperatures and drought stress.

Till now, litter information is available on the physiological and biochemical responses of food legumes to combined heat and drought stress. In lentil, very limited research studied the impact of the combined heat and drought stress on the different processes (Sehgal et al., 2019, 2017). The development of drought and heat tolerant cultivars is one of the ways to tackle the problem of high temperature and water deficit in lentil under the current weather fluctuations of climate change. However, heat and/or drought tolerance is a complex trait in nature because many morphological, physiological and biochemical and metabolic changes are responsible as reported earlier in different crop plants (Samineni et al., 2022; Sinha et al., 2021; Zhang et al., 2021). Continuous efforts have been made to study the drought and/or heat stress tolerance in lentil, and to characterize promising accessions based on morpho-physio-biochemicals traits (Kumar et al., 2020). This might be an important step to sustain the production and productivity of lentil under changing climatic conditions and develop ideal germplasm well adapted to the drastic and frequent heats and droughts that affect the region in which ICARDA operates, including North Africa.

Aim of the Study

Lentil as many crops is very sensitive to drought stress and/or heat stress, in consequence, breeding for drought and heat tolerant varieties is becoming very urgent needs under the continuous increase of climate change.

This study is aimed to:

1. Investigate the individual and combined effects of terminal heat and drought stress during the reproductive phase in lentil, and to use selection indices to identify promising accessions with tolerance to heat stress and combined heat-drought stress for future use in breeding.
2. Investigate the impact of high temperature and drought stress on traits associated with phenology, grain yield, nutritional quality and canopy temperature under field conditions.
3. Examine the possible genotypic variation in lentil for transpiration response to high vapor pressure deficit (VPD) conditions over controlled environments.
4. Determine the enzymatic (Catalase, Ascorbate peroxidase, Superoxide dismutase) and total phenolic content, total flavonoids content, tannins content, proline content, total soluble sugars, reducing sugars, total antioxidant activity responses to heat stress and the combined heat-drought stress.

Chapter's Description

Chapter I- Literature review: This chapter represents generalities about lentil, its global status and the major environmental constraints that are limiting its production and productivity.

Chapter II- Screening the FIGS Set of Lentil (*Lens Culinaris* Medikus) Germplasm for Tolerance to Terminal Heat and Combined Drought-Heat Stress: In this chapter, we screened a set of 162 accessions of lentil under heat stress and combined heat-drought stress conditions at two experimental stations in Morocco. The best promising accessions were identified using numerous drought and heat tolerant indices. This work was published in Agronomy MDPI journal (impact factor: 3.95) in July 2020. This scientific paper was cited 21 times (till December 2022).

Chapter III- High-Temperature and Drought Stress Effects on Growth, Yield and Nutritional Quality with Transpiration Response to Vapor Pressure Deficit in Lentil: This chapter described the impact of high-temperatures and the combined high-temperature and drought stress on the phenology, growth, yield and nutritional quality of lentil selected accessions. Further, the accessions were tested also for limited transpiration rate under controlled conditions. This work was published in Plants MDPI journal (Impact factor: 4.66) in December 2021.The paper was cited 11 times till now (December 2022).

Chapter IV- Enzymatic and Non-Enzymatic Responses to High-Temperatures and Combined High-Temperature-Drought Stress in Lentil (*Lens culinaris* Medikus): This chapter describes the enzymatic and non-enzymatic responses of the selected lentil accessions to high-temperatures and the combined heat-drought stress. The work of this paper was published in Plants MDPI journal (Impact factor: 4.66) in August 2023.

Chapter V- General Discussion and Perspectives: This chapter summarizes a general discussion of the global results obtained during this study, and the perspectives were presented.

Chapter I : Literature Review

I.1. History of Lentil

Lentil (*Lens culinaris* Medikus) is an old world grain legume food crop with remains found alongside human habitation up to 13,000 year BC (Sandhu and Singh, 2007). Its domestication is equally old and was probably one of the earliest crops domesticated in the old world (Smartt, 1990). Lentil co-evolved simultaneously with wheat and barley, and became a staple crops for the Neolithic agriculture more than 7,000 years ago (Muehlbauer and McPhee, 2005). Small seeded (2-3 mm) types were found at Tell Mureybit in Syria and have been dated to 8500-7500 BC (Zohary, 1972). Lentil traces have been discovered in Neolithic, aceramic farming villages which were occupied in the 7^{th} milliennium BC in the near east arc. Carbonized lentil seeds have been recovered from broadly dispersed sites such as Tell Ramand in Syria (6250–5950 BC), aceramic Beidha in Jordan, ceramic Hacilar in Turkey (5800–5000 BC) and Tepe Sabz in Iran (5500–5000 BC) (Helbæk, 1970; van Zeist and Bottema, 1971). In Greece, lentils dating back to 6000–5000 BC have been found in Neolithic settlements such as Argissa-Magula Tessaly and Nea Mikomedeia, Macedonia and in the same period lentil was also found in Egypt. The archaeobotanical remains of lentil have been found in the excavations of the Harappan civilization covering the period of 3300–1300 BC (Yadav et al., 2007).

I.2. Origin and Distribution of Lentil

The genus *Lens* Miller is part of the family *Fabaceae* (Leguminosae), subfamily *Faboideae*, tribe *Fabeae*, or alternatively in subfamily *Papilionaceae*, tribe *Vicieae*. *Lens culinaris* subsp. *culinaris* (Bioss.) Ponert is divided into two groups: the small- seeded, red cotyledon var. *microsperma* and the large-seeded, red, yellow, green var. *macrosperma*. *Lens culinaris* is indigenous to the near East and Central Asia, and its putative progenitor is *Lens culinaris* subsp. *orinetalis*. The origin of lentil is still debated. Lentil seeds were found in Syria, Lebanon, Turkey, Iran, Iraq, Jordan, Afghanistan, Uzbekistan and Greece (Zohary and Hopf, 1973). Initially Cubero (1981) classified the genus *Lens* into five species *Lens culinaris*, *Lens orientalis*, *Lens ervoidis*, *Lens nigricans*, and *Lens montbretti*. Later this genus was divided in seven species/subspecies (Ferguson, 2000) as following:

(a) L. culinaris.

subsp. *culinaris,*

subsp. *orientalis* (Boiss.) Ponert

subsp. *tomentosus* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson,

subsp. *odemensis* (Ladizinsky) Ferguson, Maxted, van Slageren & Robertson.

*(b) L. ervoides (*Brign.*)* Grande.

(c) L. nigricans (M.Bieb.) Godron.

(d) L. lamottei Czefr.

Most of the West Asian lentils have a flattened lens-like aspect, whereas South Asian lentils have a convex shape on both sides. Renfrew (1973) reported that *L. nigricans* is the progenitor of the cultivated species *L. culinaris* based on his belief of the domestication of lentil in southern Europe, however, recent investigations have assumed that *L. orientalis* is the progenitor of the cultivated species based on the fact that the wild species were found in the fields of the farmers where lentil crops were cultivated in the Middle East (Ladizinsky, 1979a; Sandhu and Singh, 2007; Zohary and Hopf, 1973). In addition, Ladizinsky (1979b) attempted the cross between *L. culinaris* and *L. orientalis* and studied the behavior of F1 and F2 populations. The results showed that pod indehiscence is governed by a single recessive gene. Together these data gave a reason to believe that domestication of lentil might have started with selection of the variants from wild populations which were non-pod dehiscence (Ladizinsky, 1979c).

Non-pod dehiscence variants made the harvesting easy due to retention of pods till harvest. *L. orientalis* might have originated first from perennial species and at the secondary level become the progenitor of cultivated species. According to Ladizinsky (1979a), *L. orientalis* is the progenitor of cultivated species based on the cytoplasmic studies. In the cytogenetic analysis of interspecific hybrids, three chromosome interchanges were identified between the cultivated *L. culinaris* and *L. nigricans* while only one was found between the cultivated species and *L. orientalis*, which accentuates the concept that *L. orientalis* is the most probable progenitor of the domestic lentil. *Lens orientalis* is the presumed progenitor of *Lens culinaris* and the two species are crossable and produce fully fertile progeny (Muehlbauer et al., 2006).

The distribution of crop wild relatives (CWR) in genus *Lens* is reported by Singh et al. (2014). *Lens culinaris orientalis* exists in the entire Fertile Crescent including Jordan, Syria, Palestine, Lebanon, and Cyprus and in Armenia, Azerbaijan, Czech Republic, Iran, Russia, Turkmenistan, Tajikistan, and Uzbekistan in open or shaded habitats on stony calcareous to basalt soils at 500–

1700 m. *L. culinaris* ssp. *tomentosus* is reported from Syria and Turkey. *L. culinaris* spp. *odemensis* is distributed in Palestine, Syria and Israel in grassy habitat and in calcareous soils of pine groves and on basaltic gravel, at 700–1400 m. in Turkey. *L. ervoides* is found in Jordan, Syria, and Palestine and westwards to Montenegro, Italy, and Croatia and northwards to Russia, Ukraine Armenia, and Azerbaijan under trees and shrubs in shady and partially shady habitat. *L. lamottei* is reported from Spain and France. *L. nigricans* extends in open or shady stony habitats in granitic or limestone soil or in terraces, settlements, and plantations up to 1200 m in Crimea, Croatia, France, Italy Montenegro, Spain, and Ukraine.

I.3. Common Names of Lentil

Lentil is known by several names in different parts of the world. The most common names are lentil (English), adas (Arabic), mercimek (Turkey), messer (Ethopia), masser or massur (India), heramame (Japanese), mangu or margu (Persian), masura, Renuka or mangalaya (Sanskrit).

I.4. Domestication of Lentil

The domestication syndrome denotes all modifications taking place in a crop when, from a wild plant, it turns to be cultivated, and, therefore, dependent on man (Meyer et al., 2012). Many are the characters involved in domestication process which depend on the species and the end use of it by man. These variations may comprise seed size, seed dispersal, seed dormancy, gigantism, plant architecture, etc (Sonnante et al., 2009). For lentils, the chief traits involved in the domestication process are pod dehiscence and seed dormancy, which are both controlled by single recessive genes. A third major trait is seed size which appears to be under more complex control (Sonnante et al., 2009). Several authors hypothesized that humans first select for the absence of seed germination constrains through cycle of cultivation/selection of wild material which lasted only few years (Blumler, 1991; Ladizinsky, 1987; Zohary, 1989). Further, the first character object of selection by humans was the dispersion of seeds because of its consequences on yield effectiveness and increase seeds efficiency (Zohary, 1999). It was suggested that seed size increase was pursued later, since archaeological remains do not display seed size increase until the Bronze age (Fuller, 2007).

I.5. Botanical Description of Lentil

The genus *Lens* is part of the subfamily *Faboideae* which is contained in the flowering plant family Fabaceae or commonly known as legume or bean family, of the order Fabales in the kingdom Plantae (Erskine, 2009). *Lens* is a small genus which consists of the cultivated L. culinaris and six related wild taxa. Among the different taxa of wild lentils, *Lens orientalis* is considered to be the progenitor of the cultivated lentil and it is now generally classified as *Lens culinaris* subsp. *orientalis* (Yadav et al., 2007). Lentil is hypogeal, which means the cotyledons of the germinating seed stay in the ground and inside the seed coat. Therefore, it is less vulnerable to frost, wind erosion, or insect attack. There are several cultivated varieties of the plant, differing in size, hairiness and colour of the leaves, flowers and seeds (Figure I.1).

Figure I.1. *Illustration of the lentil plant (Thomé, 1985)*

Lentil is a diploid, annual, bushy herb of erect, semierect, or spreading and compact growth and normally varies from 30 to 50 centimeters (12 to 20 in) in height. It has many hairy branches, and its stem is slender and angular. The rachis bears 10 to 15 leaflets in five to eight pairs. The leaves are alternate, of oblong-linear and obtuse shape and from yellowish green to dark bluish green in

colour. In general, the upper leaves are converted into tendrils, whereas the lower leaves are mucronate, with small stipules. The flowers are small and ranged from one to four in number, and the colour of flowers might be white, pink, purple, pale purple, or pale blue. Flowers emerge from the axils of the leaves, on a slender footstalk almost as long as the leaves. The pods are oblong, slightly inflated, and about 1.5 centimeters (5⁄8 in) long. Each of them holds normally two seeds, about 0.5 centimeters (1⁄4 in) in diameter, in the characteristic lens shape. The seeds can also be mottled and speckled. The several cultivated varieties of lentil vary in size, hairiness, and colour of the leaves, flowers, and seeds. Lentils are self-pollinating. The flowering initiates from the lowermost buds and gradually moves upward, so-called acropetal flowering. A duration of around two weeks are required for all the flowers to open on the single branch. At the end of the second day and on the third day after the opening of the flowers, they close entirely and the colour starts to fade. The setting of the pods takes place after three to four days (Yadav et al., 2007).

Lentils groups numerous types based primarily on cotyledon and seed coat colour. Currently, green and red lentils are the dominate lentil forms grown, consumed and traded around the world. Green lentils have a yellow cotyledon and pale green seed coat and red lentils have an orange cotyledon and usually a dark seed coat, although the dominant seed coat colour varies between countries. Green lentils are typically cooked and consumed whole and red lentils split for use in products such as soups and dhal. Red lentils constitute 70–80% of world production. These two clusters may be further subdivided based on size (small, medium and large). Generally, the red lentils are also smaller sized than green lentils, however, there are small green and medium-large red lentils. In addition, there are a series of minor niche varieties (low tannin, black, dark green, speckled and brown) which may be locally important (Yadav et al., 2007).

I.6. Status of Genetic Resources Conservation of Lentil

I.6.1. Ex-situ Conservation

The ex-situ collection of lentils is reported at 58,405 accessions held in several national and international genebanks with a sizeable number of duplicates (FAO, 2010). At present, ICARDA genebank conserves a collection of 14,577 *Lens* accessions, which include 11,203 landraces, 602 wild accessions, and 2,755 breeding lines (https://www.genesys-pgr.org) (Table I.1). Despite being the largest collection, there are major germplasm gaps at species and accession levels, and a continuum is very much required to fill these gaps in wild gene pool from the unrepresented areas

of diversity in the genebank. The other larger lentil germplasm collections include the European Cooperative Programme for Plant Genetic Resources (ECPGR) (4,598 accessions, https://www.ecpgr.cgiar.org), the NBPGR, New Delhi, India (7,712 accessions, http://www.nbpgr.ernet.in), AGG, Horsham, Australia (5,254 accessions, https://grdc.com.au), USDA-ARS (3,187 accessions), SPII, Karaj, Iran (3000 accessions, http:// http://en.spii.ir/seSPII) and Vavilov Institute, Russia (2,556 accessions, http://www.vir.nw.ru). Till now, ICARDA has characterized 11,165 accessions of lentils for various morphological and phenological traits. This was possible because of the formation of core, mini-core, and Focused Identification of Germplasm Strategy (FIGS) sets of lentil germplasm, which have been a very advantageous for systematic evaluation (Coyne et al., 2020).

Table I.1: *Genetic resources conserved at ICARDA*

Name of taxon	**Number of accessions**	**No. of countries of collection**
Lens culinaris ssp. *culinaris*	13,958	
Landraces	11,203	78
Breeding lines (ICARDA)	2,755	
Wild species	602	
Lens culinaris ssp. *orientalis*	263	15
Lens culinaris ssp. t*omentosus*	21	2
Lens culinaris ssp. *odemensis*	66	5
Lens ervoides	174	16
Lens nigricans	68	8
Lens lamottei	10	8

I.6.2. In-situ Conservation

The number of accessions preserved ex situ from the zones of origin and diversity has been increasing. Seed has been collected from each taxon and used in further study to determine within-population diversity. This will aid to launch the potential of in situ conservation for wild *Lens* species. However, many areas of greatest interest for in situ conservation (e.g. Turkey and other Mediterranean countries) are threatened by fast loss of precious genetic resources because of habitat destruction. The relatively poor competitive capacity and high palatability of *Lens* species, together with the fact that they happen in small disjunct populations, strengthens this hazard. Central parts to target for in situ conservation comprise west Turkey for *L. nigricans*, southeast Turkey, northwest Syria, south Syria and Jordan for *L. culinaris* ssp. *orientalis*, south Syria for *L.*

culinaris ssp. *odemensis* and the coastal border region between Turkey and Syria stretching along the Syrian coast for *L. ervoides* (Coyne et al., 2020).

I.7. Production, Productivity and Area of Lentil

Lentil ranks fifth in the global production of pulses and its yield varies between 850 to 1,100 kg ha^{-1}. According to FAO, the global production of lentils was 7,130.7 thousand metric tons in 2017, which has decreased by 8.32% and reached 6,537.5 thousand metric tons in 2020 (Figure I.2). Average annual global production of lentil was reported to be 4.57 million metric tonnes during 2000 to 2020. Lentil is relatively drought-enduring plant that is grown worldwide, however, major lentil producing countries are Canada, India, Australia, Turkey, and USA contributing more than three fourth of the total world's lentil production. In 2020, the top two producers were Canada and India with 43.8% and 18.0%, respectively, of the total global world production (FAOSTAT, 2020).

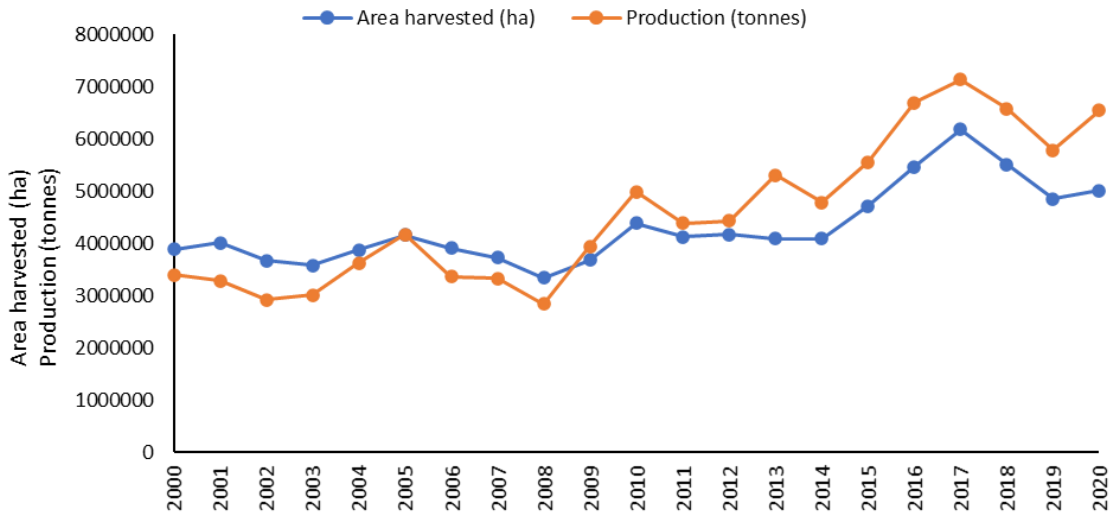

Figure I.2. *Production and yield quantities of lentil in the world from 2000 to 2020*

The global lentil production is decreasing due to various reasons such as extremely high climatic factors (i.e., drought and heat stress) and reducing demand. However, a few major producers such as Canada, Australia, and USA have witnessed 20.39%, 46.3%, and 37.5% growth in 2020 when compared to 2019. The production of India has decreased for the current crop season 2020 due to farmers shift to other crops, pandemic impact (Covid-19) and weather conditions. In Morocco which is ranked as 17th worldwide producer, lentil production was cultivated on 40,560 hectares in 2020, while the production was 9,044 tons decreased by 24% compared to 2019 (37,095 tons). The cultivation of lentil is strongly controlled by several biotic and abiotic factors, mainly drought

stress, which explains the high fluctuation in its production over years (Figure I.3). The yield average of lentil in Morocco reduced from 9,226 kg ha^{-1} in 2019 to 2,230 kg ha^{-1} in 2020 because the extremely severe drought. Average annual global yield of lentil in Morocco was reported to be 5,615 kg ha^{-1} during 2000 to 2020, which is less 40% than Canada, 28% than India and 52% than Ethiopia.

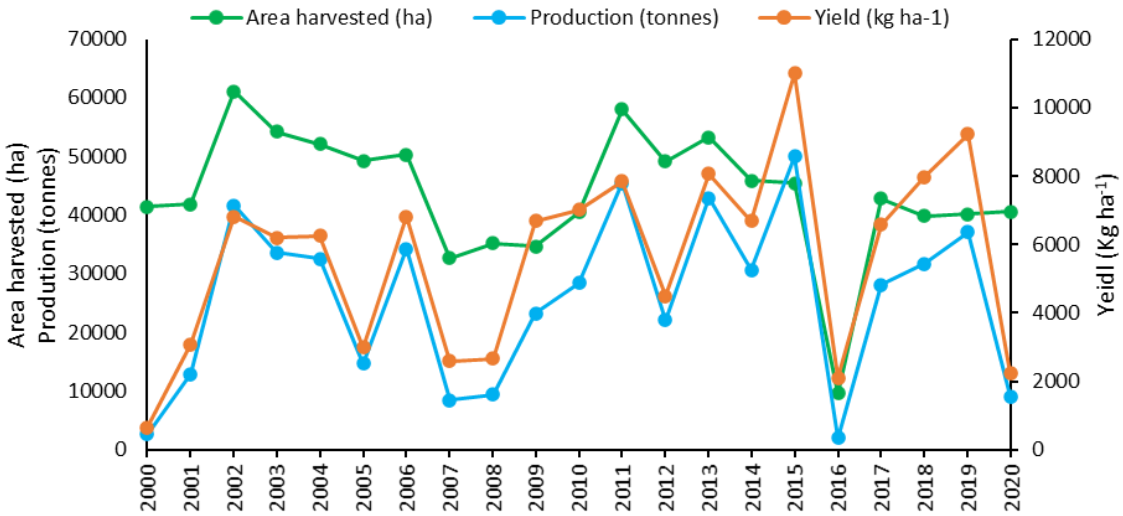

Figure I.3. *Area harvested, production and yield of lentil in Morocco from 2000 to 2020*

I.8. Nutritional Value of Lentil

Lentil composition differs significantly with genetic and environmental factors, but overall, the crop contains a high number of nutritional components and is gluten free. In details, lentil is known as poor meat for human, since it is a cheap source of proteins (21–31%) (Saricaoglu, 2020) representing an important food resource for developing countries, low-income people, vegetarian and vegans. Lentil proteins contain all the essential amino acids (39.3 g of essential amino acids per 100 g of proteins) and are rich in lysine, leucine, arginine, aspartic and glutamic acid. However, they are limited in sulfur-containing amino acids (methionine and cysteine) and tryptophan, and thus the consumption of lentils mixed with other plant protein sources, such as cereal grains, represents an efficient way to obtain an adequately well-balanced amino acid profile (Monnet et al., 2019). The total carbohydrate content in lentil seeds (62–69%) includes primarily starch (35–53%), with low glycemic index value (21–22), followed by high concentrations of dietary fibres (5–20%) and oligosaccharides (Elango et al., 2022; Graf et al., 2020; Revilla et al., 2019). Moreover, lentils are rich in micronutrients such as vitamins (mainly vitamin B9/folate), zinc (4.8 mg/100 g) and iron (7.5 mg/100 g). Lentils also represent an abundant source of

phytochemicals, many of which have been identified as potential chemopreventive candidates. Among these, phenolics (760 mg GAE/100 g), which exist in much higher concentrations than in other legume species can contribute to their high antioxidant, antidiabetic, antiobesity, anticancer and antiinflammatory properties (Faris et al., 2020). These properties are not only connected to the phytochemicals but also to bioactive peptides (e.g. lectins, defensin), proteins (e.g. trypsin and protease inhibitors) and saponins (34 mg/100 g of lentils). Most of these compounds are classically recognized as antinutritional factors (ANFs) which can inhibit the activity of digestive enzymes or sequester nutrients, thereby making them unavailable for digestion (Nosworthy et al., 2018). The amount of ANFs in lentils is reported to be reduced or inactivated by diverse preprocessing and processing procedures (e.g. cooking, fermentation, soaking, germination, or mechanical methods such as dehulling and milling), thus helps to eliminate the major difficulties in the consumer's mind about including lentil-based products in their diet. In particular, the greatest part of ANFs (e.g. tannins) can be found in the seed coat and thus it could be reduced in LF by pre-treatment methods such as dehulling (physical removal) and grinding/milling (size reduction) of whole or decorticated seeds. More recently, a number of these compounds (e.g. saponins, phytic acid, lectins) are attracting large attention in the fields of biochemistry, medicine, pharmacology, and nutrition as a result of their beneficial anticancer, antimicrobial properties in humans (Mattila et al., 2018; Romano et al., 2021).

I.9. High-Temperatures and/or Drought Stress Constraints on Lentil

Lentil production as almost all cool-season legumes is impacted by several biotic and abiotic factors that results in low profitability leading to grave reduction in cultivated areas dedicated to this crop. On global scale, high temperatures and drought stress conditions are considered today as the most important environmental features, which frequently restrict the growth and productivity of major crop species including lentil (Sehgal et al., 2017). Changes in temperature thresholds, even by a single degree beyond the threshold level, are sure to affect the production potential of crops. Global climate change is an added concern, increasing overall temperatures, altering the distribution of precipitation and aggravating drought conditions in semi-arid and arid areas and, ultimately, compromising crop productivity in numerous regions worldwide (Awasthi et al., 2014). The prevalence of drought, accompanied by high temperatures, are anticipated to amplify in the near future (IPCC, 2022).

I.9.1. High-Temperatures Stress

High-temperature stress or heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development (Wahid et al., 2007). In general, a transient elevation in temperature, usually 10-15 °C above ambient, is considered heat shock or heat stress. However, heat stress is a complex function of intensity (temperature in degrees), duration, and rate of increase in temperature. The extent to which it occurs in specific climatic zones depends on the probability and period of high temperatures occurring during the day and/or the night. Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperatures (Wahid et al., 2007).

Lentil like many cool-season legumes, is a sensitive crop to the rising temperature and its growth and development are hindered because of high temperature. At various stages, temperature requirement varies, and it ranges from 18 to 30°C, cooler temperature is required at vegetative stage, while warmer temperatures are needed at the time of maturity (Choudhury et al., 2012). Under the changing climatic scenario, the length of heat period has been increased in comparison to chilling period. Some of the responses of plants exposed to heat stress are sterility of pollen grain and flower drop crops, which are exposed to increasing temperature stress specifically at reproductive stage, the most critical period for all the crops (Hasanuzzaman et al., 2013).

The maximum temperature above 32/20°C (the ratio represents max/min temperature) at flowering and pod filling stage in lentil can sharply deteriorate the quality of the grain and also reduce the grain yield. Heat stress during legume reproduction causes significant loss of seed yield, primarily by compromising seed setting and/or subsequent seed filling (Patriyawaty et al., 2018). It should be noted that failure of seed setting by heat stress imposed at the early reproductive stage cannot be rescued, usually leading to fatal and irreversible yield loss, while compromised seed filling by heat stress imposed at the late reproductive stage may be to some extent recovered by subsequent proper cultivation management.

According to the reports, heat waves (35 °C) consecutively for 6 days influence lentil production, and results in 70% reduction across southeastern Australia (Delahunty et al., 2015). Heat stress during germination decreases the germination rate in lentil. Heat stress during the critical period, i.e., bracketing flowering, disrupts sexual reproductive processes including microsporogenesis and megasporogenesis, pollen production and viability, stigma receptivity, pollen germination and

tube elongation, fertilization, early embryogenesis inducing massive damage of seed number, increase in lipid peroxidation, and reduction in photosynthetic efficiency. For example, pre-anthesis heat stress impairs anther development and decreases pollen production and fertility in chickpea, while heat stress during anthesis and/or post-anthesis reduces stigma receptivity, pollen germination, and pollen tube growth, restricting the success of double fertilization (Devasirvatham et al., 2012ab).

Studies in lentil report that accessions tolerant to heat stress exhibit superiority in functionality of pollen and greater antioxidants level and reduced reproductive period, reduced seed set, caused pod abortion, and forced maturity (Bhandari et al., 2016). Therefore, for this crop, temperatures >35/25°C is identified as harmful for growth and yield and can be used as critical temperature for differentiating the heat tolerant and sensitive lentil accessions (Gaur et al., 2014; Sita et al., 2017b).

I.9.2. Drought Stress

Drought is a meteorological term and is commonly defined as a period without significant rainfall. Generally, drought stress occurs when the available water in the soil is reduced, and atmospheric conditions cause continuous loss of water by transpiration or evaporation. Drought stress tolerance is seen in almost all plants but its extent differs from species to species and even within species. Today, drought is known as the single most devastating environmental stress, which deteriorates crop productivity more than any other environmental stress (Farooq et al., 2012). Alarmingly, it is predicted that 10% of the total land will suffer from drought stress during early of 21st century that can be increased up to 40% by the end of this century (Burke et al., 2006).

To date, much has been done in research for drought resistance/tolerance, however the outcome of these efforts has not met the demand for crops production. This could be explained with the extreme variability and complexity of drought stress impacts. Firstly, drought can affect plants in differential stages of their growth; either early during plant germination, in vegetative developmental stage (intermittent drought) or at the end of growing season in reproductive stage (terminal drought). Out of these, terminal drought contributes to the greatest severe yield losses as it decreases fertility of the plants (Bernier et al., 2007). Secondly, drought has diverse intensities and effects and plants have developed several strategies to deal with them on different levels of their phenological, morphological and anatomical structure as well as on the levels of various physiological and biochemical processes.

Among all the legumes, lentil has been characterized by moderate tolerance to drought and can be grown in dry areas (Abraham, 2015). During its growth and development, minimal water is needed, however, the productivity is limited due to variable annual rainfall which increases with the occurrence of prolonged drought periods (Dai, 2011). Drought stress also disturbs the metabolism, osmoregulation, and concentration of photosynthetic pigments, in lentil (Biju et al., 2017). The impact of drought stress differs depending on the stages of crop development. At flowering or podding period, drought influences negatively vegetative and reproductive growth resulting to reduced leaf area (48–55%), flower production (22–55%), number of pods and seeds (27–66%), and total dry matter (32–50%) with significantly higher abortion of pods and flower drop and aborted pods (Shrestha et al., 2006).

Drought avoidance and tolerance are the two important mechanisms engaged in lentil for combating water stress. Traits linked to root are the central parameters of drought avoidance mechanism, and selection for root-associated traits offers drought tolerance to the crop and ultimately increases the yield and productivity of the crop. In lentil, varieties with short cycle and early-maturity such as Precoz, Bakria, BARI M4, BARI M5, SBARI M6, and Idlib 3 display a good adaptation to drought stress and reflect drought avoidance. Early or delayed flowering and pubescence play a crucial part in protecting plants exposed to water-deficient conditions. Singh et al. (2014b) revealed the correlation of agro-morphological characters with drought tolerance. To breed for traits for drought tolerance, two approaches can be used: short-term approach for evaluating the genetic variability for drought tolerance in lentil germplasm and long-term approach to insert necessary traits from CWR species to the cultivated varieties.

I.9.3. Combination High-Temperatures and Drought Stress

Increased ambient temperatures and frequently occurring water deficit stress episodes are now the most important risk to yield, chiefly since large range of agricultural land used for legumes and cereals cultivation are under rainfed conditions. The synchronized occurrence of drought stress events with high-temperatures waves subjects crops to a combination of water-deficit and heat stress in the field, and this combination intensifies the impacts of water-deficit stress, as higher temperatures rise water loss due to higher evapotranspiration and, by consequence, affecting soil water resources (Reza et al., 2022).

The impact of high temperature and drought stress on grain yield are complex and involve several processes such as nutrient assimilation and their mobilization to various reproductive organs,

accumulation of stem reserves, gametogenesis, fertilization, embryogenesis, and endosperm as well as seed development. The existence of heat stress associated with drought stress at any growth stage can disturb the production of the crop, however, the seed filling phase is decisive for determining average seed weight, seed composition and, consequently, the final quantitative and qualitative yield (Prasad et al., 2017).

Heat and drought stresses often occur in combination, yet their interactive impacts on crop production and productivity have received a little consideration (Barnabás et al., 2008), apart from a few studies (Awasthi et al., 2017; Canci and Toker, 2009; Hamidou et al., 2013; Sehgal et al., 2017). Very limited effort has been made entirely on the influences of the combined stress on seed filling and nutritional quality. The combination heat-drought stress damage several molecular, biochemical and physiological functions, to more severely impair growth, quality, and yield, compared with their individual impacts (Pradhan et al., 2012). Likewise, the combined heat-drought stress have different effects on cellular processes in plants, compared to their individual impacts, indicating stress-specific responses (Sehgal et al., 2018, 2017).

Grain filling is a key growth period for all crops, which comprises mobilization and transport processes essential for importing many elements, and numerous biochemical processes for the synthesis of proteins, carbohydrates and lipids in the developing seeds (Awasthi et al., 2014). It was reported that seed filling processes and the accumulation of reserves in the developing and maturing seeds are extremely sensitive to environmental variations, which impact the qualitative and quantitative characters of the final yield (Yang and Zhang, 2006). High-temperatures and drought stress can affect the accumulation of various seed constituents, primarily starch and proteins via inhibiting the enzymatic processes, synthesis of starch and proteins (Ahmadi and Baker, 2001; Sehgal et al., 2018).

When drought and heat stress are applied together, this decrease leaf water availability more harshly, leading to accelerate senescence, acute chlorosis and membrane damage, severe inhibition of photosynthesis and synthesis of assimilates in leaves were revealed by numerous studies, for instance, in chickpea (Awasthi et al., 2014); lentil (Sehgal et al., 2017; Sita et al., 2018) and barley (Roohi et al., 2013). The effects of each of these stresses, when applied individually on plants may vary, depending upon its intensity and duration, however, some common symptoms appear as general decrease in vegetative biomass, reproductive growth and yield. The impacts of heat and drought stresses may differ, when applied singly; for example, heat stress through seed filling may

accelerate or even inhibit the seed filling process, and diminish the interval of filling and restrain the accumulation of numerous reserves (Chakrabarti et al., 2013). Drought stress slowed down the seed filling process due to water limitations, and by consequence lead to inhibitory effects on seed size and numbers. Whereas these two stresses influence negatively the seed filling in a different way, each stress leads to decreased seed size due to decrease in the cell number in endosperm/cotyledons, inhibitions or acceleration of seed filling rate and many biochemical pathways. Differences may also happen in the relative composition of seed reserves in response to either of these stresses, still both stresses affect severally seed quality (Choukri et al., 2020; El Haddad et al., 2022; Sehgal et al., 2017).

The combined impacts of heat and drought stress are an excellent example of two abiotic stresses occurring in the field simultaneously. Little is known about on the combined effects of heat and drought stress on crops, with most studies reporting very severe effects on crop growth and yield (Awasthi et al., 2017; Hamidou et al., 2013; Sehgal et al., 2019). The impact of the synchronized stresses is more pronounced during early part of reproductive processes, especially micro-and mega-sporogenesis, pollen and stigmatic function, anthesis, pollination, pollen tube growth, fertilization, and early embryo development, compared to individual drought or heat stress (Prasad et al., 2015). Malfunction in any of these processes will considerably decline the fertilization amount or result in early embryo abortion, leading in lower seed or grain numbers and consequently to lower crop yield. Significant reduction showed in crop yields under combined stress environment have been attributed to several explanations, such as metabolic variations, decreases in the period of crop developmental phases (Reddy et al., 2004). Reduction in light perception, reduced phenology, and perturbation of processes involved in carbon assimilation such as transpiration, photosynthesis and respiration may lead to fewer, malformed and smaller seeds (Awasthi et al., 2017, 2014), which are economically less effective.

Grain-filling period declines more under combined heat-drought stress than individual stress, as evidenced in wheat (Farooq et al., 2017a,b), chickpea (Awasthi et al., 2014) and lentil (Sehgal et al., 2019, 2017). Both stresses can occur at the same time after anthesis to restrict seed-filling duration, and by consequence in poor-quality grains in wheat, due to the substantial decrease in seed dry weight, seed numbers, and starch content. The proportion of transport of non-structural carbohydrates in endosperm tissue declines, as in wheat, in response to dual heat and drought stress (Plaut et al., 2004). In addition, there is a high decrease in starch accumulation because of

combined heat-drought stress applied at seed filling period, which has led to more extreme inhibition of starch synthesizing enzymes, compared to individual stress treatments. Combined heat-drought stress can also decrease nitrogen pool due to a decline in free amino acids holding numerous transfer elements related to metabolism of nitrogen and other osmotic compounds (Awasthi et al., 2014).

The response to dual stress situation is crop specific, for example, in soybean plants, exposed to combined stress, seeds exhibited higher protein contents, but inferior oil contents compared to controls/individual stresses (Dornbos and Mullen, 1992). In cereals such as barley and wheat, the combined stress treatment decreased starch accumulation but augmented protein content than single stress treatment (Savin and Nicolas, 1996). In Brassica species, exposed to combined heat and drought stress, seed protein content increased while seed weight declined at higher temperature (35/18°C), compared to moderate temperature (28/18°C) (Gan et al., 2004) (Figure I.4). Considering that heat and drought stress are often experienced together and would be a major hazard for crops production in future, extra studies are required to assess the impacts of these two stresses on mechanisms at various organizational levels affecting seed yield and quality in various crops.

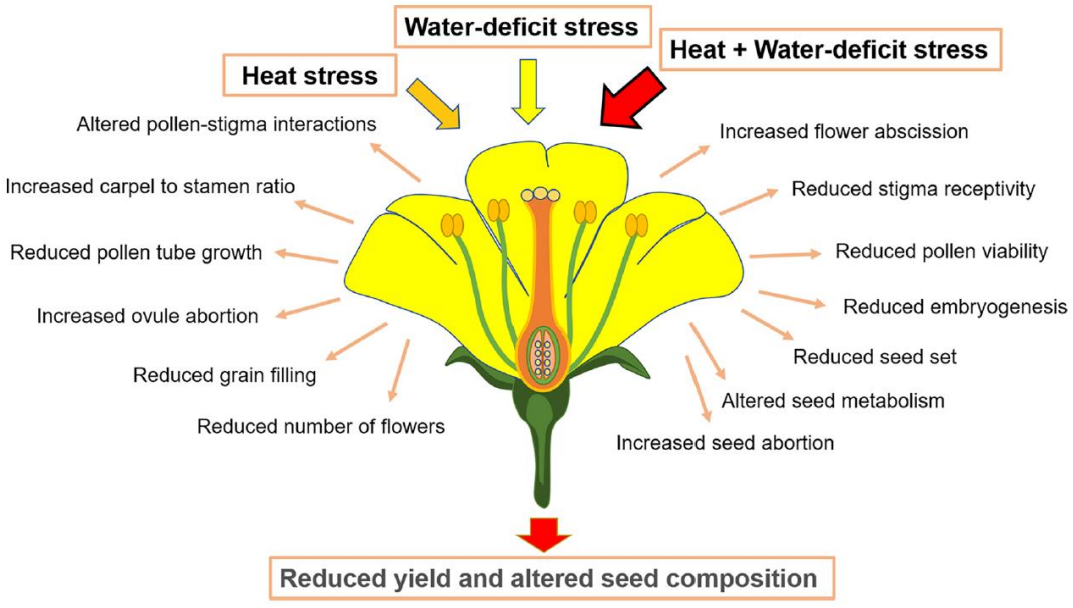

Figure I. 4. *Different reproductive processes of plants affected by water-deficit, heat stress and/or water-deficit and heat stress combination. The impact of water-deficit, heat and/or water-deficit and heat combination on these processes is shown to result in yield reduction (Sinha et al., 2021)*

I.10. Impact of Abiotic Stress on Metabolic Function of Legumes

I.10.1. Temperature and Drought Induced Oxidative Stress

High-temperatures and drought stress alter normal metabolic functioning of plants, which developed some regulation mechanisms to stress conditions such as anti-oxidative machinery. Reactive oxygen species (ROS) such as superoxide anion (O_2-), hydrogen peroxidase (H_2O_2), hydroxyl (OH), alkoxy radicals (RO) and singlet oxygen ($^1O_2$) are normally synthetized as byproducts of various cellular oxidation process and act useful as secondary messengers. However, there is a delicate balance between ROS generation and its scavenging and over-production can be destructive. Excessive concentration of ROS results oxidative damage to lipids, proteins, nucleic acids and hereditary material (like DNA) leading to altered intrinsic properties of biomolecules and cell death (Farooq et al., 2017a).

Each plant has its own anti-oxidative system where ROS-scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), peroxidase (POX) and glutathione reductase (GR) come into play along non-enzymatic antioxidants such as ascorbate, glutathione, tocopherols, carotenoids, ascorbic acid and phenolics. Among the enzymatic antioxidants, SOD acts as a first line of defense via detoxification of superoxide radicals and H_2O_2 and molecular oxygen thereby preventing oxidative damage. APX is a key enzyme in the glutathione ascorbate pathway and helps to regenerate NADP+ and converts H_2O_2 to water (Nadeem et al., 2019, Figure I.5). APX helps also to remove H_2O_2 whereas GR and DHAR assist by providing a substrate for the reactions. During oxidative stress, the concentration of antioxidants may be increased more in the recovery phase in the stress period, as observed in pea (Mittler and Zilinskas, 1994; Osman, 2015), soybean (Guler and Pehlivan, 2016), green bean (Yasar et al., 2013), chickpea (Patel et al., 2011) and lentil (Sita et al., 2022).

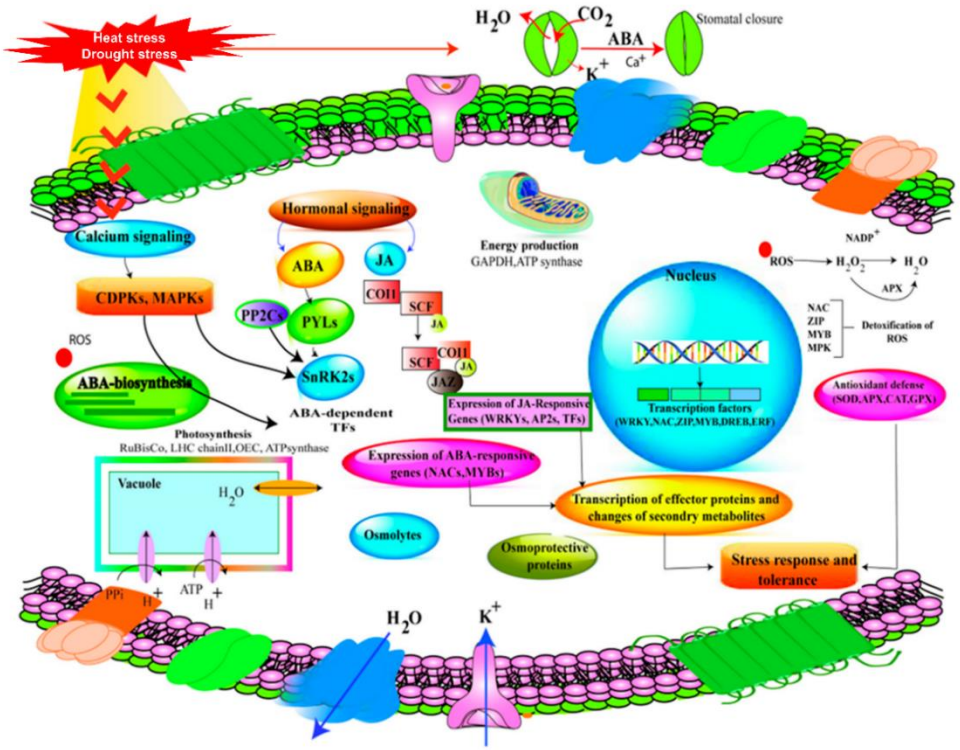

Figure I.5. *Schematic representation of heat and drought tolerance in legumes. Reactive oxygen species (ROS), Ca2+, ABA, and JA are activated under abiotic stress. Stress induces biosynthesis of ABA and JA, which, in turn, up-regulate the transcription of ion transporter genes. Overexpression of transcription factors (WRKY, GmNACs, DREB, ZIP, AP2/ERF, MYB) has been reported under drought and heat stress. ABA, abscisic acid; JA, Jasmonic acid (Nadeem et al., 2019)*

ROS generation is an indicator of cellular damage due to lipid peroxidation and altered membrane permeability under heat stress conditions (Potters et al., 2007). Under high temperatures, antioxidative system gears up for easing heat-induced oxidative stress as observed in selected fabaceous plants such as chickpea (S. Kumar et al., 2013), soybean (Djanaguiraman et al., 2011) and lentil (Mansoor and Naqvi, 2013). Chickpea plants exposed to 45/40 °C (D/N) displayed wide-ranging expression of enzymatic (SOD, CAT, APX, GR) and non-enzymatic antioxidants (Ascorbate, glutathione and proline) (Kaushal et al., 2011). In addition, Kaushal et al (2011) reported that the stress injury (as membrane damage) increased with elevation of temperatures while cellular respiration, chlorophyll content and relative leaf water content reduced as the temperature increased to 45/40 °C. Similarly, it has been noted that SOD, APX, GR, GST, GPX and POD activities under drought stress are amplified in resistant cultivars of common bean (Saglam et al., 2011), chickpea (Mohammadi et al., 2011; Patel and Hemantaranjan, 2012) and soybean (Akitha Devi and Giridhar, 2015).

In chickpea, the endogenous proline was raised to 46 μmol g^{-1} dw at 40/35 °C but declined to 19 μmol g^{-1} dw at 45/40 °C that was linked with significant inhibition of growth at this temperature (Kaushal et al., 2011). The enzymes associated with carbon fixation (RUBISCO), sucrose synthesis (sucrose phosphate synthase) and sucrose hydrolysis (invertase) were highly inhibited at 45/40 °C. It was noted also that the application of endogenous proline on plants enhanced their proline content up to 63 μmol g^{-1} dw and presented less injury to membranes, and enhanced chlorophyll and water contents, especially at 45/40 °C. Moreover, the oxidative injury was strongly decreased coupled with elevated levels of enzymatic and non-enzymatic antioxidants. An important improvement was also observed in the activities of enzymes of carbon metabolism in proline-treated plants. Kaushal et al (2011) concluded that proline improve heat tolerance to chickpea's growth by diminishing the cellular injury and protection of dynamic enzymes correlated to carbon and oxidative metabolism and exogenous application of proline appears to have a countering consequence against elevated high temperatures on chickpea.

In lentil, Chakraborty and Pradhan (2011) reported similar observations in their heat treatment experiments, where tolerant varieties had higher anti-oxidative properties than sensitive ones when exposed to high temperatures (35–45 °C; D/N). Recently, Bhandari et al. (2020) observed that the expression of enzymatic antioxidants (SOD, CAT, APX, GR) and non-enzymatic antioxidants declined remarkably with heat stress, more so in lentil than in chickpea. Carbon fixation (assessed as Rubisco activity) and assimilation (assessed as sucrose concentration, sucrose synthase activity) were also decreased more in lentil than in chickpea, at all the stressful temperatures, resulting in more inhibition of plant biomass (shoot and roots), damage to reproductive function and severe reduction in pods and seeds.

Mungbean exposed to high temperatures (50 °C for 2 h) also expressed enhanced levels of anti-oxidative enzymes such as CAT, POD, SOD and APO in thermo-tolerant accessions (Mansoor and Naqvi, 2013). Ascorbic acid (Kumar et al., 2011) and glutathione (Nahar et al., 2015) applications to heat-stressed mungbean declined the oxidative damage by improving the endogenous levels of ascorbic acid, glutathione, proline and activating the enzymes of glyoxalase system. Bhardwaj et al. (2021) tested the effectiveness of heat priming (HPr, 6 h at 35 °C) the lentil seeds and foliar treatment of γ-aminobutyric acid (GABA; 1 mM) under heat stress (32/20 °C). The outcomes exhibited that observed the combined HPr+ GABA significantly improved the photosynthetic function, measured as photosynthetic efficiency, chlorophyll concentration, and

sucrose synthesis; and significantly reduced the oxidative damage, which was associated with a marked up-regulation in the activities of enzymatic antioxidants. The combined treatment also facilitated the synthesis of osmolytes, such as proline and glycine betaine, by upregulating the expression of their biosynthesizing enzymes (pyrroline-5-carboxylate synthase; betaine aldehyde dehydrogenase) under heat stress. The HPr+GABA treatment caused a considerable enhancement in endogenous levels of GABA in leaves, more so in the two heat-sensitive accessions of lentil.

I.10.2. Sugar Metabolism in Flowers During Stress Combination

Sugars play a vital role in many reproductive and developmental processes. Flower development is energy demanding as evident by the high respiration rates and increased mitochondrial activity of floral organs (Goetz et al., 2017). Sugars are imported from leaf tissues and/or sepals symplastically or apoplastically and distributed to floral reproductive organs. The conservation of the imported sugars can be takes place in the vacuole or cytosol or metabolized. The developmental stage of the flower determines the percentage of sugar import by flower tissues. Developing anthers and filling grains for example show strong sink activity (De Storme and Geelen, 2014).

The process of sugar import requires enzymes such as cell wall invertases (cwINVs) and sucrose synthases (SUSs), as well as sugar transporters (STPs) have vital role in sugar import process to make available sugar monomers for metabolism. Being a nutritive tissue, the tapetum needs high percentage of carbohydrates which are later converted into the locular fluid (upon tapetal degeneration) to support pollen cell wall synthesis and accumulation of carbon reserves in pollen for tube development. Sugar is indispensable for the viability of pollen as declined sugar accumulation in walls of anther and pollen correlates with reduced pollen viability. Also, higher activities of cwINVs in developing pollen walls during pollen development facilitate the formation of male gamete (Goetz et al., 2017).

Deficiencies in sugar metabolism under abiotic stresses are accordingly expected to negatively influence the reproductive development and fertilization at several points (Jain et al., 2007). Jin et al. (2013) observed that starch granules were abnormally positioned outside of anthers, in the lemma and palea in water-deficit-stressed rice, indicating impaired tapetal functions under water-deficit stress. The expression of INVs, hexokinases (HXKs) and glycoside hydrolase were also downregulated in rice flowers demonstrating impaired utilization of sugars in anthers under water-deficit stress. Likewise, in wheat, gene expression and activity of cwINVs and vINVs are decreased under water-deficit and pollen development is affected (Koonjul et al., 2005). Several

studies also reported decrease in sucrose contents and reduced gene expression and enzymatic activity of cwINV in microspores and anthers under heat stress in mungbean (Kaur et al., 2015), chickpea (Kaushal et al., 2013) and sorghum (Jain et al., 2007). Recently, Santiago et al. (2021) reported a significant decrease in sucrose and glucose concentrations along with reduced expression of the sucrose transporter PvSUT1.1 in whole flowers and anthers of a heat susceptible accession of common bean exposed to high temperatures.

Sita et al. (2018) indicated that heat stress inhibited the enzymes related to sucrose and starch metabolism in lentil seeds, which might further restrict sucrose import into seeds. In addition, acid invertases (for the lysis of sucrose into glucose and fructose) declined more in heat sensitive lentil accessions, and therefore had less decrease in tolerant accessions. The minor acid invertase activity in heat-stressed seeds result to decline the capacity of sucrose utilization, which might reduce the import of sucrose into seeds. High-temperatures inhibited also the starch-synthesizing enzyme to a greater extent than sucrose synthase, suggesting impaired diversion of hexoses for starch formation (Sita et al., 2018).

Further, Sehgal et al. (2017) revealed that sucrose synthesis in lentil leaves was enhanced under heat stress, especially in tolerant accessions, but not under drought stress or the combined stress, and matched the activity of the sucrose-synthesizing enzyme (sucrose-P-synthase). In contrast, sucrose content and sucrose-P-synthase activity declined in seeds under all stress treatments, but less so under heat stress. In addition, drought stress affected starch accumulation more than heat stress in both leaves and seeds. The reduction in seed weight due to stress is principally attributed to a reduction in starch accumulation, which was associated to a decrease in the activity of the starch-synthesizing enzyme as well as poor availability of sucrose to seeds. Moreover, sucrose hydrolysis by acid invertase increased in leaves under drought and heat stress, but not under the combined stress. In seeds, sucrose hydrolysis increased only under heat stress. However, starch hydrolysis by b-amylase increased under drought as well as heat stress in both leaves and seeds, with some exceptions in sensitive accessions. The end products of both enzymes are reducing sugars, which increased in leaves and seeds, more so under drought stress than heat stress, unlike the expression of these enzymes, suggesting differences in the response of two organs. Nevertheless, the combined stress was highly detrimental to carbohydrate metabolism in both leaves and seeds, resulting in stunted plants, and fewer and smaller seeds. Leaf and seed sucrose concentrations were strongly correlated with seed yield than leaf and seed starch concentrations.

Sucrose synthase in seeds was highly correlated to seed yield than starch phosphorylase. The activity of enzyme acid invertase and the concentration of reducing sugars in leaves and seeds in lentil were also correlated with seed yield (Sehgal et al., 2017).

I.10.3. Hormone Regulation under Heat and Drought Stress

Phytohormones namely abscisic acid (ABA), gibberellic acid (GA), auxin, jasmonic acid (JA), brassinosteroid (BR), ethylene (ET), and cytokinin, play central roles in flower development period (Itai, 2018). Although, GA plays an important part during the transformation of a shoot apical meristem to a floral meristem, GA is also indispensable for anther and pollen development. Auxins are essential for organogenesis and are crucial for carpel development. Likewise, auxin, GAs and JA are essential for petal development, and GA along with BR, cytokinins, auxins, and JA are involved in anther and pollen development (Chandler, 2011).

Heat or water-deficit stress disrupt the hormonal balance of plants and reduce flowers' fertility. Hormones help in alleviating the damage to pollen resulted by high-temperature stress. A large number of genes involved in the biosynthesis and signal transduction of hormones such auxin, GA, ABA and ET display differential expression in flower tissues under heat stress (Liu et al., 2020). Unlike vegetative tissues, heat stress was reported to disrupt the reproductive function by altering auxin biosynthesis in the anthers, decreasing auxin levels which lead to pollen abortion (Sinha et al., 2021). Auxin can also regulate ROS homeostasis by improving the expression of enzymes involved in ROS detoxification.

Similarly, water-deficit stress alters GA biosynthesis and signaling in flower tissues which has been associated with male sterility. Since GA-deficiency leads to abnormal anther development and male sterility, heat or water-deficit-induced declines in GA could be an additional important factor during heat/water-deficit-induced male sterility (Sakata et al., 2014). Likewise, ABA is a central hormone enhanced during drought stress and increased ABA and JA signaling in flowers under water-deficit stress is known to cause male sterility and damage reproductive development (Jin et al., 2013). ABA was found to play a key role in vegetative plant responses to water-deficit and heat stress combination (Zandalinas et al., 2016). It is thought that enhanced ABA levels are driving an increase in ROS levels that could disrupt different reproductive processes during water-deficit or heat stress, and that ABA accumulation suppressed tapetum PCD during heat stress (Zhang et al., 2021). Cytokinins were reported to have defensive roles during heat stress or water-

deficit (Rivero et al., 2007), however, heat stress reduces decreases cytokinin contents (Wu et al., 2016).

Phytohormones have both synergistic and antagonistic interactions. For example, the increase in cytokinin concentration under drought in plant xylem sap promotes stomatal opening by reducing its sensitivity to ABA. The endogenous concentration of auxin, gibberellin and cytokinins decreases during drought while ABA and ethylene tend to increase in almost all plants (Weyers and Paterson 2001). An increase in ABA concentration during drought is due to a reduction in ABA catabolism preventing its symplastic entry from both rhizosphere and phloem. Increased xylem pH (alkalinization) during drought also promotes ABA loading to root xylem (Hartung et al. 2002). For instance, in kidney bean (*Phaseolus vulgaris* L.), reduced stomatal conductance was linked with an increase in ABA accumulation promoted by rewatering (Miyashita et al. 2005). ABA enhances root hydraulic conductivity which is responsible for increased water uptake and transport in the plants. ABA also improved the production of super radicals and H_2O_2, improving the activities of antioxidant enzymes such as GR.

I.11. Adaptation Mechanisms to High-Temperatures and Drought Stress of Lentil

Lentil is a widely adapted crop over different environments including arid and semi-arid areas frequently influenced by high-temperatures and/or low moisture (Delahunty et al., 2015). The following three classes comprise the adaptative responses to drought stress and high temperatures:

i. Heat and drought escape: The success of plant in water deficient and heat stress environment can be dependent only on its phenology (Kumar et al., 2013). Before stress arises, plants will complete their reproductive cycle during the wet season (early flowering). Tough flower initiation is susceptible to increasing temperatures and drought stress in lentil (Sehgal et al., 2019), and early flowering and maturity is an scape mechanism especially in the Mediterranean spring-season environments.

ii. Heat and drought avoidance: Plant morphological, anatomical, and/or biochemical adaptation are important strategies for avoiding heat stress and/or water stress. (e.g. development of succulence of leaves and roots, reducing of transpiring surfaces, sunken stomata, presence of specialized photosynthetic pathways, thick cuticule, extensive root growth, efficient water use). These mechanisms can be either induced by stress (avoidance)

or could have constitutive character e.i. are also present in non-stressed conditions (adaptation).

iii. Heat and drought tolerance: Is commonly referred to as a sequence of biochemical changes caused by heat stress and/or water deficiency. (e.g. osmoprotection, anti-oxidative enzymes induction, chlorophyll degradation, membrane stability).

I.12. Strategies to Improve Breeding for Heat and Drought Tolerance in Lentil

Visual selection, selection for physiological traits associated to plant response to high-temperature and/or drought stress, empirical selection for yield and marker assisted selection (MAS) are four important selection methods widely used to improve heat and drought tolerance through breeding (Choudhary et al., 2018). However, the initial step in the breeding procedure is to identify genetic diversity for economically important traits. Quantifying variation in morphological characters targeted for selection and adaptation can be used to determine genetic diversity. This approach has been used in lentil under heat stress (Delahunty et al., 2015) and drought stress (Babayeva et al., 2014). In addition, new DNA based fingerprinting technologies can be used to assess the degree of diversity among potential parental lines.

Several breeders utilize late planting to induce high levels of temperatures from anthesis to the grain filling stage in order to cause heat stress. (Krishnamurthy et al., 2011). The selection of heat-tolerant accessions for lentils can be done at a temperature over 35 °C at the reproductive stage. (Delahunty et al., 2015; Sehgal et al., 2019a, Sehgal et al., 2017). However, due to the difficulty of screening under field conditions, accurate phenotyping approaches can be used to assess heat-tolerant traits in the field. (Basu et al., 2015). Nevertheless, combining field and controlled screening is the most effective method for determining heat-tolerant accessions since field and controlled screening under controlled environments would not reproduce similar results in field condition. Other researchers used a different approach in which plants were first cultivated in field conditions in pots before being moved into controlled environments at the flower initiation stage to expose them to the proper temperature during anthesis. (Choudhury et al., 2012; Sehgal et al., 2018).

High precision laboratory methods have been used to assess lentil accessions for traits such as rate of photosynthetic activity, pollen viability, and stomatal conductance. (Bhandari et al., 2016; Sita et al., 2017b). Heat-tolerant accession has been also identified by evaluating the accessions in

controlled conditions under high-temperature conditions (Guiguitant et al., 2017). The main factor to take into consideration when picking a screening procedure is relevance to the target environment. If quantitative trait loci (QTL) linked to superior heat tolerance have been identified, then molecular markers associated with these QTL can be used at any time during the selection process to conserve these chromosomal regions in the progeny. Only a few number of research has been done to find molecular indicators of heat stress in lentil.. Singh et al. (2017) found two major QTL markers (qHt_ss and qHt_ps) associated with seeding survival and pod set. This would present further opportunities to investigate potential genes and develop of molecular markers for enhancing lentil cultivars with heat tolerance.

For drought stress, screening techniques based on biochemical, morphological, and physiological traits have been widely used to find promising accessions that are well adapted to water stress. Pubescence and early or delayed flowering are crucial for protecting plants from water deficient conditions. Drought tolerance can be estimated by assessing characteristics like relative water content, stomatal conductance, and water usage efficiency (Ghanem et al., 2017; Haworth et al., 2018). A number of studies reported that the main characteristics associated with drought tolerance that assist in maintaining the transpiration demand of plants are those of the root, shoot, and leaves. (Mishra et al., 2018; Singh et al., 2013). However, limited studies were able to characterize legume root system and transpiration rate, and in lentil, few researchers reported their work on root and shoot attributes (Idrissi et al., 2015; Sarker et al., 2005). Recently, Priya et al. (2021) investigated root system architecture in lentil germplasm in response to drought, and identified promising drought tolerant accessions based on seedling survival and biomass traits.

I.13. Focused Identification Germplasm Strategy (FIGS)

'FIGS' – or the Focused Identification of Germplasm Strategy is an innovative technique searching germplasm accessions that intends to increase the likelihood of capturing specific adaptive traits in subsets, flexible in size and make-up that come from one or more genebanks. (Street et al., 2016). FIGS is based on the premise that numerous accessions in ex situ collections, or genebanks, have been gathered from the environment in which they evolved, frequently spanning millennia. Therefore, the selection pressures that existed in their environment of origin, which have been demonstrated to affect gene flow and consequently spatial/geographic differentiation, will have played a major role in determining their adaptive features. (Spieth, 1979;

Wu et al., 1975). Nikolai Ivanovich Vavilov was one of the first pioneers to comprehend the importance of ecoclimatic conditions when searching for the sources material to use in plant improvement (Kurlovich et al., 2000; Murzayev, 1988). Vavilov used the term ‘climatic analogy’ for the selection of suitable strains guided by climate and soil data. His ‘differential phytogeographic method’ also has elements that link the morphophysiological trait characters of species and strains to specific environments (Vavilov, 1922).

This innovative trait mining approach for germplasm selection aims to increase international access to relevant information and, consequently, the utility of plant genetic resources conserved in ex situ gene banks. (Endresen et al., 2012). The FIGS strategy presupposes a predictive correlation between a targeted adaptive trait, such as disease resistance, and some eco-geographic characteristics of the original germplasm collection site (with a focus on landraces and crop wild relatives). Recent works have suggested the algorithms and methods for implementation of trait mining using FIGS (Dadu et al., 2019; El Bouhssini et al., 2021; Vikas et al., 2020).

Since FIGS relies on linkage between adaptive traits within in situ populations and long-term environmental conditions, only georeferenced landraces, crop wild relatives, pastures and rangeland species are suitable for the FIGS. While there are approximately 5 million accessions classified as landraces and wild relatives conserved in genebanks worldwide (FAO, 2020), a large proportion of them, especially older ones, lack collection site latitude and longitude data. This does not mean the data necessary to capture georeferences is not available. Many collection missions, even before positioning system (GPS) devices were available, were documented rigorously with good descriptions of collections sites: the NI Vavilov institute (VIR) collection is an excellent example of this, with records dating back to the 1920s.

Recently in May 2022, the ICARDA Morocco genebank is inaugurated constituting a symbol of the regeneration, resilience, and expertise of the people in this region who are working so hard to build a climate smart future. The genebank provide global crop research with vital ‘building blocks’ to develop high-yielding, resilient crops that are adapted to increasingly hostile conditions, making the world a safer place by improving food and nutrition security. The ICARDA Morocco genebank conserves and researches an impressive collection of more 95,000 extra accessions. These plants and seeds are the progenitors of today's crops; they have evolved in the wild alongside them in a harsh environment of heat and water scarcity, and they hold the secrets to traits that can

help global crops become more resilient to stresses including elevated temperatures, pest, and water scarcity. And many of these plants are threatened in the wild (ICARDA, 2022).

Using gazetteers (e.g. the Getty Thesaurus of Geographic Names and the GEOnet Names Server) and various GIS software packages, reasonably accurate coordinates can be determined from collection missions notes. Efforts to develop software tools to automate georeferencing have been made. The BioGeaomancer Project is one such initiative (Guralnick et al., 2006; Hill et al., 2009). Today, multiple methods have also been developed to check the accuracy of captured coordinates like satellite sensors through smart-phone application or website giving the spatiotemporal coordinates details of large scale zones (Kirchhoff et al., 2021). Regardless of the tools used to collect geocoordinates, it is a laborious procedure that needs a large investment of time and resources. However, the utility of recording collection site geocoordinates is becoming clearer with recent developments in GIS software and the availability of agroclimatic and soil GIS surfaces, and more attempts are being made to georeference collections.

FIGS can use a wide variety of data classes, such as anecdotal data from farmers on agricultural attributes, binary data like "irrigated" or "non-irrigated," scalar data like "aspect" or "slope," percentage data like "cloud cover," and continuous data like "precipitation" or "temperature.". However, the procedure is likely to be more effective the finer the data resolution. For instance, climate data can be hourly, annual, or simply a few square meters to 30 km2 in size. Daily data with a resolution of a few meters would be considerably more helpful for the FIGS method than yearly averages at a scale of 30 km^2.

FIGS has been used successfully to identify novel alleles, including new sources of resistance to powdery mildew (Bhullar et al., 2010; Vikas et al., 2020), Sunn pest (Bouhssini et al., 2009), Russian wheat aphid (El Bouhssini et al., 2011), drought resistance in faba bean (Khazaei et al., 2013), stem rust resistance in wheat (Bari et al., 2012) and net blotch in barley (Angessa and Li, 2016) using long-term average monthly data at 1 km^2 gride size. The climatic variables included monthly average minimum temperature, monthly average maximum temperature, monthly average precipitation, monthly average evapotranspiration and monthly average aridity index point data extracted from continuous GIS surfaces modelled from data collection sites (De Pauw, 2008). Since 2012, the ICARDA genebank has used FIGS subsetting to select germplasm when responding to seed requests. More than 75 FIGS subsets were constructed for various traits in bread wheat, barley, durum wheat, lentil, chickpea, grass pea and faba bean. In this context, FIGS

has helped the ICARDA genebank to rationalize the distribution of the genetic resources by sending smaller sets of germplasm whilst maximizing the user's likelihood of receiving the trait variation required. In the long term, this will reduce the resources needed to maintain adequate supplies of seed for requested accessions.

To advance FIGS, it is necessary to keep pace with advances in fundamental eco-evolutionary theory. First, it is essential to identify and incorporate conflicting or concurrent selection pressures imposed by other agents into the ecogeographic profile in order to enhance the prediction of adaptation processes. (Figure I.6) (Stenberg and Ortiz, 2021). Second, following previous suggestions (Khazaei et al., 2013), biogeographic proxies of nonadaptive processes (gene flow and genetic drift) should ideally be regularly taken into account in future FIGS that deal with elusive features. Therefore, breeders should be able to find the most promising evolutionary hotspots for trait mining with the assistance of these adjustments to FIGS. Additionally, FIGS-identified resistant genebank accessions can be used as training populations for genomic prediction based on characterization and genotyping by sequencing. This may lead to identification of useful diversity hotspots hosting accessions that can be advantageously used introgressively in breeding programs. This strategy was suggested to increase production of crops (Longin and Reif, 2014), and subsequently shown to have promising potential to raise biomass yields of sorghum (Yu et al., 2016). Such 'turbocharging' of genebanks offers a cost-effective strategy to tap their valuable plant genetic resources by shifting from 'gene mining' to estimating accessions' breeding values through genomics (Stenberg and Ortiz, 2021).

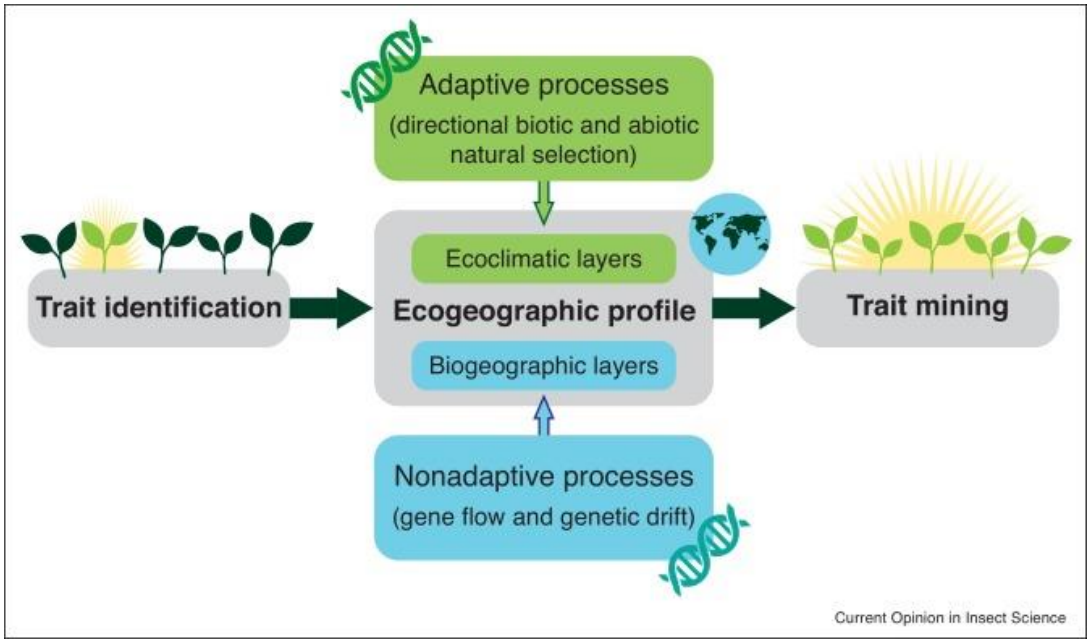

Figure I.6. *Conceptual framework for Focused Identification of Germplasm Strategy (FIGS). The first step in the FIGS process is to identify the type of trait that would be most ideal to confer a desired outcome (e.g. reduction in damage pests cause to a particular plant). Then information about the adaptive and nonadaptive evolutionary processes shaping this trait and its geographic distribution should be obtained and applied in the form of ecoclimatic and biogeographic layers in an ecogeographic profile that highlights the most intense evolutionary hotspots in the plant's range. Focusing trait mining efforts on germplasm from these hotspots should be considerably more fruitful than focusing on other areas (Stenberg and Ortiz, 2021)*

Chapter II : Screening the FIGS Set of Lentil (*Lens culinaris* Medikus) Germplasm for Tolerance to Terminal Heat and Combined Drought-Heat Stress

II.1. Introduction

Lentil (*Lens culinaris* Medikus) is an annual, diploid (2n = 14) and self-pollinated crop. Its seeds are rich in protein (22–35%), fiber, prebiotic carbohydrates and minerals, such as iron and zinc (Kumar et al., 2015). It plays a major role in alleviating malnutrition and micronutrient deficiencies of people living in Central and West Asia and North Africa (CWANA), South Asia, East Africa, and North America (Migliozzi et al., 2015). Being a legume crop, it enhances nitrogen in the soil through symbiotic nitrogen fixation and, hence, plays a crucial role in the diversification and intensification of cereal-based cropping systems, worldwide (Hamwieh et al., 2005). In 2020, the total world area under lentil production was 5.1 million hectares, with a production of 6.5 million tons; in the African continent, Morocco ranked second in lentil production after Ethiopia. Nevertheless, the average production of lentil in Morocco was recorded as 9,044 tons in 2020, reduced by up to 24% compared to 2019 (37,095 tons) due to the extremely severe drought stress that hit the regions. Further, the productivity of lentil in Morocco during last ten years (from 2010 to 2020) still very low with an average of 665 kg ha^{-1}, compared to the world average of 1180 kg ha^{-1} (FAOSTAT, 2020).

Lentil has been mainly cultivated under rainfed conditions in the marginal areas where abiotic stresses, such as drought and heat, significantly reduce crop yield and productivity (S. K. Kumar et al., 2013). During 2007–2008 season, a severe drought struck the Mediterranean region, and caused huge yield reduction of lentil in Morocco, France, the Russian Republic, Spain, the Syrian Arab Republic and Turkey (FAOSTAT, 2020). Next, there was a steep fall in lentil production and productivity in Morocco during 2016, which was declared as the warmest year of this century, and eventually the severe drought stress damaged the total cropped area of about 9581 ha (NASA/GISS, 2022). It is predicted that the global mean surface temperature during mid and late 21st century will increase by 2°C, leading to an extreme variation in precipitation events and creating more heat waves that menace crop cultivation (Giorgi, 2006). In lentil, the reproductive phase is very sensitive to changes in the external environment, and exposure to heat and drought stress during this stage reduces crop productivity significantly (Ibrahim, 2011; Öktem et al., 2008).

Lentil performs well when its reproductive stage coincides with the average day/night temperatures of 15–25°C /8–10°C (Bhandari et al., 2016). However, heat waves (temperatures >32°C) during the flowering and pod-filling stages cause damage to reproductive organs, leading to flower drop, pollen sterility, pod abortion and reducing the total number of seeds in lentil (Delahunty et al., 2015; Kumar et al., 2016c; Sita et al., 2017a; Wahid et al., 2007). On the other hand, the terminal drought stress caused by irregular and deficient precipitation during the reproductive phase shortens the duration of the seed filling phase, by accelerating the process of senescence and maturity and reducing the seed size in lentil (Idrissi et al., 2013). As the combined stress reduces both total number of seeds and seed size, and cause more yield reductions than the individual stresses, the interaction between heat and drought stress is considered the most serious challenge, which has a more significant negative impact on crop yield and productivity than each stress individually (Mittler and Blumwald, 2010; Sehgal et al., 2019; Suzuki et al., 2014). Seed development is a crucial growth period under heat or drought stress in all grain crops; however, their combination affects adversely seed filling by suppressing the transfer of the assimilates needed, leading to low grain yields and poor grain quality (Sehgal et al., 2017).

The combined effects of heat and drought have been studied in some crops such as groundnut (*Arachis hypogaea* L.) (Hamidou et al., 2013), chickpea (*Cicer arietinum* L.) (Awasthi et al., 2017, 2014; Canci and Toker, 2009), barley (*Hordeum vulgare* L.) (Hossain et al., 2012; Savin and Nicolas, 1996) and wheat (*Triticum aestivum* L.) (Prasad et al., 2011; Zhang et al., 2013), but only to a limited extent in lentil (Sehgal et al., 2019). Commonly, traits including early flowering, early maturity and yield under stress conditions were employed as the key traits to identify heat and drought tolerant germplasm in many crops, such as lentil (Kumar et al., 2012) and chickpea (Krishnamurthy et al., 2010). For instance, a heat tolerance index (HTI), based on the yield under heat stress, yield potential and flowering time, has been used effectively to evaluate the heat response in chickpea under heat conditions (Devasirvatham and Tan, 2018; Krishnamurthy et al., 2011). Likewise, several quantitative drought tolerance indices, such as the stress tolerance index (STI), geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HARM) and stress tolerance (TOL) have been used widely to assess accessions with better drought stress tolerance in many crops (Ali and El-Sadek, 2016; Kakaei, 2019; Naveed et al., 2019). Siahsar et al. (2010) reported that STI, GMP and HARM were the best indices for the selection of lentil lines

under drought stress. Additionally, these tolerance indices have been used in selecting superior accessions under heat stress conditions (Jha et al., 2018; Khan and Kabir, 2014).

The adaptation of a accession to terminal drought and heat stress is a sought-after strategy to minimize the economic impact of climate change on agriculture (Benhin, 2006; Dube et al., 2016; Mano and Nhemachena, 2007). The development of new heat and drought tolerant lentil cultivars would improve yield stability and facilitate an increase in area under sustainable cropping systems (Gaur et al., 2013). Nevertheless, it requires extensive screening of the germplasm being conserved in gene banks which demands huge investment and time. To facilitate this process, the 'Focused Identification of Germplasm Strategy (FIGS) approach was developed by the International Center for Agricultural Research in the Dry Areas (ICARDA). FIGS creates 'best-bet' trait-specific subsets of germplasm, by passing accession-level information, especially agro-climatic site information, through a series of filters, which increase the chances of finding the adaptive trait of interest (Khazaei et al., 2013). This approach is based on the premise that the environment highly influences the natural selection process and consequently, the geographical distribution of crop species (Mackay et al., 2012). Previous studies confirm the effectiveness of the FIGS approach in the identification of desirable germplasm in wheat for major biotic stresses, such as Russian wheat aphid (*Diuraphis noxia*) (El Bouhssini et al., 2011), stem rust (*Puccinia graminis* Pers.) (Bari et al., 2012; Endresen et al., 2012) and Sunn pest (*Eurygaster intergriceps* Put.) (Bouhssini et al., 2009), as well for drought stress in faba bean (*Vicia faba* L.) (Khazaei et al., 2013). The present chapter aims to assess genetic variability for heat and drought tolerance in the ICARDA lentil germplasm using the FIGS approach.

The objectives of this chapter are: (*i*) to investigate the individual and combined effects of terminal heat and drought stress during the reproductive phase; and (*ii*) to use indices and identify promising accessions with tolerance to heat stress and combined heat-drought stress for future breeding.

II.2. Materials and Methods

II.2.1. Plant Material and Study Area

A FIGS set comprising 162 germplasm accessions of lentil developed by the ICARDA gene bank in 2013 was evaluated (Annex II.1). These accessions were originated from Pakistan (66), Nepal (68), Ethiopia (13), India (4), Yemen (3), Russia (3), Sudan (2) and Iran (2). The FIGS set, along with the Moroccan cultivar, Bakria as a local check, was evaluated in an alpha lattice design, with

two replicates at two locations: Tessaout (31.42° N, 6.47° W, 68 m altitude) in the 2013–2014 cropping season, and Marchouch (33.56° N, 6.63° W, 392 m altitude) in the 2014–2015 cropping season in Morocco. At both stations, each germplasm was grown in a 2-row plot of 1 m length, with a spacing of 30 cm between rows. In each row, seeds were sown by hand at a 2 cm depth maintaining a 10 cm space between plants, and the total plot size maintained was 0.6 m^2. In general, the Tessaout research station represents a typical Mediterranean semi-arid environment, characterized by a hot dry summer with an annual rainfall of 266 mm (Elhaddoury et al., 2012). The Marchouch station also represents a Mediterranean semi-arid environment, but with higher annual precipitation (400 mm) (Moussadek et al., 2014). The experimental site in Tessaout is silty-clay soil, whereas in Marchouch, it is vertisol.

II.2.2. Treatments

At each of the two locations, three experiments involving the same set of FIGS germplasm were conducted by manipulating the planting date and water supply, in order to impose heat and water stress at the reproductive phase of the plant growth. These three experiments were considered to represent three treatments, namely the normal date of planting (treatment A), late planting with irrigation at field capacity throughout the crop period (treatment B) and late planting without irrigation during the reproductive phase (treatment C), which were referred to as treatments. Treatment A resulted in optimal growing conditions (>150 mm well-distributed rainfall and below 27°C temperature) without any heat and water stress to the plants. Treatment B (planted 50 days after normal planting date with irrigation at field capacity throughout the crop duration) imposed heat stress, as the plants were exposed under field conditions to a temperature above 32°C during the reproductive phase, while regular irrigation at field capacity avoided any water stress to the plants. Treatment C (planted 50 days after normal planting date without irrigation during the reproductive phase) imposed a combined heat and water stress. Treatment A was sown on 20 December, and no irrigation was applied during the crop period as the crop received well distributed enough rainfall at Tessaout (157.9 mm) and Marchouch (167.8 mm). Treatments B and C were planted on 8 February. Irrigation was applied to maintain water supply at field capacity using sprinkler system throughout the crop duration in treatment B, whereas irrigation was stopped from the flowering initiation stage onward in treatment C, to impose water stress in addition to the heat stress.

All the three treatments were kept weed-free throughout the growing season. A wide temperature variation was recorded between normal and late planted treatments at the Tessaout and Marchouch research stations. During the reproductive stage, the averages of the maximum and minimum temperatures were 26.33°C and 12.72°C in Treatment A. However, the maximum temperatures during the flowering stage reached the threshold level of 42°C in treatments B and C at Tessaout and 34°C at Marchouch (Figure II. 1). Therefore, late planting with irrigation at field capacity was successful in imposing heat stress during the reproductive phase of test accessions in treatment B, and late planting without irrigation in imposing drought and heat stress in treatment C.

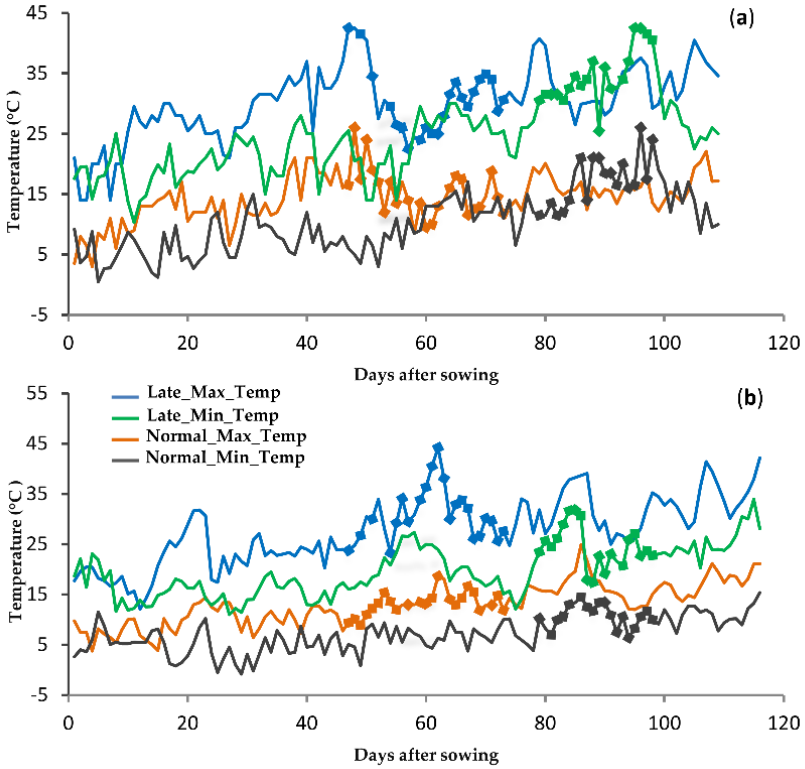

Figure II.1. *Averages of the maximum and minimum temperatures in normal and late planting at Tessaout (a) and Marchouch (b) during the crop season. The square icons indicate the flowering period for both normal and late sown crops.*

II.2.3. Investigation and Calculation of Agronomic Traits

Data were recorded for the phenological traits (days to 50% flowering and maturity) on plot basis, whereas five plants were selected randomly from each plot for the assessment of the morphological (numbers of primary, secondary and tertiary branches, and plant height) and yield (total numbers of filled and unfilled pods, biological yield, grain yield and 100-seed weight) traits following the lentil ontology (Kumar and Rajendran, 2016). Heat tolerance index (HTI) was calculated for each

accession following the multiple regression approach as suggested by Bidinger et al. (1987) and as used in chickpea (Devasirvatham and Tan, 2018). Grain yield under stressed and non-stressed conditions was used to assess tolerance of accessions against the stress. This approach considers grain yield under heat stress conditions (Ys) to be a function of yield potential (Yp), days to 50% flowering (F), and heat tolerance index (HTI) such that the yield of a accession can be expressed as:

$$Ysi = a + bYp + cFi + HTIi + E,$$

where E is the random error with zero mean and variance σ, and a, b, c are regression parameters estimated by least square methods. Heat Tolerance Index (HTI) was calculated for each accession as the difference between the estimated late-season grain yield and estimated optimal-season grain yield plus standardized residuals from regression.

On the basis of grain yield under stress (YS) and normal conditions (YP), the following quantitative tolerance indices were estimated to assess the combined effect of drought and heat stresses:

Stress tolerance index $STI = \frac{Y_{pi} * Y_{si}}{Y_p^2}$ (Fernandez, 1992);

Tolerance index $TOL = Y_{pi} - Y_{si}$ (Rosielle and Hamblin, 1981);

Geometric mean productivity $GMP = \sqrt{Y_{pi} * Y_{si}}$ (Fernandez, 1992);

Mean productivity $MP = \frac{Y_{pi} + Y_{si}}{2}$ (Rosielle and Hamblin, 1981);

Harmonic mean $HARM = \frac{2(Y_{pi} * Y_{si})}{Y_{pi} + Y_{si}}$ (Schneider et al., 1997)

where, Ysi = Yield of a accession under stress condition, Ypi = Yield of a accession under normal sown condition, Ys = Overall genotypic mean under stress condition, and Yp = Overall genotypic mean under normal condition.

II.2.4. Statistical Analysis

Analysis of variance (ANOVA) was performed using the general linear model (GLM) using IBM SPSS statistics 23. Treatment means were compared by least significant difference (LSD). Correlation coefficients (Pearson's) were calculated by multivariate analysis for heat stress condition, while Spearman's correlation coefficient was used for the combined heat-drought stress. Hierarchical cluster analysis using Ward's squared Euclidean distance method was performed for accession grouping.

II.3. Results

II.3.1. Effects of Heat Stress on Morphological, Phenological, and Yield Contributing Traits

The results of ANOVA revealed significant differences among accessions for all traits under stressed and non-stressed conditions at both the locations, Tessaout (Table II.1) and Marchouch (Table II.2). Furthermore, a highly significant variation ($p < 0.001\%$) was observed for all traits among treatments in Tessaout, as well as at Marchouch. The analysis also showed significant accession x treatment interactions for all the traits at both locations, except for plant height, number of primary branches per plant, number of tertiary branches per plant and biomass yield per plant at Tessaout. There existed a wide range of variability among lentil accessions for phenological traits at both Tessaout and Marchouch.

Table II.1. *Analysis of variance (ANOVA) expressed in mean square for different traits among 162 lentil accessions at Tessaout during 2013–2014.*

Source	df	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP	HSW
Accession (A)	161	3179.9 **	9020.6 **	7926.79 **	85.73 *	2809.87 **	3116.53 **	134681.09 **	17979.63 **	97275.47 **	6602.61 **	240.04 **	128.57 **
Treatment (T)	2	8801.5 **	81401.0 **	194468.02 **	87.07 **	4447.67 **	7474.59 **	221759.77 **	6956.78 **	169051.73 **	17534.56 **	847.36 **	153.11 **
A × T	322	1373.3 ns	3564.9 **	9245.98 **	109.86 ns	2796.58 *	2834.41 ns	158553.22 *	30640.38 **	101053.29 *	9055.03 ns	235.05 **	84.96 **
Error	486	2734.1	1675.9	4595.96	181.53	3506.01	3745.33	180293.5	31008.66	125776.68	11795.35	258.51	53.9
R^2		0.83 **	0.98 **	0.98 **	0.61 **	0.74 **	0.78 **	0.74 **	0.66 **	0.75 **	0.74 **	0.84 **	0.87 **

PH, plant height; DF, days to 50% flowering; DM, days to 95% maturity; PBPP, number of primary branches per plant; SBPP, number of secondary branches per plant; TBPP, number of tertiary branches per plant; NTPP, number of total pods per plant; NUPP, number of unfilled pods per plant; NFPP, number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight; df, degrees of freedom. Significant difference at: * $p < 0.01$, ** $p < 0.001$, ns denotes a non-significant difference.

Table II.2. *Analysis of variance (ANOVA) expressed in mean square for different traits among 162 lentil accessions at Marchouch during 2014–2015.*

Source	df	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP	HSW
Accession (A)	161	5998.87 **	12577.23 **	8763.72 **	179.10 **	4794.79 **	2163.08 **	217688.81 **	16449.68 **	164517.17 **	4042.86 **	297.13 **	168.53 **
Treatment (T)	2	23422.11 **	235449.92 **	223732.48 **	103.03 **	46726.49 **	33818.93 **	638843.72 **	45673.66 **	344672.12 **	18250.69 **	976.43 **	67.07 **
A × T	322	6986.42 **	6242.97 **	5907.75 **	222.12 **	7827.42 **	4388.39 **	324520.74 **	35015.62 **	238902.17 **	6559.96 **	382.04 **	86.57 **
Error	486	4714.99	503.09	952.59	122.45	4694.98	2275.94	160769.5	14911.52	121599.93	2839.61	217.94	74.55
R^2		0.88 **	0.99 **	0.99 **	0.80 **	0.93 **	0.95 **	0.88 **	0.87 **	0.86 **	0.91 **	0.88 **	0.81 **

PH, plant height; DF, days to 50% flowering; DM, days to 95% maturity; PBPP, number of primary branches per plant; SBPP, number of secondary branches per plant; TBPP, number of tertiary branches per plant; NTPP, number of total pods per plant; NUPP, number of unfilled pods per plant; NFPP, number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight; df, degrees of freedom. Significant difference at: * $p < 0.01$, ** $p < 0.001$, ns denotes a non-significant difference.

Under normal planting, plant height ranged from 17 to 37 cm, with the overall mean of 25 cm at Tessaout and from 14 to 52 cm, with a mean of 31.51 cm at Marchouch. Heat stress reduced plant height by 18% at Tessaout and by 31% at Marchouch. However, the combined heat-drought stress reduced plant height more than the heat stress: 29% at Tessaout and 35% at Marchouch (Table II.3).

Heat stress and combined heat-drought stress reduced the number of primary, secondary and tertiary branches per plant significantly. The reduction was more pronounced under combined stress conditions at both locations, particularly at Marchouch. The number of primary branches per plant were reduced by 24% at Tessaout and 30% at Marchouch. Likewise, the number of secondary and tertiary branches per plant were reduced by 38% and 63% at Tessaout, whereas in Marchouch, there was an 80% reduction in the number of secondary branches per plant, and a 91% reduction in the number of tertiary branches per plant (Table II.3).

Days to 50% flowering in normal planting conditions ranged from 61 to 78 days at Tessaout, and from 79 to 98 days at Marchouch. The range of days to 95% maturity varied from 100 to 119 days at Tessaout, and from 113 to 126 days at Marchouch under normal planting conditions. Heat stress decreased the crop duration by 23% at Tessaout, and 26% at Marchouch, while the combined heat-drought stress caused 28% reduction in crop duration at Tessaout and 27% at Marchouch. At each location, days to 50% of flowering was almost similar under both stress conditions: 46 days at Tessaout and 55 days at Marchouch (Table II.3).

Under normal planting, the number of total pods per plant ranged from six to 160 at Tessaout, which decreased by 47% under heat stress and 62% under combined heat-drought stress conditions. In contrast, the number of total pods per plant ranged from three to 230 at Marchouch but declined by 72% due to heat stress and 91% as a result of combined heat-drought stress. Likewise, heat stress and combined heat-drought stresses reduced the number of filled pods by 58% and 65% at Tessaout, while it was 69% and 91% at Marchouch, compared to normal planting. The mean numbers of total pods and filled pods per plant were higher under stress conditions at Tessaout than at Marchouch (Table II.3).

Biomass per plant ranged from 1.4 to 39.6 g at Tessaout and from 1.07 to 36.2 g at Marchouch under normal planting conditions. The heat stress reduced biomass by 69% at Tessaout and 77% at Marchouch. However, the reduction in biomass was higher under combined stress at both locations: it was recorded 71% at Tessaout and 85% at Marchouch. Similarly, the combined heat-

drought stress decreased the seed yield by 76% at Tessaout and 90% at Marchouch, more than the heat stress alone (68% at Tessaout; 71% at Marchouch). The mean grain yields under heat stress and combined heat-drought stress were more at Tessaout than at Marchouch. Under the normal planting, the hundred-seed weight ranged from 0.50 to 3.70 g at Tessaout and 0.50 to 3.85 g at Marchouch. The combined heat-drought stress increased the hundred-seed weight by 21% at Tessaout and 10% at Marchouch. However, the increase was higher under heat stress by 38% at Tessaout and 27% at Marchouch (Table II.3).

Table II.3. *Minimum, maximum and mean (±SD) values of traits of 162 lentil accessions under normal planting, heat stress and combined heat-drought stress in the field experiments at Tessaout during 2013–2014 and Marchouch during 2014–2015.*

Trait	Heat Stress Conditions				Heat-Drought Conditions				Normal Conditions			
	Tessaout		Marchouch		Tessaout		Marchouch		Tessaout		Marchouch	
	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD
PH	15.00–28.0	20.32 ± 2.40	15.00–34.0	21.79 ± 3.13	12.75–28.90	17.67 ± 2.91	15.00–30.0	20.52 ± 2.83	17.00–37.0	24.95 ± 2.87	14.00–52	31.51 ± 6.09
DF	41.00–67.0	45.98 ± 4.28	47.00–73.0	54.58 ± 4.15	40.00–67.0	45.92 ± 4.70	48.50–73.0	54.62 ± 4.11	61.00–78.0	65.37 ± 1.90	79.00–98	87.62 ± 5.07
DM	80.00–103	88.21 ± 4.70	81.00–109	87.09 ± 4.82	77.00–101.0	82.97 ± 4.26	72.00–109.0	86.22 ± 4.39	100.00–119.0	115.63 ± 2.66	113.00–126	118.83 ± 2.48
PBPP	1.00–3.67	2.53 ± 0.58	1.00–4.67	2.35 ± 0.71	1.00–3.67	2.29 ± 0.59	1.00–5.00	1.81 ± 0.69	2.00–4.00	3.01 ± 0.68	1.00–5.00	2.59 ± 0.80
SBPP	2.00–17.10	9.42 ± 3.30	1.00–20.00	6.30 ± 3.31	2.00–16.50	8.02 ± 3.39	1.00–17.00	3.82 ± 2.62	4.00–25.00	13.10 ± 0.68	5.33–34.70	19.61 ± 5.98
TBPP	0.15–16.50	6.22 ± 3.43	0.20–8.90	2.54 ± 1.45	0.10–12.30	3.88 ± 3.22	0.00–9.00	1.22 ± 0.94	3.00–21.00	10.57 ± 2.79	1.00–30.70	14.34 ± 4.95
NTPP	2.29–126.0	29.88 ± 19.94	2.00–140.0	18.50 ± 15.89	1.00–70.75	21.22 ± 14.08	1.00–36.00	6.09 ± 3.79	6.00–160.45	56.68 ± 24.08	3.00–230.00	65.60 ± 40.69
NUPP	0.00–83.50	10.83 ± 9.13	0.00–38.0	3.44 ± 2.63	0.00–33.50	5.55 ± 3.47	0.00–14.00	1.82 ± 1.63	0.00–57.20	11.54 ± 7.65	0.00–82.67	17.11 ± 11.31
NFPP	0.50–96.20	19.06 ± 15.19	1.00–102.0	15.06 ± 12.11	0.00–62.75	15.66 ± 11.03	1.00–30.50	4.26 ± 2.13	2.00–129.45	45.14 ± 20.61	1.00–195.33	48.50 ± 35.15
BPP	0.25–15.80	3.99 ± 1.59	0.45–11.89	2.55 ± 1.92	0.50–32.21	3.72 ± 2.34	0.50 – 11.30	1.63 ± 1.03	1.40–39.60	12.86 ± 6.19	1.07–36.20	11.25 ± 4.08
GYP	0.13–3.94	0.88 ± 0.69	0.16–4.89	0.74 ± 0.68	0.12–2.78	0.66 ± 0.55	0.13–2.49	0.27 ± 0.20	0.15–7.71	2.74 ± 1.22	0.39–7.33	2.59 ± 1.49
HSW	0.90–5.00	2.50 ± 0.58	1.10–5.00	2.36 ± 0.59	0.70–4.20	1.94 ± 0.46	0.90–4.30	1.93 ± 0.52	0.50–3.70	1.54 ± 0.51	0.53–3.85	1.73 ± 0.62

PH, Plant height; DF, Days to 50% flowering; DM, Days to 95% maturity; PBPP, Number of primary branches per plant; SBPP, Number of secondary branches per plant; TBPP, Number of tertiary branches per plant; NTPP, Number of total pods per plant; NUPP, Number of unfilled pods per plant; NFPP, Number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight.

II.3.2. Correlations among the Traits under Heat Stress and Combined Heat-Drought Stress

The correlations between the variables under normal planting were highly significant positive correlation at 0.01 level of grain yield with the number of the secondary and tertiary branches per plant and number of filled pods per plant at Tessaout and Marchouch (Tables A2 and A3). A highly positive correlation ($p < 0.01\%$) was also noticed between the grain yield and biomass at

Marchouch. However, grain yield was negatively correlated with days to 50% flowering at Marchouch. Days to 50% flowering were positively correlated with plant height, days to 95% maturity, number of secondary and tertiary branches and hundred-seed weight at Tessaout (Table II.4). Nevertheless, it was positively correlated only with plant height and days of 95% of maturity at Marchouch under normal planting (Table II.5).

Under heat stress, grain yield was positively correlated ($p < 0.01\%$) with the number of the secondary and tertiary branches per plant, the number of total pods and biomass at both stations, Tessaout and Marchouch. Furthermore, grain yield had a positive correlation with days to 95% maturity at only Tessaout. A highly positive correlation of 0.01% was identified among plant height, days to 50% flowering, days to 95% maturity, biomass and hundred-seed weight in heat stress conditions at both stations. In combined heat-drought stress conditions, grain yield was positively correlated with all variables at both stations, except with days to 50% flowering at Marchouch. Additionally, positive associations ($p < 0.01\%$) existed among days to 50% flowering, days to 95% maturity and hundred-seed weight at Tessaout and Marchouch (Tables II.4 and II.5).

Table II.4. *Pearson's correlation coefficients among various traits based on162 accessions of lentil under normal planting (A), heat stress (B) and combined heat-drought stress (C) at Tessaout.*

Trait	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP
				A.	**Under normal planting**						
DF	0.16**										
DM	0.02ns	0.14*									
PBPP	-0.07ns	-0.07ns	-0.05ns								
SBPP	0.24**	0.15**	0.01ns	-0.06ns							
TBPP	0.16**	0.15**	0.02ns	-0.06ns	0.57**						
NTPP	0.08ns	-0.01ns	-0.05ns	0.06ns	0.43**	0.37**					
NUPP	0.09ns	-0.05ns	-0.13*	0.07ns	0.20**	0.16**	0.52**				
NFPP	0.06ns	0.01ns	-0.01ns	0.04ns	0.41**	0.36**	0.93**	0.17**			
BPP	-0.01ns	0.10ns	0.08ns	-0.09ns	0.09ns	0.09ns	0.13*	0.14**	0.09ns		
GYP	0.06ns	0.06ns	0.01ns	0.03ns	0.40**	0.40**	0.87**	0.15**	0.94**	0.11*	
HSW	0.12*	0.12*	-0.12ns	-0.03ns	0.08ns	0.04ns	0.06ns	0.03ns	0.06ns	-0.06ns	0.07ns
				B.	**Under heat stress condition**						
DF	0.23**										
DM	0.15**	0.36**									
PBPP	0.07ns	0.11ns	0.09ns								
SBPP	0.05ns	0.05ns	0.12*	0.15**							
TBPP	0.11*	0.03ns	0.11ns	0.23**	0.66**						
NTPP	0.02ns	0.01ns	0.11ns	0.13*	0.51**	0.52**					
NUPP	0.04ns	0.04ns	0.12*	0.13*	0.29**	0.28**	0.69**				

NFPP	0.03ns	-0.02ns	0.05ns	0.09ns	0.50**	0.51**	0.88**	0.28**			
BPP	0.18**	0.22**	0.25**	0.21**	0.44**	0.37**	0.47**	0.34**	0.40**		
GYP	0.07ns	0.02ns	0.12*	0.11ns	0.49**	0.52**	0.85**	0.37**	0.90**	0.48**	
HSW	0.18**	0.14*	0.09ns	-0.01ns	-0.11ns	-0.03ns	-0.02ns	-0.06ns	0.01ns	0.04ns	0.02ns
				C.		**Under heat-drought conditions**					
DF	0.12*										
DM	0.19**	0.43**									
PBPP	0.08ns	0.01ns	0.15**								
SBPP	0.31**	0.09ns	0.20**	0.13*							
TBPP	0.21**	-0.08ns	0.11ns	0.06ns	0.69**						
NTPP	0.27**	0.14**	0.22**	0.17**	0.66**	0.64**					
NUPP	0.18**	0.23**	0.14**	0.04ns	0.34**	0.24**	0.52**				
NFPP	0.24**	0.08ns	0.20**	0.18**	0.63**	0.65**	0.94**	0.21**			
BPP	0.36**	0.39**	0.34**	0.18**	0.46**	0.29**	0.43**	0.38**	0.35**		
GYP	0.28**	0.12*	0.24**	0.19**	0.60**	0.60**	0.90**	0.22**	0.95**	0.39**	
HSW	0.09ns	0.16**	0.14**	0.02ns	-0.15**	-0.13*	0.02ns	0.11*	-0.02ns	0.07ns	0.01ns

*. Correlation is significant at the 0.05 level, **. Correlation is significant at the 0.01 level, ns denotes non significant difference. PH, Plant height; DF, Days to 50% flowering; DM, Days to 95% maturity; PBPP, Number of primary branches per plant; SBPP, Number of secondary branches per plant; TBPP, Number of tertiary branches per plant; NTPP, Number of total pods per plant; NUPP, Number of unfilled pods per plant; NFPP, Number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight.

Table II.5. *Pearson's correlation coefficients among various traits based on 162 accessions of lentil under normal planting (A), heat stress (B) and combined heat-drought stress (C) at Marchouch.*

Trait	**PH**	**DF**	**DM**	**PBPP**	**SBPP**	**TBPP**	**NTPP**	**NUPP**	**NFPP**	**BPP**	**GYP**
				A.		**Under normal planting**					
DF	0.20**										
DM	0.32**	0.46**									
PBPP	0.04ns	-0.01ns	-0.01ns								
SBPP	0.05ns	0.02ns	0.03ns	0.03ns							
TBPP	0.14*	-0.01ns	0.06ns	0.03ns	0.67**						
NTPP	-0.01ns	-0.09ns	0.07ns	0.07ns	0.27**	0.22**					
NUPP	-0.09ns	-0.06ns	-0.01ns	-0.05ns	0.29**	0.29**	0.54**				
NFPP	0.02ns	-0.08ns	0.08ns	0.11ns	0.21**	0.15**	0.95**	0.27**			
BPP	0.14*	0.01ns	0.07ns	0.11*	0.52**	0.47**	0.41**	0.35**	0.34**		
GYP	0.02ns	-0.13*	0.06ns	0.09ns	0.18**	0.11ns	0.86**	0.23**	0.91**	0.30**	
HSW	0.22**	-0.05ns	0.03ns	0.14**	0.02ns	0.05ns	-0.11ns	-0.13*	-0.08ns	0.15**	-0.06ns
				B.		**Under heat stress condition**					
DF	0.16**										
DM	0.36**	0.62**									
PBPP	0.03ns	0.04ns	0.15**								
SBPP	0.15**	0.05ns	0.15**	0.40**							
TBPP	0.07ns	0.07ns	0.07ns	0.28**	0.67**						
NTPP	0.22**	0.03ns	0.11*	0.36**	0.53**	0.43**					

NUPP	0.12*	0.14*	0.26**	0.29**	0.41**	0.31**	0.68**				
NFPP	0.23**	-0.01ns	0.06ns	0.3**	0.50**	0.42**	0.97**	0.50**			
BPP	0.29**	0.25**	0.34**	0.36**	0.53**	0.44**	0.65**	0.62**	0.58**		
GYP	0.25**	-0.02ns	0.04ns	0.31**	0.51**	0.42**	0.93**	0.51**	0.95**	0.58**	
HSW	0.21**	0.19**	0.27**	0.23**	0.11*	0.10ns	0.09ns	0.14**	0.06ns	0.28**	0.11ns
				C.		**Under heat-drought condition**					
DF	0.09ns										
DM	0.12*	0.56**									
PBPP	0.22**	-0.04ns	0.12*								
SBPP	0.18**	0.04ns	0.20**	0.59**							
TBPP	0.11*	-0.01ns	0.11*	0.36**	0.54**						
NTPP	0.19**	-0.01ns	0.15**	0.53**	0.46**	0.42**					
NUPP	0.14*	-0.04ns	0.06ns	0.49**	0.43**	0.44**	0.77**				
NFPP	0.17**	0.01ns	0.18**	0.44**	0.38**	0.31**	0.91**	0.44**			
BPP	0.24**	0.07ns	0.22**	0.34**	0.42**	0.46**	0.44**	0.35**	0.39**		
GYP	0.19**	0.02ns	0.17**	0.42**	0.40**	0.37**	0.89**	0.48**	0.94**	0.41**	
HSW	0.19**	0.15**	0.17**	0.16**	0.20**	0.18**	0.14*	0.14*	0.11ns	0.23**	0.12*

*. Correlation is significant at the 0.05 level, **. Correlation is significant at the 0.01 level, ns denotes non significant difference. PH, Plant height; DF, Days to 50% flowering; DM, Days to 95% maturity; PBPP, Number of primary branches per plant; SBPP, Number of secondary branches per plant; TBPP, Number of tertiary branches per plant; NTPP, Number of total pods per plant; NUPP, Number of unfilled pods per plant; NFPP, Number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight.

II.3.3. Classification of Accessions Based on Heat Tolerance Index

Germplasm accessions were classified into representative groups based on the heat tolerance index (HTI). Hierarchical cluster analysis using Ward's incremental squared Euclidean distance method resulted in five clusters. These genotypic clusters differed significantly in HTI and defined as highly heat tolerant (HTI >1), heat tolerant (HTI means 0.68 in Marchouch and 0.66 in Tessaout), moderately heat tolerant (0.25 and 0.10), heat sensitive (-0.08 and -0.25) and highly heat sensitive (-0.37 and -0.55).

Based on the HTI, four accessions (ILL 7833, ILL 6338, ILL 7835 and ILL 6104) showed good performance under heat stress at Marchouch and three accessions (ILL 7835, ILL 7814 and ILL 8029) as highly heat tolerant at Tessaout. ILL 7835 emerged as heat tolerant at both the locations, showing its stable performance under heat stress. These accessions were characterized by a short phenological cycle with 53.5 days to achieve 50% flowering and about 86 days to 95% maturity at Marchouch, however, the duration was less at Tessaout with 45 days to 50% of flowering and 82 days to 95% maturity (Table II.6). Additionally, 14 and 26 accessions were categorized as heat tolerant at Marchouch and Tessaout (Annex II.2), respectively. The moderately-heat tolerant group comprised 26 accessions at Marchouch and 60 accessions at Tessaout. In this study, heat sensitive

and highly heat-sensitive clusters were included of 76 and 42 accessions, respectively, at Marchouch, whereas it was 39 and 34 accessions at Tessaout (Annex II.3).

Table II.6. *Day to 50% flowering, days to 95% maturity, grain yield per plant and heat tolerant index of highly heat tolerant accessions of lentil at Marchouch and Tessaout.*

		Marchouch				Tessaout			
Location	**Accession**	**DF**	**DM**	**GYP**	**HTI**	**DF**	**DM**	**GYP**	**HTI**
Marchouch	ILL 7833	53	85.00	4.33	1.97	45	80.00	0.23	−0.68
	ILL 6338	54	86.00	3.16	1.52	47	83.00	0.59	−0.12
	ILL 7835	54	86.00	3.02	1.39	43.5	81.00	3.52	1.95
	ILL 6104	53	85.00	2.52	1.11	44	82.20	0.43	−0.27
	Mean	53.5	85.5	3.26	1.49	44.87	81.55	1.19	0.22
	SD	0.5	0.5	0.66	0.31	1.34	1.14	1.34	1.02
Tessaout	ILL 7835	54	86.00	3.02	1.39	43.5	81.00	3.52	1.95
	ILL 7814	54	85.00	0.2	−0.38	43.5	82.50	3.26	1.83
	ILL 8029	55	85.00	1.43	0.44	47.5	83.50	2.65	1.36
	Mean	54.33	85.33	1.55	0.48	44.83	82.33	3.14	1.71
	SD	0.47	0.47	1.15	0.72	1.88	1.02	0.36	0.25

DF, days to 50% flowering; DM, days to 95% maturity; GYP, grain yield per plant; HTI, heat tolerance index

II.3.4. Response to Combined Heat-Drought Stress

Based on the stress tolerance index (STI) and the geometric mean productivity (GMP), accession ILL 7835 was identified as tolerant to heat and drought at Marchouch and three accessions, ILL 7814, ILL 7835 and ILL 7804 at Tessaout. Accession ILL 7835 showed tolerance to drought and heat stresses at both locations. The three highly tolerant accessions at Tessaout achieved 50% flowering in around 43 days, while 50% flowering was achieved in about 60 days for the ILL 7835 at Marchouch. Furthermore, these accessions reached 95% maturity in 83 days at Tessaout and in 90 days at Marchouch (Table II.7). Using Spearman's correlation coefficient, the GMP was strongly correlated to STI ($r = 1$; $p < 0.01$) in Tessaout and Marchouch. Furthermore, highly positive correlation ($p < 0.01$) was recorded between yield potential (Yp) and yield under stress condition (Ys) ($p < 0.05$) at both stations. The Ys was positively correlated with the Yp, STI, GMP, MP and HARM indices, and negatively correlated with stress tolerance (TOL) at Tessaout and Marchouch (Table II.8). Additionally, a non-significant correlation was observed between Ys and TOL under combined heat-drought stress in Marchouch.

Table II.7. *Days to 50% flowering, days to 95% maturity, grain yield and stress tolerance indices of the ten best tolerant accessions of lentil grown under normal planting and combined heat-drought stress at Marchouch and Tessaout.*

Location	Accession	DF	DM	Ys	Yp	STI	GMP	MP	TOL	HARM
Marchouch	ILL 7835	59.50	89.50	1.88	3.98	1.12	2.73	2.93	2.10	2.55
	ILL 6075	58.00	87.00	1.99	2.15	0.64	2.07	2.07	0.16	2.07
	ILL 6362	59.00	87.50	0.77	5.30	0.61	2.02	3.04	4.53	1.34
	ILL 7819	55.00	87.50	1.54	2.43	0.56	1.93	1.98	0.89	1.88
	ILL 7266	56.00	88.00	1.20	2.20	0.40	1.62	1.70	1.00	1.55
	ILL 6361	59.00	89.00	0.60	4.21	0.38	1.59	2.40	3.61	1.05
	ILL 880	64.00	98.00	0.67	3.66	0.37	1.56	2.16	2.99	1.13
	ILL 4605	49.00	83.30	0.59	4.07	0.36	1.55	2.33	3.48	1.03
	ILL 6088	57.50	87.50	0.90	2.45	0.33	1.48	1.67	1.55	1.31
	ILL 7815	58.50	85.50	0.48	4.16	0.30	1.41	2.32	3.69	0.85
	Mean	57.55	88.28	1.062	3.461	0.51	1.796	2.26	2.4	1.476
	SD	3.64	3.64	0.53	1.02	0.23	0.38	0.43	1.38	0.51
Tessaout	ILL 7814	42.50	83.00	2.71	4.63	1.68	3.54	3.67	1.92	3.42
	ILL 7835	42.50	81.50	2.28	5.30	1.61	3.47	3.79	3.02	3.19
	ILL 7804	43.00	82.50	2.53	3.37	1.14	2.91	2.95	0.84	2.89
	ILL 6101	45.00	86.00	2.20	3.11	0.91	2.62	2.66	0.91	2.58
	ILL 6100	45.00	83.50	2.22	3.20	0.95	2.67	2.71	0.98	2.62
	ILL 7807	45.50	83.50	1.40	4.86	0.91	2.61	3.13	3.46	2.18
	ILL 6091	47.00	82.50	1.23	4.09	0.67	2.24	2.66	2.86	1.88
	ILL 8029	46.50	83.50	1.11	4.25	0.63	2.17	2.68	3.14	1.76
	ILL 4605	51.58	81.07	1.15	3.81	0.59	2.09	2.48	2.66	1.77
	ILL 8061	66.50	97.00	1.16	3.76	0.58	2.08	2.46	2.60	1.77
	Mean	47.51	84.41	1.79	4.04	0.97	2.64	2.92	2.24	2.41
	SD	6.82	4.39	0.61	0.69	0.38	0.51	0.45	0.95	0.59

DF, days to 50% flowering; DM, days to 95% maturity; Ys, seed yield in combined heat-drought condition; Yp, seed yield in normal condition; STI, stress tolerance index; GMP, geometric mean productivity; MP, mean productivity; TOL, tolerance index; and HARM, harmonic mean.

Table II.8. *Spearman's correlation coefficients between stress tolerance parameters in lentil under stressed and non-stressed environments at Tessaout and Marchouch.*

Location	Indices	Ys	Yp	STI	GMP	MP	TOL	HARM
Tessaout	Yp	0.19 *	1					
	STI	0.86 **	0.61 **	1				
	GMP	0.86 **	0.61 **	1.00 **	1			
	MP	0.47 **	0.94 **	0.82 **	0.82 **	1		
	TOL	−0.16 *	0.90 **	0.29 **	0.29 **	0.73 **	1	
	HARM	0.98 **	0.33 **	0.94 **	0.23 **	0.60 **	0.05 ns	1
Marchouch	Yp	0.14 *	1					
	STI	0.54 **	0.83 **	1				
	GMP	0.54 **	0.83 **	1.00 **	1			
	MP	0.24 **	0.98 **	0.91 **	0.91 **	1		
	TOL	0.30ns	0.98 **	0.73 **	0.73 **	0.92 **	1	
	HARM	0.95 **	0.37 **	0.73 **	0.73 **	0.48 **	0.26 **	1

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level, ns denotes non-significant difference. Ys, seed yield in combined heat-drought condition; Yp, seed yield in normal condition; STI, stress tolerance index; GMP, geometric mean productivity; MP, mean productivity; TOL, tolerance index; and HARM, harmonic mean.

II.4. Discussion

The present study demonstrated delayed sowing as an effective approach to screen lentil germplasm for heat tolerance. A similar methodology was used to assess heat tolerance responses in chickpea (Devasirvatham and Tan, 2018; Krishnamurthy et al., 2011), lentil (Choudhury et al., 2012; Sehgal et al., 2017) and mung bean (Kaur et al., 2015; Sharma et al., 2016) successfully. High temperature and low vapor pressure deficits (VPD) decrease soil moisture and increase transpiration rate, resulting in combined heat and drought stress (Sita et al., 2017b). Hence, frequent irrigation must be provided to remove the confounding effects of drought stress, to assess the effect of heat stress. Even the photoperiod may change during late planting, and previous studies by Summerfield et al. (1985) and Erskine et al. (1990) reported that temperature had a much bigger effect on the flowering time than the photoperiod in lentil.

Our findings showed that heat stress adversely affected plant height, number of branches and pods, grain yield and biomass, which agrees with several investigations in chickpea (Jumrani and Bhatia, 2014; Upadhyaya et al., 2011), lentil (Kumar et al., 2016c) and faba bean (Abdelmula and Abuanja, 2007). However, the influence of the combined heat-drought stress was more severe when compared to heat stress only, due to the reduction in water use efficiency. Heat and combined heat-drought stresses shortened vegetative and reproductive periods by accelerating the rate of plant growth and development. Similar findings were reported in previous studies (Gan et al., 2004;

Gaur et al., 2014). The combined heat-drought stress largely decreased the number of pods more than the individual heat stress, leading to severe yield losses and biomass. This is in agreement with the previous research of Sehgal et al. (2019, 2017), which has also shown the impact of combined heat-drought stress in lentil.

The days to 50% flowering under stressed conditions have shown quite similar results in both locations: approximately 46 days at Tessaout and 54 days at Marchouch. Lentil responded in the same way to heat stress and combined heat-drought stress by forcing early flowering (Figure 1) in both locations. Awasthi et al. (2014) reported a very similar response in days to 50% flowering eventually, accelerated markedly in chickpea accessions under heat and combined heat-drought stresses environment.

Overall, grain yield decreased under stress conditions, with a more pronounced effect under the combined heat-drought stress condition. High temperature stress led to yield loss by altering pollen and stigma development, pollination and pod set, while drought directly influenced the seed filling stage (Fang et al., 2010; Ohnishi et al., 2010; Sehgal et al., 2017). Moreover, yield is always influenced by photosynthetic ability and the performance of reproductive organs under stressed conditions (Hendrix, 2001). In this study, the number of primary, secondary and tertiary branches were significantly positively correlated with seed yield ($p < 0.01$) under stressed environments, at both stations, except heat stress of Tessaout, which was not correlated with seed yield (Tables II.4 and II.5). Previous studies agreed with our findings and demonstrated a positive direct effect of primary branches on seed yield in lentil (Ul Hussan et al., 2018; Younis et al., 2008).

Recently, Ahmadi et al. (2019) suggested that if lentil accessions have a greater number of branches mainly secondary branches, the final potential would increase the plant yield under stress conditions. It appears that plant biomass was low, mostly due to combined heat-drought stress that demands high water use efficiency. Further, heat stress, in combination with drought, increases leaf temperature and decreased net photosynthetic rate and stomatal conductance, resulting in a more damaging impact under the combined stress condition (Prasad et al., 2015). Overall, biomass reduction largely affected the number of total pods and seed yield per plant (Tullu et al., 2001). It was also positively correlated with number of total pods per plant, the numbers of primary, secondary and tertiary branches, and seed yield under heat stress and combined heat-drought stress at Tessaout and Marchouch (Table II.9). The present study showed increased 100-seed weight under heat stress and the combined heat-drought stress condition, due to the reduction of seed

numbers per plant. Similar results were reported by Chakherchaman et al. (2009) in lentil under drought stress.

In the present study, three accessions, namely, ILL7833, ILL6338 and ILL6104, were selected as potential sources of heat tolerance at Marchouch and two accessions (ILL7814 an ILL8029) at Tessaout. Tolerant accessions produced significantly more pods under heat stress, as compared to heat sensitive accessions. Under the combined stress of heat and drought, the most tolerant lines were ILL7835, ILL6075 and ILL6362 (STI >1) at Marchouch and ILL7814, ILL7835 and ILL7804 (STI >1) at Tessaout. Altogether, ILL7835 was identified as a good source of tolerance under heat stress and combined heat-drought stress at Tessaout, as well as at Marchouch, while ILL7814 was identified as promising accession under both stresses at Tessaout. These selected lines are characterized by early flowering, which helped them to escape from heat and drought stress and shorter crop cycles. Usually, early flowering and maturity are the stress escape mechanism that can help lentil to perform well under stress conditions, and the development of accessions with short duration is one of the major strategies used in breeding programs (Kumar et al., 2016a). Although, early duration is considered to be the best adapted trait for chickpea accessions to Mediterranean (spring sown) and south Indian environments (Berger et al., 2011; Saxena, 1987). Furthermore, the identified lines had a greater number of filled pods per plant, representing an excellent source for the lentil breeding program, and can be directly used in the crossing program to transfer heat and drought tolerance in a high yielding genetic background.

The selection of superior germplasm against combined stress was carried out by using a stress tolerance index (STI) and geometric mean productivity (GMP). STI and GMP have been used earlier for screening accessions of chickpea (Erdemci, 2018; Jha et al., 2018; Shabani et al., 2018), lentil (Mishra et al., 2016; Rad et al., 2010), bread wheat (Grzesiak et al., 2019), barley (Ajalli and Salehi, 2012; Zare, 2012), fenugreek (Ahari et al., 2009), oat (Akcura and Ceri, 2011) and maize (El-Sabagh et al., 2018; Papathanasiou et al., 2015) against abiotic stresses. Higher GMP and STI values indicate more tolerance to drought stress (Farshadfar and Elyasi, 2012). The stress tolerance index (STI) showed a highly positive correlation with both yield under stress conditions (Ys) and yield potential (Yp), GMP, MP and HARM at both locations. These results are in conformity with those of Ganjali et al. (2009) and Siahsar et al. (2010) in chickpea under stress and non-stress conditions. However, stress tolerance (TOL) was negatively correlated with Ys at Tessaout and non-significantly correlated at Marchouch, while it was positively correlated with potential yield

at both locations. Similar results were reported also by Chakherchaman et al. (2009) and Rad et al. (2010) for lentil under drought stress conditions. Majidi et al. (2011) also reported non-significant correlation between TOL and Ys, and a highly significant correlation between TOL and Yp, confirming that selection based on TOL should decrease yield in the moisture stress environment and increase grain yield under normal conditions. The limitations of using the TOL index have been described previously in several studies (Abd El-Mohsen et al., 2015; Siahsar et al., 2010). In general, our findings confirmed the effectiveness of using STI and GMP as reliable selection criteria for terminal heat or drought tolerance, also reported earlier by Fernandez (1992), Farshadfar et al. (2013) and Talebi et al. (2011).

II.5. Conclusion

In the present chapter, our findings show that heat, as well as combined heat-drought stress, severely affect flower production and pod set, leading to a substantial loss in grain yield in lentil. The field screening technique has been demonstrated as an effective way for evaluating and selecting promising lines under heat or drought stress. The adaptation by selecting the promising accessions can be a strong approach to mitigating the impact of climate change. Our study suggests that the FIGS approach in identifying heat tolerant germplasm in lentil is a successful approach under heat and combined heat-drought stress. This research identified a group of highly heat tolerant accessions using HTI based on flowering time, and grain yield under stress and non-stress conditions. The accessions ILL7833, ILL6338 and ILL6104, were identified as potential sources of heat tolerance at Marchouch and two accessions (ILL7814 and ILL8029) at Tessaout. Tolerant accessions produced significantly more pods under heat stress, as compared to heat sensitive accessions. However, the most tolerant accessions under the combined stress of heat and drought were ILL 7835, ILL 6075 and ILL6362 (STI >1) at Marchouch and ILL7814, ILL7835 and 7804 (STI >1) at Tessaout. Altogether, ILL7835 was identified as a good source of tolerance under heat stress and combined heat-drought stress at Tessaout, as well as at Marchouch, while ILL7814 was identified as promising accession under both stresses at Tessaout. Furthermore, this chapter showed that STI, GMP and MP are the most suitable criteria, not only for selecting the high yielding accessions under individual heat and drought stresses, but also in combined heat-drought stress. These lines would be an excellent source of stress tolerance to increase the adaptation of lentil under climate change, a major issue that currently affects agriculture.

Chapter III : High-Temperature and Drought Stress Effects on Growth, Yield and Nutritional Quality with Transpiration Response to Vapor Pressure Deficit in Lentil

III.1. Introduction

Lentil (*Lens culinaris* Medikus) is an important cool-season food legume that plays a significant role in human and animal nutrition as well as in maintaining and improving soil fertility (Erskine et al., 2011; Sarker and Kumar, 2011). Lentil grains are highly digestible and nutritious, representing a true staple crop in the dryland of many developing countries with affordable levels of dietary proteins (22–35%), vitamins, fiber, carbohydrates and essential micronutrients such as zinc and iron (Kumar et al., 2015). As lentil is highly nutritious and can be cooked more quickly than other pulses, it is the pulse most preferred by poor rural households worldwide, particularly those living in developing nations such as India, Bangladesh, Pakistan and Ethiopia (Reda, 2015). However, micronutrient deficiencies are currently becoming more serious due to the high consumption of carbohydrate-rich cereal-based diets, which are low in micronutrients and vitamins (Bouis et al., 2013; Jha and Warkentin, 2020). Micronutrient malnutrition, commonly known as hidden hunger, affects more than 2 billion people in developing countries, while all over the world, >3 billion people suffer from micronutrient deficiencies (Cakmak et al., 2010; Depar et al., 2011; Kumar et al., 2016b; Rehman et al., 2020, 2018). In the last decade, efforts have been made to reduce the risks of malnutrition using several approaches including nutrient supplementation, diet diversification and food fortification. However, genetic biofortification using conventional and transgenic breeding methods has been recognized as the most effective and sustainable method for developing new crop varieties to combat hidden hunger and provide essential micronutrients and vitamins to the poor population through daily diet (Bouis and Saltzman, 2017; Jha and Warkentin, 2020; Saltzman et al., 2013).

Highly nutritional accessions have been developed through genetic biofortification in lentil (Choukri et al., 2020), chickpea (Ullah et al., 2020) and wheat (Devendra Singh et al., 2017), helping to minimize micronutrient deficiencies substantially in recent decades. However, the increase in the world population to around 10 billion by 2050 will further escalate malnutrition (Shah et al., 2019; Steiner et al., 2019), and more efforts will be required to meet the human population's nutritional needs for a healthy life. Enhancing the production of lentil would support

food and nutritional security and improve the livelihoods of resource-poor farmers in developing nations. Still, lentil productivity in rainfed regions is expected to suffer from fluctuations in climate change, mainly due to the increased incidence of drought and higher temperatures (Johnson et al., 2020).

High-temperature and drought stress have been reported as the major environmental factors that can markedly affect plant productivity and the quality of many cultivated crops (El Haddad et al., 2021), however their impact on cool-season food legumes appears to be more serious (Awasthi et al., 2017; Sultana et al., 2014). A temperature higher than 30 °C causes stress in most cool-season food legume crops. Both high-temperature and drought stress hamper plant growth by disturbing the normal physiology and morphology, thereby influencing an array of processes including growth, floral development, carbohydrates, protein content in grains, and micronutrient concentration (zinc and iron), which ultimately affect grain yield and nutritional quality (Johansson et al., 2020; Maqbool et al., 2020).

Furthermore, the combined effect of high-temperature and drought stress on crops could be more severe than the individual stress impact. The reproductive stages of crops are more vulnerable to drought, high temperature and combined stress than the vegetative stages (Choukri et al., 2020; Sehgal et al., 2017). During anthesis and flowering periods, both stresses led to fertilization failures because of reduced pollen and ovule function and inhibited pollen development and sterility in legumes (Kumari et al., 2021; Sita et al., 2017b) and cereals (Fábián et al., 2019; Zahra et al., 2021). While considering the current change in climate, it is expected that both the severity of high temperatures and the risk of drought will increase mainly in the subtropical region, impacting food security (Raza et al., 2019).

The impacts of high temperatures (Bhandari et al., 2016; Sita et al., 2018), water stress (Ahmed et al., 2020; Salehi et al., 2008) and combined temperature-drought stress on lentil growth, yield and composition have been documented (El Haddad et al., 2021, 2020; Sehgal et al., 2019, 2017). However, more information is needed on the physiological responses of lentil and their mechanisms to develop climate-resilient management strategies and new varieties. Since lentil is cultivated mainly in environments with high vapor pressure deficit (VPD) conditions (hot and dry areas) during the post-rainy season, where light rainfall is often noted, the study of the limited transpiration (TR_{lim}) trait could be crucial in water-deficit conditions (Ghanem et al., 2017). VPD is defined as the difference between the amount of moisture in the air and the moisture the air can

hold when saturated. Temperature and relative humidity are the two major factors controlling VPD variation (Devi and Reddy, 2018).

The high VPD, which usually occurs due to exacerbation of plant water stress in the middle of the day, affects plant photosynthesis, growth, yield and physiology (Grossiord et al., 2020). The intensification of atmospheric water demand highly influences plant hydraulic status, and the key behind these impacts is plant stomatal behavior (Kulkarni et al., 2017; Li et al., 2017). Regulation of stomatal conductance in high VPD conditions by limiting transpiration restricts the rate of water use and increases transpiration efficiency, helping to conserve water and support plant growth later in the season when drought develops (Ghanem and Sinclair, 2019; Sinclair et al., 2017). TR_{lim} in response to high VPD has been reported in several crop species including wheat (*Triticum aestivum* L.) (Li et al., 2017; Medina et al., 2019), maize (*Zea mays* L.) (Shekoofa et al., 2016; Shrestha et al., 2021), sorghum (*Sorghum bicolor* L.) (Gholipoor et al., 2012; Karthika et al., 2019), soybean (*Glycine max* L.) (Gilbert et al., 2011; Sadok and Sinclair, 2009), peanut (*Arachis hypogaea* L.) (Pradhan et al., 2019; Shekoofa et al., 2015), cowpea (*Vigna unguiculata* L.) (Belko et al., 2013; Manandhar et al., 2017), chickpea (*Cicer arietinum* L.) (Sivasakthi et al., 2017; Zaman-Allah et al., 2011) and lentil (Guiguitant et al., 2017).

Based on the available literature, the study by Guiguitant et al. (2017) is the only investigation that examined lentil accession differences in transpiration response to high VPD conditions. In the study by Guiguitant et al. (2017), 17 lentil accessions were tested for the expression of TR_{lim} and almost all tested lines showed a VPD breakpoint at approximately 3.4 kPa. In addition, Guiguitant et al. (Guiguitant et al., 2017) reported a possible yield benefit of developing accessions expressing the TR_{lim} trait, especially in regions with low rainfall, and that the impact of this trait on lentil productivity differs with geography and environments. Furthermore, several studies have shown increases in crop yield by 25 to 75% in water-limited areas based on crop simulation models (Drake et al., 2013; Ghanem et al., 2015; Messina et al., 2015; Shekoofa et al., 2016). Thus, developing and/or identifying drought- and heat-tolerant varieties is crucial in improving lentil yield under water-limited and high-temperature environments, which would directly contribute to food and nutritional security in the near future.

Based on the results from preliminary field screening presented the chapter II, 20 lentil accessions were selected as primary candidates for temperature and drought stress studies. This chapter aims (i) to investigate the impact of high temperature and drought stress on traits associated with

phenology, grain yield, nutritional quality and canopy temperature under field conditions, and (ii) to examine the possible genotypic variation in lentil for transpiration response to high VPD conditions over controlled environments. The generated information may help to inform selection strategies and decisions within breeding programs and identify options to mitigate the impacts of high temperatures and water deficit on lentil productivity.

III.2. Materials and Methods

III.2.1. Plant Material

The material consisted of 20 lentil accessions selected from our previous field screening study (El Haddad et al., 2020), where we evaluated a focused identification of germplasm strategy (FIGS) set against drought and temperature stresses. In the present study, we included moderate heat tolerant, moderate drought tolerant, heat tolerant, heat sensitive, drought tolerant and drought sensitive lines (Table III.1).

Table III.1. *Description of origin, IG number and classification of lentil accessions used in the current study. MHT: Moderately heat tolerant; MDT: Moderately drought tolerant, HT: heat tolerant, HS: heat sensitive, DT: drought tolerant, DS: drought sensitive.*

Accession	IG number	Origin	Classification
ILL 3484	3484	Nepal	MT
Bakria	4605	Morocco	MT
ILL 5919	69528	Ethiopia	HS; DS
ILL 6075	70130	Pakistan	DT
ILL 6104	70159	Pakistan	HT
ILL 6338	71270	Pakistan	HT
ILL 6359	71291	Pakistan	MT
ILL 6362	71294	Pakistan	HDT
ILL 6363	71295	Pakistan	MT
ILL 7223	75942	Nepal	MT
ILL 7286	76005	Nepal	HT
ILL 7804	114892	Nepal	HDT
ILL 7813	114929	Nepal	HS; HDS
ILL 7814	114931	Nepal	HT; HDT
ILL 7819	114948	Nepal	MT
ILL 7820	114951	Nepal	HS; HDS
ILL 7833	115006	Nepal	HT
ILL 7835	115010	Nepal	HT; HDT
ILL 8025	117726	Pakistan	MT
ILL 8029	117734	Pakistan	HT

III.2.2. Field Experiment

The germplasm was assessed under three independent experiments at the ICARDA experimental station Marchouch (33.56°N, 6.63°W, 392 m altitude) during the 2016–17 cropping season. These three experiments were deemed to represent three treatments, namely (i) normal date of planting (Treatment A), (ii) late planting with irrigation (Treatment B) and (iii) late planting without irrigation (Treatment C). All of the treatments were planted in an alpha lattice design with two replications. In the three treatments, each accession was planted in a three-row plot of 1 m length, with a spacing of 0.30 m between rows. In each row, seeds were sown at 2 cm depth, maintaining 10 cm space between plants. Treatment A resulted in optimal growing conditions (150 mm well-distributed rainfall and temperature below 27 °C, without any heat or water stress to the plants). Treatment B (planted 65 days after normal planting date with irrigation at field capacity throughout crop duration) imposed high-temperature stress.

In both late planting treatments, the plants were synchronized with temperatures above 32 °C during the reproductive stage. Regular irrigation at field capacity avoided any water stress to the plants during their growth and development. Treatment C (planted 65 days after normal planting date without irrigation during the reproductive stage) imposed combined high-temperature and drought stress. Treatment A was planted on 27 December 2016, without any supplementary irrigation during the cropping period, as the crop received enough well-distributed rainfall during the cropping season. Treatments B and C were planted on 1 March 2017. Irrigation was performed regularly to sustain water supply at field capacity using a sprinkler system throughout the crop duration in treatment B. In contrast, irrigation was stopped at the flower initiation stage onward in treatment C to impose water stress (<5 mm rainfall during the reproductive stage) combined with the temperature stress. All field management followed standard agricultural practices in lentil production (Kumar and Rajendran, 2016).

III.2.3. Data Collection

Data were collected on early growth vigor (EGV) after 1 month of sowing, plant height (PLH), days to first flowering (DFF), days to 50% of flowering (D50F), days to 50% of podding (D50P) and days to physiological maturity (DM) on a plot basis. Five plants were randomly selected from each plot to measure number of total pods plant−1 (NTPP), number of filled pods plant−1 (NFPP) and number of unfilled pods plant−1 (NUPP), grain yield plant−1 (GYP) and hundred-seed weight

(HSW). Canopy temperature (CT) was determined using a thermal infrared camera FLIR T4xx-series (Model T420-KIT-15). Thermal images were captured between 11:00 and 13:00 GMT time on a sunny day and analyzed using FLIR tools+ software (FLIR systems, Teledyne FLIR, Wilsonville, OR, USA). The seeds harvested from these experiments were analyzed for protein content and micronutrient contents (zinc and iron).

III.2.4. Crude protein Content

A 0.3 g amount of ground lentil seeds from each treatment was digested with sulfuric acid and selenium mixture following the modified Kjeldahl procedure (Baethgen and Alley, 1989). Based on the color reaction between ammonium and a weakly alkaline mixture of sodium salicylate and sodium hypochlorite, a UV visible spectrophotometer was used to determine the color development in the samples at 560 nm. Next, crude protein content (CP) was measured using nitrogen values multiplied by 6.25, and triplicate analyses were performed for each sample.

III.2.5. Iron and Zinc Determination

Seeds were ground by a Cyclone mill (Twister, 10 mm–250 um, Retsch). Iron (Fe) and zinc (Zn) concentrations were measured using a modified HNO_3 and H_2O_2 method (Gupta et al., 2016). In the digestion block (QBlock series, Horiba), 0.5 g of each ground sample was placed in individual tubes and digested with 6 mL nitric acid (HNO_3), followed by heat treatments at 90 °C for 1 h. To each tube, 3 mL of 30% hydrogen peroxide (H_2O_2) was added, and sample digestion was continued by heating for 15 min at 90 °C, and then 3 mL of 6 M hydrochloric acid (HCL) was added. Once samples were cooled, the solutions were filtered and diluted to 10 mL with distilled water. The mineral content analysis was carried out by inductivity coupled plasma-optical emission spectrometry (ICP-OES); (iCAP-7000 Duo, Thermo Fisher Scientific, Waltham, MA, USA) at the Cereals and Legumes Quality Laboratory, ICARDA, Rabat, Morocco.

III.2.6. Plant Growth conditions in the Greenhouse

An independent experiment was conducted during summer 2018 in the greenhouse at ICARDA, Rabat, Morocco. Three seeds were sown at a depth of 2.5 cm in plastic pots (15 cm in diameter and 20 cm in height) filled with 1.5 kg of 50% sandy loam soil and 50% of compost garden soil (Floragard Vertriebs-GmbH product, Oldenburg) that included 18-10-20 N-P-K fertilizer. Here, the same set of 20 accessions assessed under field conditions were studied under controlled

conditions. Five replications were used for each accession. The pots were positioned in a randomized design on steel grid platform installed at mid-height of 1.3 m. In each pot, a small hole was opened at the bottom of the end cap to facilitate drainage. Plants were grown under well-watered conditions, and the temperature in the greenhouse was maintained at 25 °C/18 °C (day/night) for 1 month. The photosynthetic photon flux density in the greenhouse was approximately 600 μmol m^{-2} s^{-1}.

The day before the measurement, pots were watered to full capacity until their water flowed from the bottom of the pots, then the pots were allowed to drain overnight. After drainage, the pots were bagged with plastic bags and covered with polyethylene balls to keep soil evaporation to a minimum. A thermo-hygrograph sensor (Tinytag Ultra 2 TGU-4500 Gemini Datalogger Ltd., Chichester, UK) was positioned over the plant to measure the temperature and RH at 5 min intervals. Hourly atmospheric VPD was calculated on the basis of average temperature and RH. Plants were exposed to each humidity and temperature treatment for 1 h and then reweighed to measure the final weight. On an hourly basis, the transpiration rate (TR) was calculated as the weight difference between successive measurements using a balance with a resolution of 0.1 g.

The measurement was conducted in the morning from 7:00 am (Morocco standard time) at low VPD until 7:00 pm, when the VPD decreased following the midday maximum. Measurements were first started with the low VPD (0–1.5 kPa) treatment, the medium VPD treatment (1.5–2.5 kPa), and finally, the high VPD (2.5–4.5 kPa) treatment. The experiment in the greenhouse allowed us to control the temperature and RH, exposing the plants to the expected ranges of VPD (1.18 to 4.5 kPa). The average temperature and RH during the experiment fluctuated from 19 to 40 °C and from 30 to 80%, respectively. A high VPD level was achieved by increasing temperature and decreasing RH inside the greenhouse. At the end of the experiment, the leaf area was measured using a leaf area meter, and the TR was expressed as water loss per unit of leaf area and time (g H_2O_2 cm^{-2} h^{-1}).

III.2.7. Statistical Analysis

III.2.7.1. Field Data Analysis

Analysis of variance was carried out using the general linear model (GLM) in IBM SPSS statistics 23. Duncan's post-hoc was applied to compare differences between the mean values at $p < 0.05$. Pearson's correlation coefficient was determined for the three treatments. Principal component analysis (PCA) was performed using the *Factoextra* and *FactoMineR* packages in R version 4.1.0

and RStudio version 1. 3.1093 (Husson et al., 2019; Kassambara and Mundt, 2017). In addition, hierarchical cluster analysis was performed using Ward's squared Euclidean distance method with the *dendextend* R package (Galili, 2015).

III.2.7.2. Vapor Pressure Deficit Analysis

The individual data for each VPD treatment of each accession were used in the regression analysis of transpiration response. The data were first tested to fit the data to a two-segment linear regression using GraphPad Prism 7 (GraphPad Software Inc.). The results of a successful regression fit to the two-segment model were the coefficients defining two intersecting linear regressions:

$$\text{If VPD} < \text{BP, TR} = \text{intercept 1} + \text{slope 1 (VPD)} \quad (1)$$

$$\text{If VPD} \geq \text{BP, TR} = \text{intercept 2} + \text{slope 2 (VPD)} \quad (2)$$

where BP is the breakpoint between the two linear segments; it is also an output of the GraphPad analysis and an estimate of the standard error for BP. The intercept represents the constant of the first- and second-line segments; the two slopes were statistically compared within GraphPad for a significant difference at $p \leq 0.05$ level. In case there was a significant difference, the two-segment model characterized the outcomes for that accession. All data for a accession were assumed to fit a single linear regression model if two slopes were not identified to be significantly different.

III.3. Results

III.3.1. Weather Data

Accumulated rainfall for normal planting was about 225 mm between December and April, while the late planting received around 26 mm mostly before the anthesis period. Under normal conditions, the minimum and maximum temperatures differed from −2.40 to 11.7 °C and 9.18 to 30.6 °C, respectively, during the vegetative stage, while they varied from 0.35 to 8.58 °C and 13.3 to 30.2 °C during the reproductive stage, respectively. Under the late planting, minimum and maximum temperatures were from 0.35 to 15.3 °C and from 13.3 to 37.5 °C respectively, before the flowering stage, and from 5.61 to 14.9 °C and from 22.6 to 36.9 °C, respectively, during the reproductive stage. Under normal planting, the RH ranged from 52 to 98% in the vegetative stage and 41 to 91% in the reproductive stage. It varied in late planting from 32 to 96% in the vegetative

stage and from 42 to 79% during the reproductive stage. The maximum VPD registered in normal planting was 2.11 kPa and 3.25 kPa in late planting (Figure III.1).

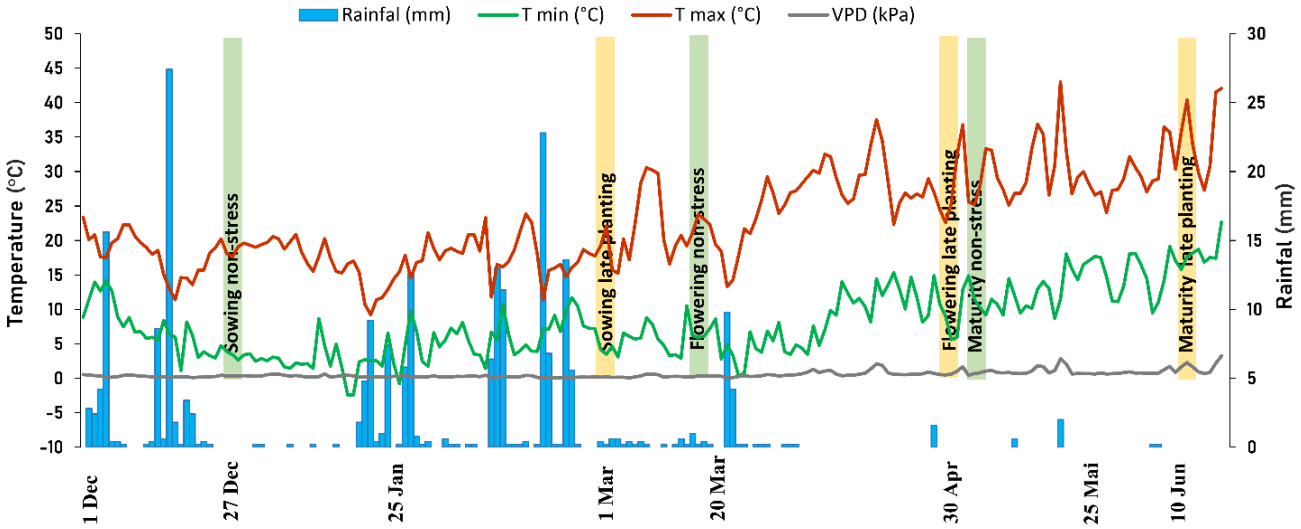

Figure III.1. *Rainfall, maximum and minimum temperatures, and vapor pressure deficit (VPD) during normal and late planting experiments in Marchouch, Morocco during 2016/17 season*

III.3.2. Combined Analysis of Variance

The combined analysis of variance indicated a significant difference among studied accessions for all traits (Table III.2). ANOVA showed a highly significant ($p < 0.001$) effect of temperature stress and combined temperature-drought stress on all measured characteristics. However, accession × treatment interaction was significant for all traits except NUPP and HSW. Furthermore, the coefficient of variation (CV) among treatments was less than 13% for most traits, but 35% for both GYP and NFPP, with 25 and 49% for NTPP and NUPP, respectively.

Table III.2. *ANOVA analysis for 20 lines of under normal and late planting trials at Marchouch.*

	PLH	EGV	DFF	D50F	D50P	DM	NFPP	NUPP	NTPP	GYP	HSW	CP	Zn	Fe
Gen (G)	**	**	**	**	**	**	*	*	*	*	**	**	**	**
Trt (T)	**	**	**	**	**	**	**	**	**	**	**	**	**	**
G*T	*	**	**	*	**	*	*	ns	*	*	ns	**	**	**
Error	2.7	2.4	0.9	3.3	2.7	1.2	859.3	19.2	868.8	1.3	0.1	0.2	15.4	16.1
CV	7.1	7.9	1.3	2.4	1.8	0.9	36.8	49.3	25.8	35.3	12.9	2.1	9.1	5.2
R^2	0.92	0.92	0.99	0.98	0.99	0.99	0.78	0.64	0.79	0.75	0.91	0.99	0.9	0.92

*, ** and ns indicate significant difference at 0.01 and 0.001, and ns denotes a non-significant. PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods per plant; NUPP, number of unfilled pods per plant; NTPP, number of total pods per plant; GYP, grain yield per plant; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content; Fe, iron content.

III.3.3. Temperature and Combined Heat-drought Impact on Plant phenology

PLH declined significantly, by 19.2%, in temperature stress and by 29.6% in the combined temperature-drought stress compared to its level in normal conditions. EGV ranged from 17.0 to 30.0 cm with an overall mean of 23.1 cm under normal conditions, whereas high-temperature and combined temperature-drought stress reduced EGV by 26.2 and 22.9%, respectively. DFF varied between 82 and 89 days in normal conditions, and a reduction of 28 days was observed for both stressed treatments. Likewise, days to 50% flowering (D50F) declined significantly, by 27 days, under both temperature and combined temperature-drought stress conditions (Table III.3).

Temperature stress alone and in combination with drought severely reduced the D50P by 26 and 27 days, respectively. Under normal conditions, plants achieved physiological maturity in about 127 days, while days to maturity decreased significantly by 19 and 20 days under temperature stress and combined temperature-drought stress, respectively. NFPP varied from 40.8 to 234.2 in normal conditions and was significantly reduced by 44 to 69% in temperature stress and by 48 to 71% in combined temperature-drought stress, respectively. Similarly, NUPP decreased substantially by 21–62% and 3–43% in temperature stress and combined temperature-drought stress, respectively. NTPP ranged from 56 to 240 pods in normal conditions with an average of 122.8, while it decreased significantly by 44–72% under temperature stress and 46–75% under combined temperature-drought stress conditions. GYP ranged from 1.59 to 8.86 g with an overall mean of 4.13 g under normal conditions. The late planting treatments showed a significant reduction in grain yield, by 43% under temperature stress and 49% under the combined stress. HSW varied from 1.93 and 4.97 g in normal conditions, with a mean of 2.64 g, whereas it was decreased significantly by 8% and 19% under the temperature stress and combined temperature-drought stress, respectively (Table III.3).

Table III.3. *Minimum, maximum and mean morphological parameters of 20 lentil accessions under normal, temperature stress and temperature-drought stress conditions.*

Trait	Normal			Temperature Stress			Temperature-Drought Stress		
	Min	Max	Mean± SE	Min	Max	Mean± SE	Min	Max	Mean± SE
PLH	24.75	34.21	28.28 ^{a} ± 3.39	19.75	27.25	22.86 ^{b} ± 0.30	14.33	25.03	19.74 ^{c} ± 0.37
EGV	17.00	30.00	23.12 ^{a} ± 0.56	13.00	21.00	17.07 ^{b} ± 0.30	12.00	21.00	17.82 ^{b} ± 0.35
DFF	82.00	89.00	84.95 ^{a} ± 0.22	60.00	66.00	61.55 ^{b} ± 0.19	590.00	64.00	61.15 ^{b} ± 0.23
D50F	86.00	100.00	93.08 ^{a} ± 0.45	65.00	74.00	68.53 ^{b} ± 0.31	630.00	74.00	68.32 ^{b} ± 0.39
D50P	98.00	106.00	101.78 ^{a} ± 0.36	71.00	80.00	76.20 ^{b} ± 0.44	700.00	80.00	74.77 ^{c} ± 0.41
DM	124.00	131.00	127.20 ^{a} ± 0.32	101.00	105.00	103.53 ^{b} ± 0.17	100.00	105.00	101.70 ^{c} ± 0.17
NFPP	40.83	234.25	111.45 ^{a} ± 7.43	12.75	129.40	57.75 ^{b} ± 4.66	12.00	119.50	52.07 ^{b} ± 4.14

NUPP	3.50	25.25	11.34 ^{a} ± 0.88	1.33	20.00	6.15 ^{b} ± 0.51	2.00	24.50	7.89 ^{b} ± 0.79
NTPP	56.00	240.00	122.79 ^{a} ± 7.23	15.75	134.00	63.90 ^{b} ± 4.71	14.00	130.00	59.96 ^{b} ± 4.17
GYP	1.59	8.86	4.13 ^{a} ± 0.28	0.43	4.56	2.35 ^{b} ± 0.17	0.66	4.32	2.12 ^{b} ± 0.14
HSW	1.93	4.97	2.64 ^{a} ± 0.09	1.59	5.23	2.42 ^{b} ± 0.11	1.42	5.51	2.15 ^{c} ± 0.13
CP	23.40	31.90	28.91 ^{a} ± 0.35	22.00	27.00	24.36 ^{b} ± 0.20	11.00	14.90	13.34 ^{c} ± 0.16
Zn	33.10	64.40	47.86 ^{a} ± 1.52	27.40	59.10	41.12 ^{b} ± 1.41	30.40	50.90	39.28 ^{c} ± 0.80
Fe	62.30	99.30	78.19 ^{a} ± 1.44	51.30	81.60	66.71 ^{b} ± 1.42	52.40	73.50	62.86 ^{c} ± 0.81

Means for each variable followed by the same letters are not significantly different ($p < 0.05$). PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods plant^{-1}; NUPP, number of unfilled pods plant^{-1}; NTPP, number of total pods plant^{-1}; GYP, grain yield plant^{-1}; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content and Fe, iron content.

III.3.4. Influence oh High-temperature and Combined Temperature-Drought Stress on Fe, Zn and Protein Contents

Crude protein (CP) varied from 23.4 to 31.9% under normal conditions, with a mean of 28.9%. CP decreased significantly to 15% due to temperature stress and 53% under combined temperature-drought stress. Under normal conditions, the mean Zn concentration was 47.9 mg kg^{-1}, ranging from 33.1 to 64.4 mg kg^{-1}. High-temperature stress and combined temperature-drought stress significantly reduced Zn concentration by 14 and 18%, respectively. Similar results were observed for Fe with a reduction of 15% due to temperature stress and 20% under combined temperature-drought stress. Fe concentration varied from 62.3 to 99.3 mg kg^{-1} under normal conditions (Table III.3).

III.3.5. Correlation Coefficient Between Measured Traits

A significant positive correlation was observed between PLH and EGV (r = 0.38) under normal conditions. However, a highly significant negative correlation ($p < 0.01$) was observed among EGV, DFF and D50P. HSW had a negative correlation with DFF, D50F and D50P, and it had a positive correlation with EGV (r = 0.62, $p < 0.01$). GYP was positively correlated with NFPP and NTPP (r = 0.97 and r = 0.96 at $p < 0.01$, respectively). For quality traits, there were no correlations between CP and phenological and yield parameters under normal conditions. Positive correlations were observed between Fe and Zn (r = 0.68), D50P (r = 0.52) and DM (r = 0.38, $p < 0.05$), and negative correlations with EGV (r = −0.61, $p < 0.01$), HSW (r = −0.39, $p < 0.05$) and CP (r = −0.35, $p < 0.05$). In addition, a highly negative correlation was observed between Zn and CP (r = −0.47, $p < 0.01$) in normal conditions (Table III.4).

A significant negative correlation was found between EGV and DFF, D50F, and D50P under both temperature stress and combined temperature-drought stress (**Table III.5**). Similarly to normal conditions, GYP had a significant positive correlation with NTPP and NFPP (r = 0.90) under the

two stressed conditions. Under temperature stress, Fe content showed a positive correlation with D50F (r = 0.37, $p < 0.05$), GYP (r = 0.34, $p < 0.05$) and Zn (r = 0.46, $p < 0.01$). A significant and positive correlation between Zn and Fe (r = 48, $p < 0.01$) was also observed in combined temperature-drought stress conditions. In addition, Zn was found to be positively correlated with HSW (r = 0.34, $p < 0.05$) and negatively correlated with CP under temperature stress (r = −0.36, $p < 0.05$) and combined temperature-drought stress (r = −0.45, $p < 0.01$). Likewise, a positive correlation was observed between CP and DM (r = 0.36, $p < 0.05$) under temperature stress.

Table III.4*. Correlation between tested traits under normal conditions conducted at Marchouch*

	PLH	EGV	DFF	D50F	D50P	DM	NFPP	NUPP	NTPP	GYP	HSW	CP	Zn	Fe
PLH	1													
EGV	**.38***	1												
DFF	-.02	**-.46****	1											
D50F	-.10	**-.53****	**.70****	1										
D50P	-.15	**-.61****	**.49****	.30	1									
D95M	-.24	-.29	-.12	.01	.13	1								
NFPP	.25	.08	-.14	.03	-.23	.04	1							
NUPP	**-.40***	-.23	.09	-.06	.15	.01	-.28	1						
NTPP	.21	.05	-.13	.02	-.22	.04	**.99****	-.16	1					
GYP	.23	.16	-.15	.01	-.29	.02	**.97****	-.23	**.96****	1				
HSW	.24	**.62****	**-.53****	**-.58****	**-.48****	-.21	.07	-.14	.05	.12	1			
CP	-.02	-.03	.03	.10	-.10	-.31	.03	.09	.05	.01	-.13	1		
Zn	-.02	-.27	.04	-.14	.30	.22	-.03	.01	-.03	-.08	.02	**-.47****	1	
Fe	-.27	**-.61****	.19	.14	**.52****	**.38***	-.21	.19	-.19	-.28	**-.39***	**-.35***	**.68****	1

* and ** indicate significant difference at 0.05 and 0.01. PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods per plant^{-1}; NUPP, number of unfilled pods per plant^{-1}; NTPP, number of total pods per plant^{-1}; GYP, grain yield per plant^{-1}; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content; Fe, iron content.

Table III.5*. Correlation for late under combined temperature-drought stress (above) and temperature stress (below) in lentil*

	PLH	EGV	DFF	D50F	D50P	DM	NFPP	NUPP	NTPP	GYP	HSW	CP	Zn	Fe
PLH	1	**.40****	-.10	-.02	-.15	-.13	-.07	.06	-.06	-.03	.16	-.14	.09	.21
EGV	.18	1	**-.43****	**-.40****	**-.39***	**-.44****	-.23	-.21	-.27	-.11	.25	-.11	.12	.26
DFF	-.06	**-.41****	1	**.66****	**.54****	**.42****	.03	.09	.05	.02	.08	.17	-.28	-.17
D50F	-.17	**-.47****	**.81****	1	**.77****	**.63****	.07	-.02	.07	.03	**.34***	.19	-.31*	-.11
D50P	-.07	**-.52****	**.68****	**.79****	1	**.74****	.16	.19	.19	.16	.30	.16	-.24	-.15
DM	-.05	-.21	.19	**.32***	**.40***	1	**.32***	.11	**.34***	.25	.12	.12	**-.40***	-.19
NFPP	.01	.14	-.20	-.09	-.07	.08	1	-.06	**.98****	**.90****	-.11	.06	-.09	-.10
NUPP	.15	-.09	.05	.13	.17	.09	.05	1	.13	-.02	-.11	.15	-.09	-.24
NTPP	.03	.13	-.19	-.08	-.05	.09	**.99****	.15	1	**.89****	-.13	.09	-.11	-.15
GYP	-.04	-.02	-.15	.01	-.03	-.07	**.88****	-.02	**.86****	1	.02	.05	-.01	-.04
HSW	-.25	-.02	**.53****	**.38***	.25	-.22	-.13	-.08	-.14	-.04	1	**.32***	-.14	.02

CP	.03	-.06	-.09	-.15	-.12	**.36***	.28	-.03	.28	.12	-.31	1	**-.45****	**-.43****
Zn	.01	.12	.29	.29	.16	-.15	-.17	.24	-.14	-.12	**.34***	**-.36***	1	**.48****
Fe	-.01	-.24	.25	**.37***	.29	-.01	.26	.03	.26	**.34***	.14	-.11	**.46****	1

* and ** indicate significant difference at 0.05 and 0.01. PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods per plant^{-1}; NUPP, number of unfilled pods per plant^{-1}; NTPP, number of total pods per plant^{-1}; GYP, grain yield per plant^{-1}; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content; Fe, iron content.

III.3.6. Principal Component Analysis for Stress and Normal Conditions

Principal component analysis (PCA) was performed to study the relationships between traits in the studied lentil accessions. Under normal conditions, the first three PCAs (PC1: 31.3%, PC2: 22.6% and PC3: 16.5%) explained 70.4% of the total variability (Table III,6). The results of PCA showed that EGV, D50P, HSW and Fe content were the most important traits contributing to PC1 of distinct origin. In PC2, which described 22.6% of the total variance, NFPP, GYP and D50F demonstrated large contributions, while Zn content and CP accounted for much of the total variance in PC3 (Table III.7). These 20 accessions were clustered into three groups based on hierarchical cluster analysis (Figure III.2). The check Bakria (ILL 4605) and three accessions (ILL 5919, ILL 7813 and ILL 3484) were identified in group 1 and had the highest GYP (5.55 g) and HSW (3.27 g), and moderate Zn, Fe and CP. In group 2, ten accessions were classified and recorded the highest Fe (85.1 mg kg^{-1}) and Zn (54.1 mg kg^{-1}), with medium GYP and HSW. Six accessions were grouped in cluster 3 and exhibited the highest CP (29.9%), medium Fe (72.4 mg kg^{-1}) and HSW (2.5 g), and the lowest GYP (Table III.8). The distribution of accessions and measured traits along the first PC axes under normal conditions are shown in Figure III.3.

Table III.6. *Eigenvalues and eigenvectors of the three PCA dimensions under normal conditions, temperature stress and the combined temperature-drought stress.*

Traits	Normal			Temperature Stress			Temperature-Drought Stress		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
PLH	−0.52 *	0.21ns	−0.07ns	−0.31ns	−0.02ns	0.01ns	−0.27ns	−0.13ns	0.19ns
EGV	−0.78 **	−0.47 *	0.05ns	−0.49 *	−0.42ns	0.38ns	−0.64 **	−0.05ns	0.14ns
DFF	0.50 *	0.40ns	−0.50 *	0.86 **	0.30ns	0.02ns	0.64 **	−0.44ns	0.18ns
D50F	0.38ns	0.62 **	−0.58 **	0.86 **	0.44ns	0.01ns	0.74 **	−0.44 *	0.44ns
D50P	0.88 **	0.13ns	0.06ns	0.79 **	0.42ns	−0.21ns	0.77 **	−0.33ns	0.30ns
DM	0.46 *	0.16ns	0.45 *	0.22ns	0.41ns	−0.70 **	0.89 **	−0.11ns	0.19ns
NFPP	−0.50 *	0.83 **	0.14ns	−0.59 **	0.76 **	0.23ns	0.55 *	0.81 **	0.16ns
NTPP	−0.44 *	0.84 **	0.13ns	−0.57 **	0.76 **	0.23ns	0.59 **	0.78 **	0.09ns
NUPP	0.48 *	−0.29 **	−0.08ns	0.05ns	0.20ns	0.04ns	0.22ns	−0.20ns	−0.13ns
GYP	−0.49 *	0.81 **	0.11ns	−0.44ns	0.79 **	0.33ns	0.49 *	0.76 **	0.19ns
HSW	−0.71 **	−0.30ns	0.42ns	0.60 **	−0.09ns	0.42ns	0.13ns	−0.50 *	0.35ns
CP	−0.14ns	−0.07ns	−0.65 **	−0.34ns	0.32ns	−0.60 **	0.52 *	−0.30ns	−0.39ns
Zn	0.35ns	0.18ns	0.77 **	0.49 *	−0.08ns	0.61 **	−0.61 **	0.14ns	0.35ns

Fe	0.74 *	0.24ns	0.51 **	0.36ns	0.47 *	0.44ns	−0.64 **	0.05ns	0.55 *
Eigenvalue	4.38	3.17	2.31	4.08	2.92	1.98	4.83	2.70	1.73
Variance explained (%)	31.29	22.65	16.49	34.00	24.32	16.52	34.51	19.30	12.42
Total variance (%)	31.29	53.29	70.43	34.00	58.32	74.85	34.51	53.81	66.23

** 0.01, * 0.05, ns not significant. PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods plant^{-1}; NUPP, number of unfilled pods plant^{-1}; NTPP, number of total pods plant^{-1}; GYP, grain yield per plant; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content; Fe, iron content.

Table III.7. *Contribution of traits to the three first PCA dimensions under normal conditions, temperature stress, and the combined temperature-drought stress.*

Traits	Normal			Temperature stress			Temperature-drought stress		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
PLH	6.21	1.45	0.20	2.36	0.01	0.00	1.55	0.64	19.81
EGV	13.82	6.89	0.11	5.78	6.05	7.37	8.35	0.09	1.16
DFF	5.69	5.13	10.94	18.01	3.05	0.02	8.46	7.05	1.95
D50F	3.23	12.15	14.53	18.00	6.66	0.00	11.38	7.30	11.18
D50P	17.59	0.51	0.15	15.23	6.12	2.27	12.29	4.13	5.21
DM	4.87	0.83	8.63	1.14	5.89	24.59	16.56	0.48	2.11
NFPP	5.83	21.55	0.85	8.51	19.57	2.72	6.21	24.03	1.48
NUPP	5.39	2.66	0.31	0.07	1.31	0.10	7.30	22.50	0.46
NTPP	4.53	22.24	0.77	8.02	19.91	2.73	0.98	1.55	14.36
GYP	5.56	20.82	0.54	4.74	21.15	5.37	4.91	21.10	2.02
HSW	11.41	2.83	7.80	8.77	0.31	8.70	0.33	6.91	7.12
CP	0.46	0.17	18.38	2.79	3.52	17.92	5.66	3.40	8.83
Zn	2.80	0.97	25.43	5.79	0.21	18.69	7.65	0.73	6.88
Fe	12.60	1.79	11.36	3.22	7.55	9.63	8.36	0.08	17.43

PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods per plant^{-1}; NUPP, number of unfilled pods per plant^{-1}; NTPP, number of total pods per plant^{-1}; GYP, grain yield per plant^{-1}; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content; Fe, iron content.

Table III.8. *Grain yield, hundred-seed weight, crude protein, zinc content and iron content of the three clusters for normal, temperature stress and combined temperature-drought stress.*

Cluster	Experiment	GYP	HSW	CP	Zn	Fe
Cluster I	Normal	5.55	3.27	29.78	44.12	69.50
	High-temperature	1.65	4.95	22.52	56.63	73.85
	Temperature-drought	1.84	2.00	12.92	41.26	65.02
Cluster II	Normal	4.06	2.44	27.94	54.13	85.15
	High-temperature	3.30	2.17	24.66	42.49	69.73
	Temperature-drought	1.28	3.56	14.25	33.85	58.65
Cluster III	Normal	3.30	2.54	29.94	39.94	72.39
	High-temperature	1.97	2.35	24.37	39.30	64.77
	Temperature-drought	2.97	1.96	13.87	37.12	59.99

GYP; grain yield, HSW; 100-seed weight, CP; crude protein, Zn; zinc content and Fe; iron content.

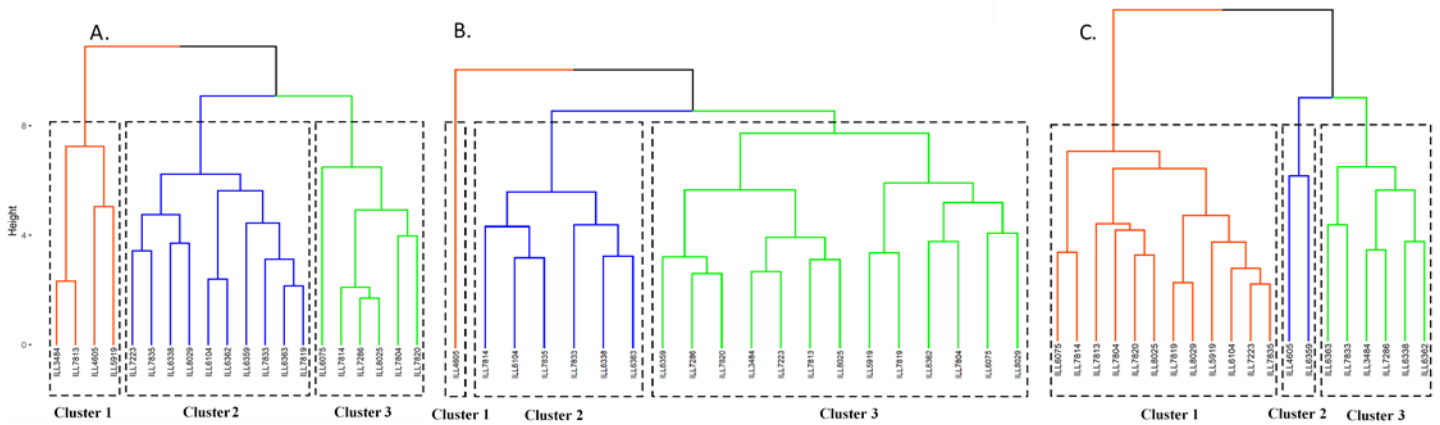

Figure III.2. Dendrogram normal (a), temperature stress (b) and the combined temperature-drought stress (c) condition.

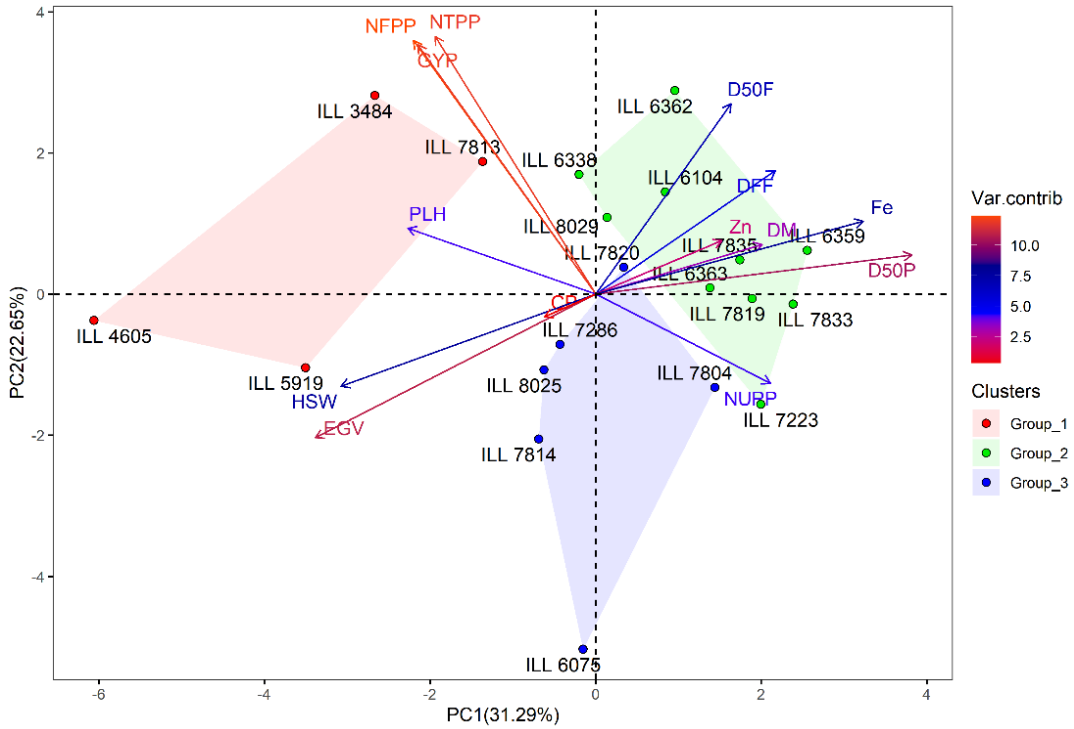

Figure III.3. *Biplot of the first two axes of the PCA analysis summarizing the relationship between the measured variables and the classification of lentil accessions under normal conditions.*

For temperature stress, PC1 and PC2 (PC1: 34.0% and PC2: 24.3%) explained 58.3% of the total variation, while PC3 explained 16.5% of the total variation. PC1 was positively correlated with DFF, D50F, D50P, HSW and Zn, whereas PC2 was positively correlated with GYP, NTPP, NFPP and Fe content. Furthermore, negative correlations were observed between PC1 and EGV, NTPP and NFPP. PC3 had a significant positive correlation with Zn content and negative association with CP and DM (Table III.6). Hierarchical cluster analysis was conducted, and the accessions

were grouped into three clusters (Figure III.2). Cluster 1 grouped only the check Bakria (ILL 4605), a moderate heat- and drought-tolerant variety, which had the highest HSW (4.95 g) and Zn and Fe contents with a mean of 56.6 and 73.8 mg kg^{-1}, respectively. Five heat-tolerant accessions (ILL 7814, ILL 6104, ILL 7835, ILL 7833 and ILL 6338) and one moderately heat- and drought-tolerant accession (ILL 6363) were identified in cluster 2. This group had the highest GYP with a mean of 3.30 g and CP with an average of 24.7%, while Zn and Fe concentrations were 42.5 and 69.7 mg kg^{-1}, respectively. Thirteen accessions including two heat-tolerant lines (ILL 8029 and ILL 7286), three combined temperature-drought tolerant accessions (ILL 6362, ILL 7804 and ILL 6075), five moderately tolerant lines and three susceptible lines were found in cluster 3 (Figure III.2). This group had moderate GYP with an average of 1.97 g, HSW with 2.35 g and CP with a mean of 24.4%, whereas Zn and Fe concentrations were 39.3 and 64.8 mg kg^{-1}, respectively (Table III.8). The biplot of PC1 and PC2 of the three clusters and traits under temperature stress conditions is shown in Figure III.4.

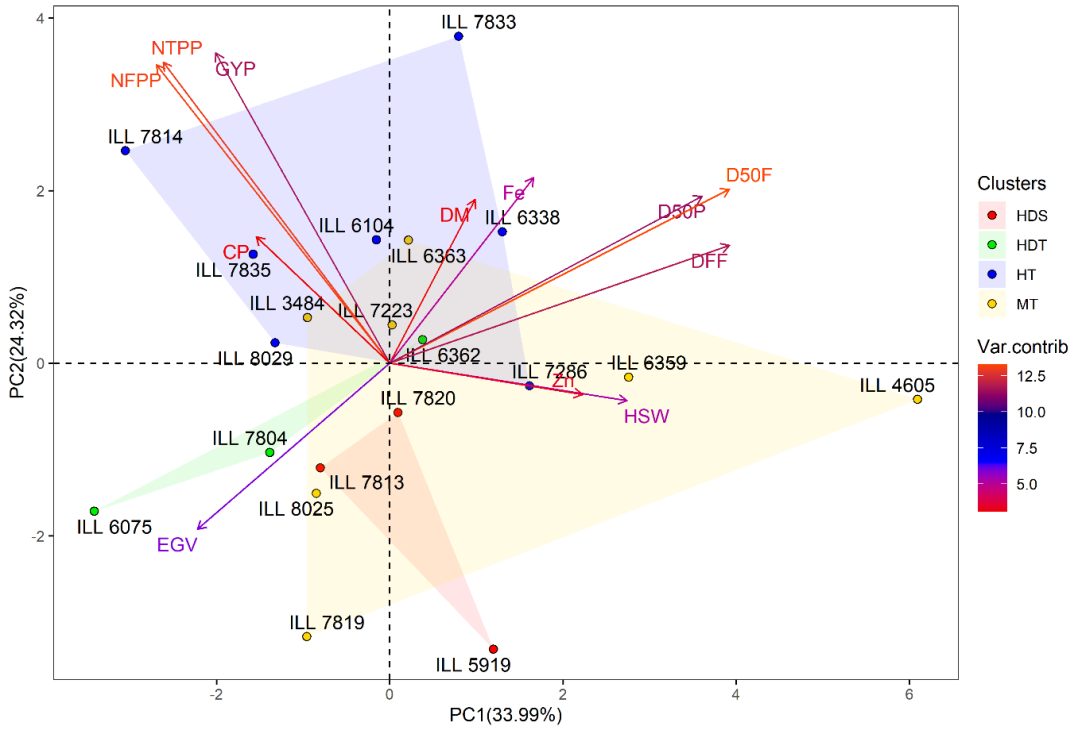

Figure III.4. *Biplot of the first two axes of the PCA analysis summarizing the relationship between the measured variables and the classification of lentil accessions under temperature stress conditions. HDS, heat stress and combined heat-drought susceptible; HDT, heat-drought tolerant; HT; heat tolerant and MT, moderately tolerant to heat stress and combined heat-drought stress.*

The first three PCAs in the combined temperature-drought stress explained 66.2% of the entire dissimilarity (PC1:34.5%, PC2:19.3% and PC3:12.4%). PC1 showed a significant positive correlation with D50F, D50P, DM, GYP, NTPP, NFPP and CP, and a negative correlation with Zn, Fe and EGV. PC2 was positively correlated with GYP, NTPP and NFPP, and negatively correlated with D50F and HSW (Table III.6). Based on hierarchical cluster analysis (Figure III.2), 11 accessions including two heat-tolerant lines (ILL 6104 and ILL 8029), four heat-drought tolerant lines (ILL 6075, ILL 7804, ILL 7835 and ILL 7814), three moderately tolerant lines (ILL 7223, ILL 7819 and ILL 8025) and three susceptible accessions (ILL 5919, ILL 7813 and ILL 7820) were found in group 1. This group was characterized by the highest Zn and Fe concentrations with an average of 41.3 and 65.0 mg kg^{-1}, respectively, and medium GYP (1.84 g). Group 2 comprised two moderately tolerant accessions (ILL 4605 and ILL 6359), and had the highest CP and HSW, with a mean of 14.2% and 3.56 g, respectively. Three heat-tolerant lines (ILL 7833, ILL 7286 and ILL 6338), two moderately tolerant (ILL 6363 and ILL 3484) and one heat-drought tolerant accession (ILL 6362) were grouped in cluster 3. This group had the highest GYP with an average of 2.97 g, and moderate concentrations of Zn and Fe with a mean of 37.1 and 59.9 mg kg^{-1}, respectively (Table III.8). The biplot of PC1 and PC2 of the three clusters and measured traits under the combined temperature-drought stress is illustrated in Figure III.5.

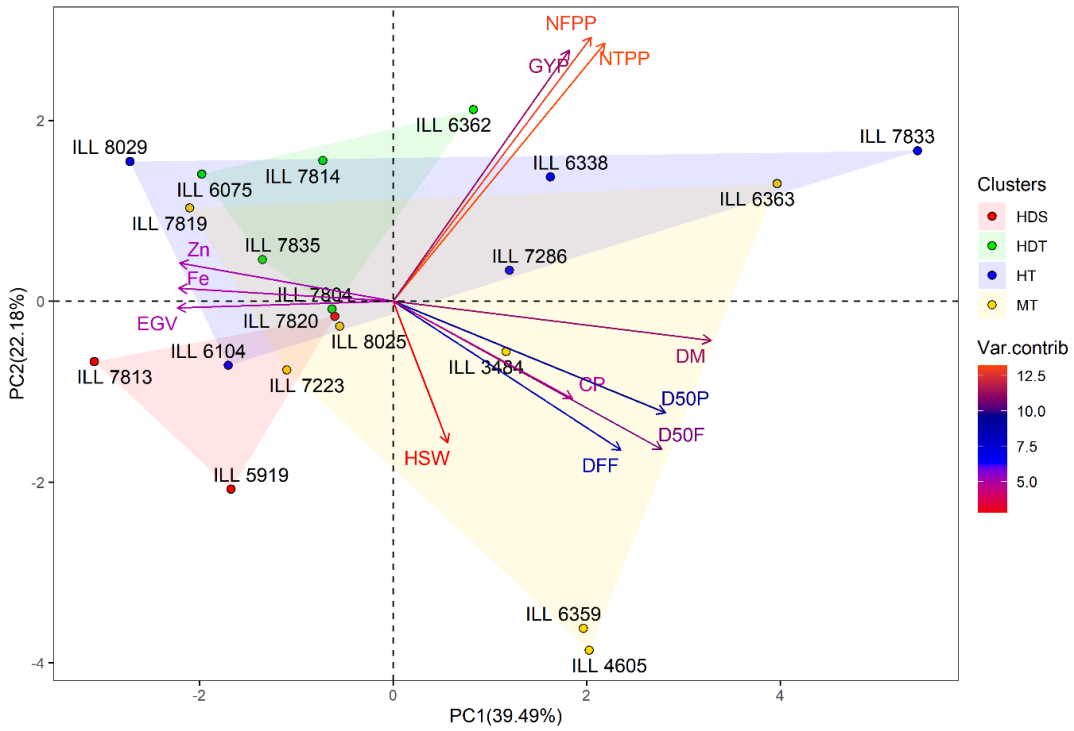

Figure III.5. *Biplot of the first two axes of the PCA analysis summarizing the relationship between the measured variables and the classification of lentil accessions under the combined temperature-drought stress conditions. HDS, heat-drought susceptible; HDT, heat-drought tolerant; HT; heat tolerant and MT, moderately tolerant to heat-drought stress.*

III.3.7. Canopy Temperature Variation Under Normal and Stress conditions

Analysis of variance for canopy temperature (CT) showed a significant difference between accessions and treatments; however, there were no significant differences for accession x treatment interaction (Table III.9). Under normal conditions, the CT of plants increased significantly from an average of 29.1 °C at day 100 to 31.8 °C at day 115 after sowing. Under normal conditions, the minimum and maximum CT were 26.6–32.7 °C at day 100 and 29.7–33.4 °C at day 115 (Table III.10). Under temperature stress, the plants recorded an average of 29.2 °C and 31.7 °C at day 65 and day 70, respectively. The average CT increased significantly from 31.5 °C at day 80 to 31.9 °C at day 90. The lowest maximum CT was recorded at day 65 with 31.3 °C, while the highest was achieved at day 90 with 33.8 °C. Under combined temperature-drought stress, the CT increased significantly from an average of 29.5 °C at day 65 to 31.9 at day 70. On day 80 and day 90, the CT was 31.6 and 31.7 °C, respectively. Plants at day 65 recorded the lowest maximum CT (31.3°C), while, the highest was recorded at day 90 with 34.0 °C.

Table III.9*. Analysis of variance for canopy temperature between normal conditions, temperature stress and the combined temperature-drought stress at Marchouch.*

Source	df	Mean Square	F
TRT	9	58.11*	64.54
Gen	19	2.99*	3.33
Rep	1	1.50ns	1.67
TRT * Gen	171	.89ns	.99

* indicates significant difference at 0.001 of probability, and ns denotes a non-significant

Table III.10. *Minimum, Maximum, Mean of canopy temperature under normal conditions, temperature stress and the combined temperature-drought stress at Marchouch*

	Temperature-drought stress				Temperature stress				Normal	
Days	**65**	**70**	**80**	**90**	**65**	**70**	**80**	**90**	**100**	**115**
Min	27.80	29.70	29.30	28.40	27.70	29.50	29.30	29.60	26.60	29.70
Max	31.30	33.60	33.90	34.00	31.30	33.10	32,40	33.80	32.70	33.40
Mean±S	29.53e	31.89ab	31.64cd	31.76abc	29.20f	31.67bc	31.50d	31.96a	29.10f	31.78ab
D	±0.8	±0.8	±1.1	±1.2	±0.8	d±0.9	±0.8	±0.8	±1.4	c±1.1
LSD	0.61	0.59	0.73	1.01	0.54	0.62	0.64	0.69	1.07	0.78
CV	2.50	2.16	2.73	4.22	2.17	2.31	2.47	2.57	4.78	2.93

Means followed by the same letters are not significantly different ($p<0.05$).

III.3.8. Transpiration Response to VPD Under Controlled Environments

The results revealed that nine lentil accessions were well represented by the two-segment regression with a breakpoint (BP) (Table III.11). However, 11 accessions exhibited a linear response and failed to express the limited transpiration (TR_{lim}) trait during the increase in VPD (Table III.12). The R^2 of the accessions with BP ranged from 0.60 to 0.87 and varied from 0.66 to 0.95 for the accessions without TR_{lim}. The results displayed high variation between the assessed accessions in TR to increasing VPD. The value of BP ± SE of the accessions expressing TR_{lim} varied from 2.76 ± 0.43 to 3.51 ± 0.54 kPa with an average of 3.14 ± 0.66 (Table III.11).

Table III.11. *Outputs from the analysis of the two-segment linear regression used for fitting the TR to the VPD variations.*

Accession	Breakpoint		Left Slope		Right Slope		R^2
	BP ± SE	CI (95%)	S1	CI (95%)	S2	CI (95%)	
ILL 3484	3.27 ± 0.95	3.01 to 4.50	44.48	32.68 to 59.19	24.95	3.45 to 39.94	0.87
ILL 6075	3.15 ± 1.14	2.73 to 4.51	28.91	21.74 to 45.84	17.37	0.25 to 28.46	0.82
ILL 6104	3.00 ± 0.25	3.00 to 3.45	36.40	25.72 to 47.06	12.26	−2.57 to 26.43	0.83
ILL 6338	3.37 ± 0.61	3.00 to 3.45	43.73	31.41 to 60.63	15.42	−9.64 to 38.61	0.81
ILL 6362	3.01 ± 0.99	2.76 to 3.46	44.71	34.40 to 71.39	28.14	2.88 to 41.59	0.87
ILL 7814	3.36 ± 0.56	2.79 to 4.17	34.42	25.90 to 77.81	5.59	−26.00 to 28.45	0.85
ILL 7833	2.81 ± 0.47	1.67 to 3.57	18.14	9.59 to 38.87	−1.63	−13.49 to 8.32	0.60
ILL 7835	2.76 ± 0.43	2.23 to 3.30	37.32	23.76 to 50.88	10.79	−3.32 to 24.91	0.79
ILL 8029	3.51 ± 0.54	3.07 to 4.01	22.69	14.10 to 31.29	13.10	−10.92 to 37.13	0.70

The BP is the breakpoint (kPa), S1 and S2 are the left slope and right slope, respectively (mg H_2O m^{-2} s^{-1} kPa^{-1}), CI is the confidence interval.

Table III.12. *Results of the 11 accessions exhibiting single linear regression fits of TR response to vapor pressure deficit. The table includes slope with standard error (SE), X-intercept and R^2 values.*

Accession	Slope ± SE	Slope CI (95%)	X-Intercept	X-Intercept CI (95%)	R^2
ILL 5919	27.23 ± 2.59	21.96 to 32.50	−0.58	−1.34 to −0.06	0.77
ILL 4605	16.53 ± 1.51	13.46 to 19.61	−0.39	−1.13 to 0.13	0.79
ILL 6359	31.18 ± 2.82	25.44 to 36.92	−0.13	−0.82 to 0.36	0.79
ILL 6363	27.07 ± 2.72	21.55 to 32.58	−0.75	−1.65 to −0.15	0.74
ILL 7223	25.95 ± 1.01	23.88 to 28.02	0.06	−0.20 to 0.28	0.95
ILL 7286	22.04 ± 2.98	15.94 to 28.13	−1.32	−2.84 to −0.44	0.66
ILL 7804	30.32 ± 2.17	25.93 to 34.71	0.12	−0.35 to 0.49	0.82
ILL 7813	29.08 ± 3.67	21.54 to 36.62	−0.15	−1.19 to 0.48	0.70
ILL 7819	24.22 ± 2.27	19.62 to 28.83	−0.20	−0.92 to 0.30	0.76
ILL 7820	23.01 ± 2.61	17.60 to 28.43	−0.61	−1.62 to 0.03	0.78
ILL 8025	36.48 ± 2.78	30.81 to 42.16	0.22	−0.27 to 0.59	0.85

High dissimilarity was distinguished for the slopes below and above BP increasing TR with further increases in VPD. The slope at VPD above BP ranged from −1.63 ± 5.34 to 28.1 ± 11.3 mg with an average of 14 mg H_2O m^{-2} s^{-1} kPa^{-1}. In contrast, the slope below BP varied from 18.1 ± 5.49 to 44.7 ± 7.37 mg with an average of 34.5 mg H_2O m^{-2} s^{-1} kPa^{-1}. Accession ILL 8029 showed the highest BP at about 3.51 kPa, while the two accessions ILL 7835 and ILL 7833 recorded the lowest BP at around 2.76 and 2.81 kPa, respectively (Figure III.6). The accession ILL 7833 expressed the lowest TR during the increase in VPD, with 18.1 and −1.63 mg H_2O m^{-2} s^{-1} kPa^{-1} for left and right slopes, respectively. Similarly, ILL 7814 and ILL 7835 were characterized by a very low TR after expressing the BP, with slopes greater than the BP ranging from 5.59 to 10.8 mg H_2O m^{-2} s^{-1} kPa^{-1}. Among accessions that did not express a BP, the check Bakria (ILL 4605) had the lowest slope with a TR of 16.5 ± 1.51 mg H_2O m^{-2} s^{-1} kPa^{-1}. Oppositely, ILL 8025 exhibited the highest slope with 36.5 ± 2.78 mg H_2O m^{-2} s^{-1} kPa^{-1}. The results also revealed that ILL 5919, ILL 7813 and ILL 8025 had the maximum TR at around 175 mg H_2O m^{-2} s^{-1} kPa^{-1}, which lost TR_{lim} trait expression during the increase in VPD. ILL 6262 recorded the highest TR with 210 mg H_2O m^{-2} s^{-1} kPa^{-1}, followed by the accessions ILL 6338 and ILL 3484, which had a TR of 190 mg H_2O m^{-2} s^{-1} kPa^{-1} (Table III.11).

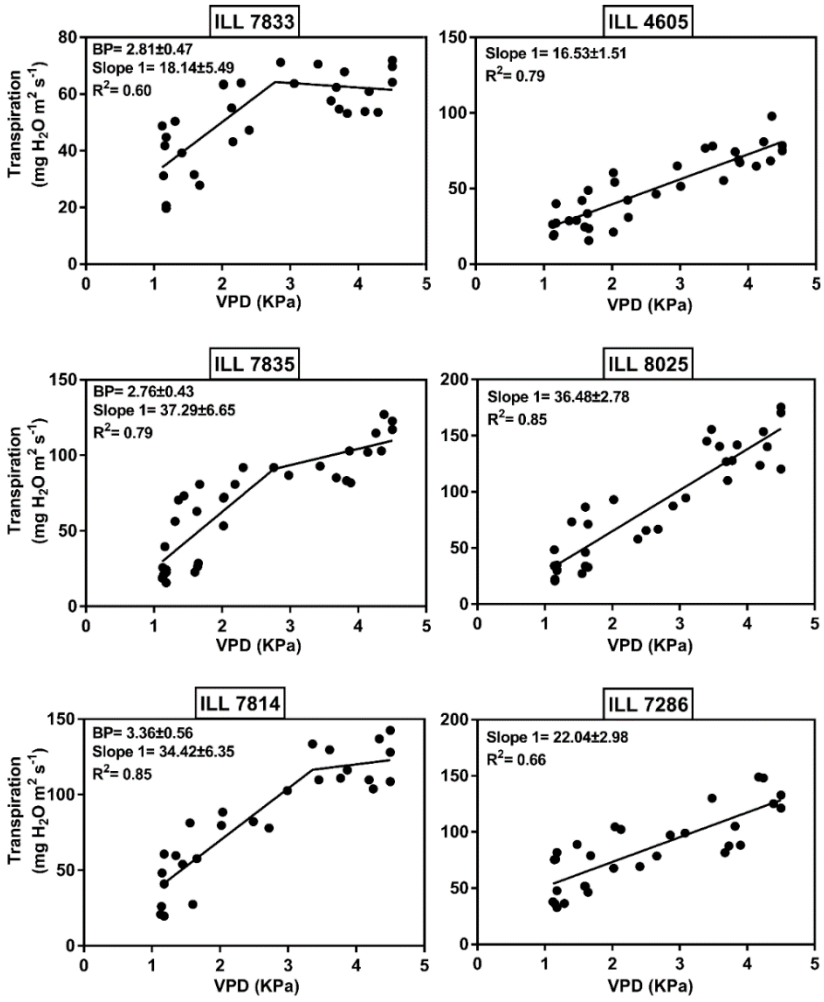

Figure III.6. *Transpiration rate (mg H_2O m^{-2} s^{-1}) response to increasing vapor pressure deficit (VPD in kPa). Accessions ILL 7833, ILL 7835 and ILL 7814 represent tolerant accessions with two segmental regression, while ILL 4605, ILL 8025 and ILL 7286 represent susceptible accessions with linear transpiration response to increasing VPD. The breakpoint (BP), slopes and R^2 are indicated.*

III.4. Discussion

III.4.1. Phenology, Yield and Nutritional Quality under High-Temperature and Drought Stress

In this study, delayed sowing to expose the reproductive stage to heat stress significantly affected phenological traits, grain yield components, nutritional quality parameters and canopy temperature in lentil accessions. However, combining drought with heat stress through limited irrigation had a more severe impact on plant growth, resulting in significantly lower grain yield and nutritional quality. These results are consistent with our previous studies that confirmed the influence of water

limitation and high temperature on lentil (Choukri et al., 2020; El Haddad et al., 2020). Similar findings were also observed in chickpea (Devasirvatham and Tan, 2018), lentil (Sehgal et al., 2019; Sita et al., 2018), soybean (Jumrani and Bhatia, 2018) and groundnut (Hamidou et al., 2013) where combined temperature-drought stress was more detrimental than temperature stress alone.

Our findings revealed that the number of filled pods decreased because of high-temperature and combined temperature-drought stress. This is in agreement with earlier studies that showed temperature and/or drought stress negatively influenced the reproductive processes, mainly pollen and ovule fertility (Mahrookashani et al., 2017; Ruan et al., 2010), resulting in the reduction of filled pods. Recently, Jiang et al. (2019) described that temperature stress reduced the number of pollen grains per anther, induced smaller pollen grains and increased reactive oxygen species (ROS) production in pollen grains in pea. Still, it did not affect ROS accumulation in ovules and ovule number per ovary. Furthermore, high-temperature exposure when young floral buds were visible at the first formed reproductive node was more destructive to flower retention, seed set, pod development and seed yield compared to heat exposure started later, when flowers at the second reproductive node were fully open. Temperature and drought stress also impact pollen vacuolization, which plays a vital role in increasing the volume of pollen grains with the accumulation of cytoplasmic components (Pacini and Dolferus, 2016). A recent study by Fábián et al. (2019) showed that the combination of high temperature and water stress altered the phenology of the plants, reduced pollen viability, shortened the duration of gametogenesis and grain filling, and modified the morphology and anatomy of the pistils. Functionality of female and male reproductive parts was reduced by 34 and 66%, respectively.

Our study showed a decrease in seed weight due to temperature stress, while combined temperature-drought stress magnified the impact. It is a fact that during the seed-filling stage, the decline in photosynthesis and leaf sucrose metabolism in response to temperature stress and drought stress led to lower carbohydrate availability for import into developing seeds (Cohen et al., 2021b). Previous investigations suggested seed weight is reduced in response to high-temperature and/or drought stress during grain filling by altering endosperm cells (Nicolas et al., 1985) and reducing starch accumulation (Balla et al., 2011). Starch is the central component in seeds for major global staple crops. A decrease in source strength and carbon availability for starch biosynthesis through the grain filling period will definitely reduce seed size and weight (X. Liu et al., 2020; Thitisaksakul et al., 2012). Recent studies revealed a decline in the activity of enzymes

involved in starch biosynthesis under high-temperature or water stress. Eventually, their combination contributed to a decrease in the starch accumulation rate and duration and starch content (Lu et al., 2019; Ning et al., 2018). Sehgal et al., (2019a) indicated a significant decrease in starch content under temperature stress and water stress in lentil seeds. However, the combined temperature-drought stress caused the most severe reduction due to the inhibition of starch synthesizing enzyme (starch synthase) and sucrose synthesizing activity (sucrose synthase), which were previously identified to have a correlated effect on seed size in chickpea (Kumar and Turner, 2009).

A significant reduction in protein content was reported under temperature stress; however, the combined temperature-drought stress caused more decrease, which is in agreement with previous observations in lentil (Choukri et al., 2020; Sehgal et al., 2019), and in chickpea (Awasthi et al., 2014) through reduced function of PSII, weakened nitrogen anabolism and strengthened protein catabolism. Previous findings by Zahedi et al. (2004) and Triboï et al. (2003) revealed a stable relation between protein content and the total quantity of nitrogen in wheat grain. They suggested that the synthesis of storage proteins is limited mainly by the availability of nitrogen. Similarly, Liu et al. (2018) also reported a decrease in crude protein fractions in drought-stressed alfalfa (*Medicago sativa* L.) due to water limitation in addition to a reduction in N fixation. For that, improvement of N fixation could lead to higher biomass production and water-use efficiency and may increase pod yield under drought stress conditions.

High temperature induced a significant reduction in grain micronutrient concentrations. The combined temperature-drought stress further aggravated the decline, by 18% for Zn and 20% for Fe, compared to that under normal conditions. Similar findings were observed in other legume crops such as lentil, where iron and zinc contents were dramatically reduced in response to temperature and drought stress due to decreased root nutrient uptake, with reduced root biomass and metabolic rate (Heckathorn et al., 2013) or by direct damage to roots (Huang et al., 2012). In addition, the reduction in transpiration rate because of water deficit may also decrease nutrient absorption and the effectiveness of their use by the plants (Bista et al., 2018; Sehgal et al., 2019). Choukri et al. (2020) suggested that the decrease in iron and zinc was attributed to decreasing water availability under heat and drought stress conditions. Furthermore, Hummel et al. (2018) reported that the variations in zinc, iron and protein under drought stress conditions are more affected by weather conditions than accession.

The present study also revealed a negative correlation between Zn and protein content in high-temperature stress and heat-drought stress conditions, and between Fe and protein content in the combined temperature-drought stress, which are in disagreement with previous studies conducted by Ghanbari et al. (2013) and Impa et al. (2019). They reported a positive correlation between protein content and micronutrients under temperature and drought stress conditions. However, Fe and Zn were positively correlated under the three treatments, which was also reported in earlier findings in lentil (Khazaei et al., 2017), chickpea (Samineni et al., 2022; Tan et al., 2018) and pea (Ma et al., 2017). Furthermore, our results showed a significant increase in canopy temperature of plants under high-temperature stress and combined temperature-drought stress. This effect might be explained by inhibition of stomatal conductance and transpiration reducing leaf water content, which resulted from an increase in leaf temperatures (Anjum et al., 2011; Sehgal et al., 2018).

III.4.2. Transpiration Response to High VPD under Controlled Conditions

Our findings showed significant genetic variation among lentil accessions in transpiration response to increasing VPD under controlled environments. Eleven accessions consistently increased TR with increasing VPD, while nine exhibited a distinct response by limiting their TR when VPD reached about 3.1 kPa. Our outcomes indicated that most of the accessions displayed lower BP compared to what was reported by Guiguitant et al. (2017), who showed a BP at approximately 3.4 kPa in the majority of lentil accessions, with the lowest BP at 3.31 kPa. In our study, two tolerant accessions (ILL 7833 and ILL 7835) limited their TR at early VPD (2.8 kPa), representing the lowest BP over all the already published VPD experiments in lentil. Restriction of TR under high VPD was also reported in many other species such as chickpea (Zaman-Allah et al., 2011), peanut (Shekoofa et al., 2015), soybean (Gilbert et al., 2011), sorghum (Shekoofa et al., 2014) and maize (Sunita et al., 2014), in which the BP values mostly fluctuated from 1.1 to 2.7 kPa. However, Schoppach and Sadok, (2012) showed high BP ranging from 2.4 to 3.9 kPa in wheat, while Sinclair et al. (2018) described a VPD threshold ranging from 2.6 to 3.38 kPa in peanut.

Over the whole range of tested VPD, almost all of the tolerant accessions showed lower TR_{lim} compared to the sensitive lentil lines, which exhibited a continuously increasing TR with increasing VPD. These results support our field findings (El Haddad et al., 2020), and they are in good agreement with the conclusion of Belko et al. (2013) in cowpea. Accessions with TR_{lim} may have considerable potential for increased soil water conservation, indicating somehow more

effective control of TR under limited-water conditions (Ghanem and Sinclair, 2019). Several findings reported that accessions with a conservative water behavior have the opportunity of using the conserved soil water to preserve physiological activity during the grain filling period and produce higher grain yield than accessions without the TR_{lim} trait in late-season water deficit conditions (Ghanem et al., 2017; Ghanem and Sinclair, 2019; Guiguitant et al., 2017; Messina et al., 2015; Sinclair, 2005; Sinclair et al., 2017).

Interestingly, the check variety Bakria, a moderately tolerant line to drought and heat stress, exhibited the lowest TR among all tested lines with 16 mg H_2O m^{-2} s^{-1} kPa^{-1} under increasing VPD, and probably had the lowest conductance among the studied accessions. Among the tolerant lines, ILL 7833 and ILL 8029 had the lowest TR presented in slope 1 with 18.1 and 22.7 H_2O m^{-2} s^{-1} kPa^{-1}, respectively. However, accession ILL 8029 registered the maximum BP at 3.51 kPa, which was likely to be disadvantageous in expressing the TR_{lim} trait under high levels of VPD (Schoppach and Sadok, 2012; Vadez et al., 2013). Variation in TR shown in the two slopes, which normalized water use efficiency, can be explained by the difference in the hydraulic conductance to water flux in the plants. Several studies have indicated that the TR_{lim} trait under high evaporative conditions of VPD is likely due to low hydraulic conductivity in leaves between the xylem and into the guard cells, which increased water use efficiency (Fan et al., 2020; Sinclair et al., 2008; Sunita et al., 2014). The data described here do not allow support for the claim of a hydraulic conductance limitation in leaves; however, the difference in VPD breakpoint values among lentil accessions may indicate variation in stomatal conductance between studied accessions.

Since the experiment was conducted under controlled conditions, the variations in temperatures and relative humidity were the main key factors to reach higher VPD levels; however, the high temperatures (>35 °C) may influence TR response of the plants. A recent study by Kar et al., (2020) revealed that an increase in VPD had a powerful positive impact on plant water loss irrespective of the effect of temperature, light and relative humidity. Although previous studies have reported similar observations, the effects of temperature, relative humidity, radiation and water on TR had minor effects compared to VPD (Chenu et al., 2018; Schoppach and Sadok, 2012; Seversike et al., 2013).

Accessions with TR_{lim} under high VPD offer an essential candidate for breeding programs to improve lentil yields in water deficit areas (Guiguitant et al., 2017). A low BP represents an imperative approach by the plants to maintain the greatest water conservation during critical

drought periods when high VPD promotes TR. Our outcomes indicate that studied lentil accessions could be used to improve yield potential over a range of drought-stressed environments. Hence, tolerant accessions such as ILL 7833 and ILL 7835, which had the lowest BP, may be desirable for drought-prone conditions and improve lentil productivity in regions affected by drought stress. The TR profiles generated from the current investigation present an opportunity to understand the physiological behaviors of tolerant and sensitive lentil accessions under controlled conditions. However, additional studies are needed to examine more physiological and genetic responses of lentil material under field and controlled conditions.

III.5. Conclusion

In the present chapter, we validated the outcomes of the previous field screening results presented in the chapter II. Almost all tolerant accessions showed a high adaptation to high temperatures and combined temperature-drought stress under field conditions and exhibited a fairly clear TR_{lim} at high VPD under controlled conditions. Eleven accessions consistently increased TR as VPD increased, whereas nine accessions responded differently by limiting TR when VPD reached about 3.1 kPa. Accessions with limited transpiration would contribute to water saving in soil and improve the productivity under terminal heat or water-limited environments. In late-season water deficit conditions, tolerant accessions can use conserved soil water to maintain physiological activity during the grain filling period and produce higher grain yield than accessions lacking the TRlim trait. Further, these accessions provide an important candidate for breeding programs aimed at increasing lentil yields in water-stressed areas. Interestingly, two tolerant accessions (ILL 7833 and ILL 7835) exhibited the lowest breakpoint found in lentil, which may be exclusively desirable for harsh regions affected by water deficit. Accessions such as ILL 7835, ILL 7814 and Bakria (ILL 4605) combine good nutritional quality with moderate to high yield under both heat and combined heat-drought stress, which is of great interest for breeding programs. The parameters of the tested lentil accessions in response to high temperatures, water deficit and higher VPD would facilitate formulating new experimental strategies to shed light on the molecular and genetic basis of heat and drought tolerance mechanisms in the future. Hence, combining crop physiology with molecular techniques could be a promising approach to improve lentils under high temperatures, water deficit and higher VPD.

Chapter IV : Enzymatic and Non-enzymatic Responses to High Temperatures and Combined High-temperature-Drought Stress in Lentil (*Lens culinaris* Medikus)

IV.1. Introduction

Under constantly changing environmental conditions, crops are exposed to various abiotic stresses simultaneously during their life cycle causing significant damage in the growth and productivity of agricultural crops (Kumar et al., 2019; Samineni et al., 2022). Among abiotic stresses, extreme temperature and drought stress are described as two most harmful environmental threats that limit the growth, productivity and crop yield of plants, and ultimately the food security under changing climate (Çelik et al., 2017). Both stresses negatively impact the production of most important staple food crops (Awasthi et al., 2017; El Haddad et al., 2021; Sarkar et al., 2021; Zahra et al., 2021), that account for 60% of global food energy supply (Hussain et al., 2019). According to the Intergovernmental Panel on Climate Change 2022 (IPCC, 2022), the frequency and intensity of drought, accompanied by high temperatures, have increased in recent decades and are expected to escalate in the future climate, increasing malnutrition and micro-nutrient deficiencies, concentrated in sub-Saharan Africa, South Asia, Central and South America and small Islands. As elevated temperatures and water deficit are expected to enlarge around the world, it is necessary to understand physiological and biochemical mechanisms to combat heat and drought stress and increase lentil production and meet the rising demand of the world population increases.

The effects of high temperature and water stress are gaining more attention, as they are a serious hazard to the productivity of leguminous crops by altering pollen viability, fertilization and ultimately lead to reduction in pod set (Gaur et al., 2014). Temperatures above 32/20 (max/min) at the flowering and pod filling negatively the growth of lentil, from germination to grain filling, and eventually altering yield and quality (Bhardwaj et al., 2021; Choukri et al., 2020). High Temperature is very often associated to declining the availability of water (Tabari, 2020). Wahid et al. (2007) reported that heat stress can be accompanied by drought due to the rapid water from the plant and soil surface under high temperature. The general impacts of high temperature and drought stress have been studies in different plant species such as wheat (Senapati et al., 2021), barley (Naz et al., 2022), maize (Liu et al., 2020), chickpea (Rani et al., 2020), lentil (Choukri et al., 2020) and soybean (Cohen et al., 2021a). However, the combined effects of heat stress and drought on plant growth, productivity and quality are more severe than the individual effects, with

the reproductive phase more susceptible than to the vegetative stage (Liu et al., 2019; Sehgal et al., 2019).

Plant exposure to heat and drought stresses causes imbalanced on equilibrium between reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide radicals ($-O_2$), hydroxyl radicals ($OH^{\cdot}$), singlet oxygen ($^1O_2$), and scavenging activities of antioxidant system elements. Excessive production of ROS because of abiotic stresses disrupt photosynthesis, increase lipid peroxidation, electrolyte leakage, and destroys cell membrane structures as well as nucleic acid and protein disruption and therefore impairing the normal functions of cells (Roy et al., 2021). In order to adapt stress conditions and obtain oxidative homeostasis, plants use several mechanisms including accumulation of solutes (e.g. proline, soluble sugars, free amino acids and proteins), protective proteins (e.g. heat shock proteins) and activation of enzymatic (catalase, superoxide dismutase, ascorbate peroxidase, peroxidase) and non-enzymatic (tocopherols, carotenoids, ascorbate and glutathione) antioxidant systems (Sharma et al., 2021; Zhao et al., 2021). Therefore, enzymatic and non-enzymatic responses to high temperatures and drought stress could be studied as an important process to enhance the stress tolerance of crops.

Lentil (*lens culinaris* Medikus) is the third-most important cool-season grain legume in the world after chickpea and pea (Sehgal et al., 2021), covering an area of 5 million ha worldwide with a production of 6.5 million tons and productivity of 1305 Kg ha^{-1} in 2020 (FAOSTAT, 2020). Lentil is mainly cultivated under rainfed environments during winter season (Venugopalan et al., 2021), where extreme temperature and drought stress conditions become more severe limiting its productivity in the arid and semi-arid areas (Ghanem et al., 2017). Lentil reported for its high susceptibility to high temperature and drought stress, principally during reproductive stage, significantly inhibit its yield potential (El Haddad et al., 2022; Sehgal et al., 2017; Sita et al., 2017a).

Till today, there are few investigations on enzymatic and non-enzymatic responses of lentil, an important defense system to adapt to the frequent events of heat and drought stresses. In this context, antioxidant systems of fifteen lentil accessions selected from our previous studies (El Haddad et al., 2022, 2020), were investigated against drought and heat stresses. In this chapter biochemical characteristics of these accessions were assessed under intensive high temperature (above 32 °C) and drought stress conditions according to the activities of enzymes as catalase,

ascorbate peroxidase and superoxide dismutase as well as for accumulation of solutes profiles such as proline content and soluble sugars.

IV.2. Materials and Methods

IV.2.1. Germplasm

Fifteen contrasting lentil accessions selected from our previous studies (El Haddad et al., 2020, El Haddad et al., 2022) were used. The material consisted of four heat tolerant, one drought tolerant, three tolerant to heat and drought, five moderately tolerant and two sensitive lines were used. The material was sourced at the International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco. Details of these of these accessions are described in Table IV.1.

Table IV.1. *Description of origin, IG number and classification of lentil accessions used in the current study. MHT: Moderately heat tolerant; MDT: Moderately drought tolerant, HT: heat tolerant, HS: heat sensitive, DT: drought tolerant, DS: drought sensitive.*

Accession	IG number	Origin	Classification
ILL 5919	69528	Ethiopia	HDS
ILL 6075	70130	Pakistan	DT
ILL 6104	70159	Pakistan	HT
ILL 6338	71270	Pakistan	HT
ILL 6359	71291	Pakistan	MT
ILL 6362	71294	Pakistan	DT
ILL 6363	71295	Pakistan	MT
ILL 7223	75942	Nepal	MT
ILL 7814	114931	Nepal	HDT
ILL 7820	114951	Nepal	HDS
ILL 7833	115006	Nepal	HT
ILL 7835	115010	Nepal	HDT
ILL 8025	117726	Pakistan	MT
ILL 8029	117734	Pakistan	HT

IV.2.2. Experiments Details

The experiment was carried out in controlled glasshouse at ICARDA-Rabat, Morocco (latitude 33°58'45.7"N longitude 6°51'44.9"W, and altitude 52 m). The seeds of tested lentil accessions were obtained from ICARDA. Seeds were planted in plastic pots (20 cm in diameter and 18 cm in height) filled with sandy-loam soil and compost garden soil in a proportion of 2:2 w/w with a total weight of each filled pot was 3 Kg. In each pot, five seeds were initially sown (mid December 2018) at 2 cm depth and thinned to three per pot after emergence. Three treatments were conducted

named control (C), drought stress (D) and heat stress (H). For each treatment, randomized complete block design with four replications was used. For control treatment, plants were maintained under normal conditions, temperature was 25°C/18°C (day/night), 90/60% relative humidities (RH), light intensity 450 umol m^{-2} s^{-1}. Plants were fully irrigated daily between 09:00 and 10:00 h during their development, however, irrigation was restricted at flower initiation stage for drought treatment to impose water stress. At the first anthesis, plants of heat treatment were exposed to temperatures above 32°C for 4 h in growth chamber at 65-70% RH for a period of 7 days, while plants under drought stress were subjected to severe water stress for 9 days by maintaining 75% of relative water content of normal conditions. Immediately after heat and drought treatment, whole plants were collected in liquid nitrogen and stored in temperature below 20°C for various analysis. Plants under control conditions were also collected.

IV.2.3. Biochemical Analysis

- **Determination of Total Phenolic Content**

The total phenolic contents were determined following the method of (Andrew L Waterhouse, 2002). 200 µL of extract and 1 mL of Folin–Ciocalteu reagent were added in 2 mL Eppendorf tube. The mixture was incubated for 5 min, and then 800 µL of aqueous sodium carbonate 20% was added. The mixture incubated at room temperature in the dark, for 60 min. The absorbance was measured at 765 nm using a Thermo scientific spectrophotometer. The total phenolic concentration was calculated using gallic acid standard curve. Results were expressed as mg gallic acid equivalents (GAE) per gram of dry weight. Total phenolic content (TPC) was calculated as follows (Makris and Kefalas, 2013).

$$\mathbf{TPC}_{\left(\mathbf{mg}\frac{\mathbf{GAE}}{\mathbf{g}}\mathbf{dw}\right)} = (\mathbf{C} \times \mathbf{V})/\mathbf{m}$$

Where C is the total phenolic concentration (mg L^{-1}), V is the volume of the extraction and m is the dry weight (g) of the plant material.

- **Estimation of DPPH_ Scavenging Activity**

The antioxidant potential of the extracts was determined on the basis of their scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, according to the method described by Brand-Williams et al. (1995). Briefly, 200 µl of each extract preparation were added to 1.8 ml of 0.1 mM DPPH dissolved in methanol. The mixture was shaken and left for 30 min at

room temperature, and the absorbance of resulting solution was read at 517 nm. A lower absorbance represents a higher DPPH scavenging activity. The scavenging effect was expressed as shown in the following equation:

% scavenging activity = (Blank absorbance - Sample absorbance)/Blank absorbance) x100)

- **Total Soluble Sugars**

The analysis of total sugars concentration of samples was carried out using the colorimetric method of (Dubois et al., 1956). 2 mL aliquot of a carbohydrate solution is mixed with 1 mL of 5 % aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid is added rapidly to the mixture. After allowing the test tubes to stand for 10 min, they are vortexed for 30 s and placed for 20 min in a water bath at room temperature for color development. Then, light absorption at 490 nm is recorded on a spectrophotometer. Reference solutions are prepared in identical manner as above, except that the 2 mL aliquot of carbohydrate is replaced by bidistilled water. The phenol used in this procedure was redistilled and 5% phenol in water (w/w) was prepared immediately before the measurements.

- **Reducing Sugars**

The analysis of residual reducing sugars from the different samples was carried out through the analysis of reducing sugars by 3,5-dinitro-salicylic acid (DNS) as described by (Miller, 1959). 1 mL of standard or test sample in a 25 mL test tube and then 3 mL of DNS reagent was added. The reaction mixture was heated in boiling water for 5 min. The absorbance spectra of the test samples were measured at 540 nm using 2 mm path length quartz cuvettes. The measured absorbance was compared to the values of a standard mannose curve (180.2 mw) at 0 to 20 mM to detect the presence of reducing sugars released in each extract.

- **Total Flavonoids Content**

Total flavonoid constituents of seed extracts were performed employing aluminum chloride colorimetric assay and Quercetine as standard (Chang et al., 2002). 1 ml extract was added to10 ml flask containing 4 ml of distilled deionized water. A reagent blank using dd H_2O instead of the extract was prepared at this stage. 0.3 ml 5% $NaNO_2$ was added to the flask and the flask was vortexed immediately and waited 5 min. Then, 0.3 ml 10% AlCl3 solution was added to the mixture and waited 5 more min. At 6th min, 2 ml 1M NaOH was added, and the total volume was diluted to 10 ml with dd H2O. The solution was vortexed vigorously and absorbance was measured

against blank at 510 nm with a spectrophotometer. All procedure was repeated to all standard solutions of Quercetine (20-100 mg/l) and a standard curve was obtained. Total flavonoid content of extracts was calculated as mg Quercetine equivalents ((CE)/ g of extract. All samples were analyzed in triplicates.

- **Total Tannins Content**

The tannin contents were determined using the vanillin/HCl method of (Price et al., 1978). The reagent was prepared by mixing equal portions of methanol solutions of 8% HCl and 1% vanillin, just before the reaction. Aliquots of 0.1 mL of lentil leaf extracts and 0.2 mL of vanillin/HCl reagent were mixed and incubated at 30° C for 20 min. the absorbances were read at 500 nm. A standard curve was obtained using catechin methanol solutions in the range 0.1-2.5 mg mL^{-1}.

- **Total Soluble Protein**

The soluble protein concentration was estimated using a method devised by Lowry et al., (1951). The plant material (100 mg) was macerated in 0.1 M phosphate buffer (pH 7.0) and centrifuged at 513.16 g for 15 min to obtain a supernatant. 5 mL of TCA (trichloroacetic acid; 15%) was added to the supernatant and kept at 4°C for 24 h. The mixture was then centrifuged at 513.16 g for 15 min to separate the precipitates. The supernatant was discarded and the precipitate dissolved in 0.1 N NaOH (1 mL), kept for 18 h for complete dissolution, and treated as an extract.

- **Proline Content**

Proline content was extracted and estimated following the method of Bates et al. (1973). 100 mg leaf was homogenized in 10 ml of 3% sulphosalicyclic acid and then centrifuged at 3000 rpm for 10 min. Filtrate was used for estimation by adding 2 ml of acid ninhydrin and 2 ml of glacial acetic acid. Mixture was kept in water bath at 100°C for 1 hour and then transferred to separating funnel. Following which 4 ml of toluene was added after that. Two layers were formed; upper layer was used for measuring absorbance at 520 nm against toluene as blank.

- **Antioxidant Enzyme Activity Assays**

Catalase (CAT) activity was measured according to the method of Chance and Maehly (1955). Sample were ground in mortars using liquid nitrogen. The enzyme was extracted from 0.5g of tissue with chilled 50 mM sodium phosphate buffer (pH= 7), containing 1% (w/v) polyvinylpyrrolidone (PVP) and centrifuged at 12,000 rpm for 20 min at 4°C. Supernatant was

used for enzyme estimation. The reaction mixture consisted of 1.9 ml of 50 mM sodium phosphate buffer (pH 7.5), 0.1 ml enzyme extract and 1 ml of hydrogen peroxide. Decrease in absorbance after every 30 s interval for 2 min was recorded at 240 nm against blank. Enzyme activity was calculated using extinction coefficient of H_2O_2 (0.036 mM^{-1} cm^{-1}) and was expressed μmol mg^{-1} protein min.

Ascorbate peroxidase (APX) activity was measured according to the method of Nakano and Asada (1981). 0.5g of powdered sample was extracted with 2 mL of 50 Mm sodium phosphate buffer (pH 7.5) containing 1% PVP and 2 mM ascorbate. The homogenized material was centrifuged at 12 000 g for 20 min at 4°C. The supernatants were used as the crude enzyme source for the enzymatic assays. The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 2 % H_2O_2, and 0.1 mL enzyme extract in a final volume of 3 mL. The decrease in absorbance at 290 nm for 1 min was recorded and the amount of ascorbate oxidized was calculated using extinction coefficient (e = 2.8 mM^{-1} APX was defined as 1 mmol mL^{-1} per min at 25°C, cm^{-1}). Enzyme activity was expressed as lmol min^{-1} g^{-1} protein.

Superoxide dismutase (SOD) activity was assayed by monitoring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium chloride (NBT). One unit of SOD was defined as the amount of enzyme necessary to cause 50% inhibition of the rate of NBT reduction at 560nm. 3 ml of the reaction mixture contained 75 μM NBT, 1.5 mM riboflavin, 50 mM sodium bicarbonate, 13 mM methionine, 0.1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.5) and 100 μl of enzyme extraction. The test cells containing the mixture were placed on a shaker under light at 78 μmol photons s^{-1} m^{-1} for 15min and absorbance at 560 nm was recorded. A non-irradiated reaction mixture that did not develop color served as the control and its absorbance was subtracted from A560 of the reaction solution (Giannopolitis and Ries, 1977).

IV.3. Results

IV.3.1. Antioxidant Enzyme Activity (CAT, APX and SOD)

Application of drought stress was conducted for seven accessions, while twelve accessions were subjected to high-temperature (>32 C) during the reproductive stage. The results revealed significant increase ($p<0.05$) in CAT activity for all accessions subjected to heat stress (Figure IV.1A). The accessions ILL6359, ILL7814 and ILL7835 recorded the highest accumulation of CAT under heat stress, while ILL5919, ILL6075 had the lowest values. Drought stress resulted in

a slight increase in CAT activity for most of the accessions, however, ILL7814 and ILL7835 increased significantly CAT compared to the control (Figure IV.1B).

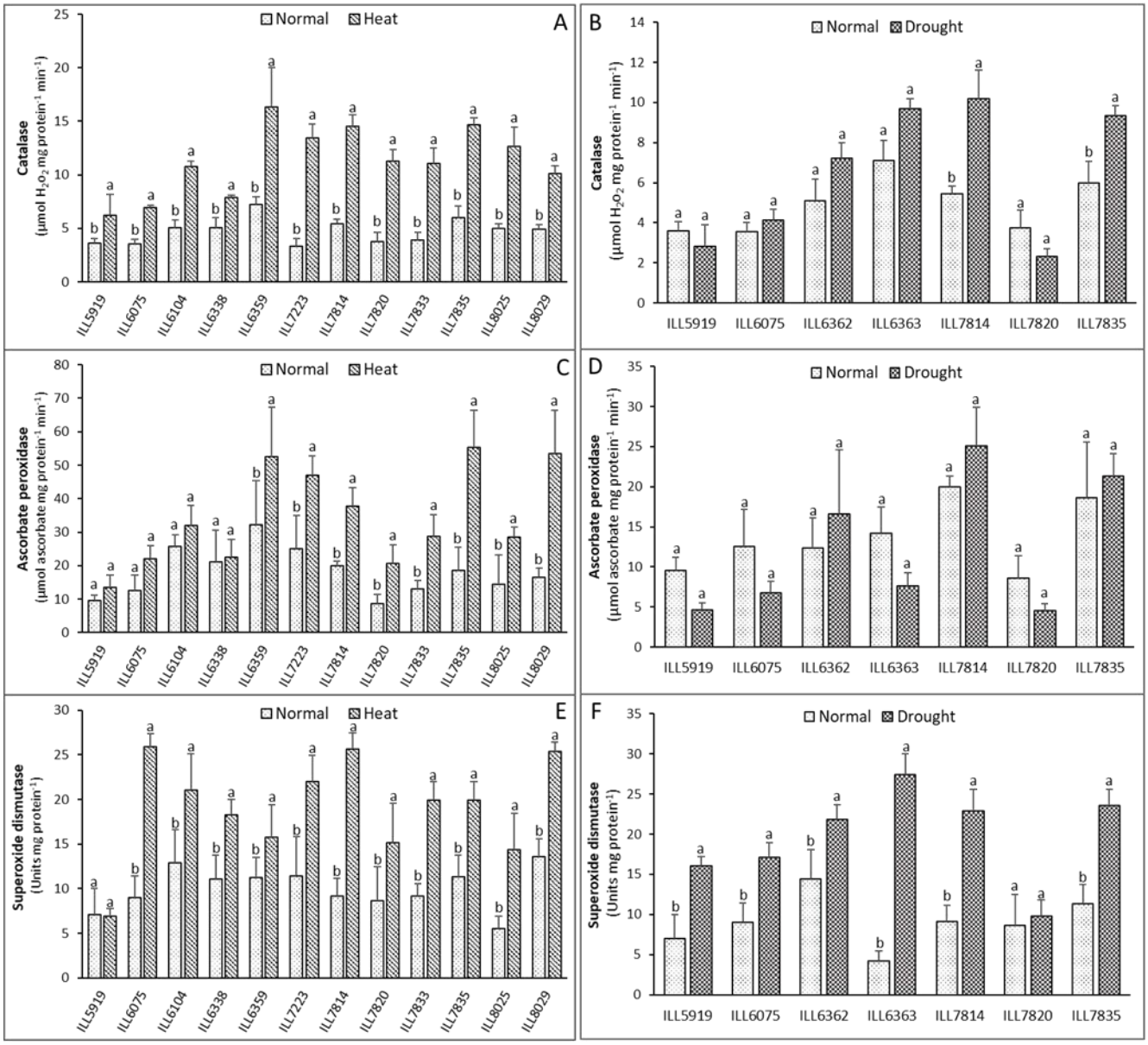

Figure IV.1. *Catalase, ascorbate peroxidase, superoxide dismutase activities against high-temperature and drought stress. Different letters on the bars indicate significant differences between means using LSD test.*

Ascorbate peroxidase (APX) significantly ($p< 0.05$) increased for all the accessions under high-temperature, except ILL5919, ILL6075, ILL6104 and ILL6338 which had values statistically similar with control (Figure IV.1C). ILL6359, ILL7835 and ILL8029 accumulated the highest values of APX under heat stress with about 50 µmol ascorbate mg protein^{-1} min^{-1}. In contrast, no

significant difference was obtained for all accessions under drought stress, the highest APX activity was recorded by the accession ILL 7814 with 25 µmol ascorbate mg protein^{-1} min^{-1} (Figure IV.1D). Superoxide dismutase (SOD) significantly enhanced for all accessions except ILL5919 in heat stress and ILL7820 in drought stress as compared to the control (Figures IV1E and F). The accessions ILL6075, ILL7814 and ILL8029 accumulated the highest value of SOD under heat stress with about 25 Units mg protein^{-1}, whereas ILL6363, ILL7814 and ILL7835 had the maximum SOD activity in drought conditions with up 22 Units mg protein^{-1}.

IV.3.2. Effect of Heat and Drought on Proline Content

As compared to control conditions, proline content (PC) increased significantly ($p<0.05$) for all the accession in response to heat and drought stress, except two accessions (ILL5919 and ILL7820) which had statistically similar PC under both stress (Figure IV2A and B). under heat stress, the maximum value of PC was registered by ILL7814, ILL7833 and ILL7835 with about 45 µmol proline/g FW. The accessions ILL6362, ILL7814 and ILL7835 had the highest PC in drought stress with 34.5, 38.1 and 35.8 45 µmol proline/g FW, respectively.

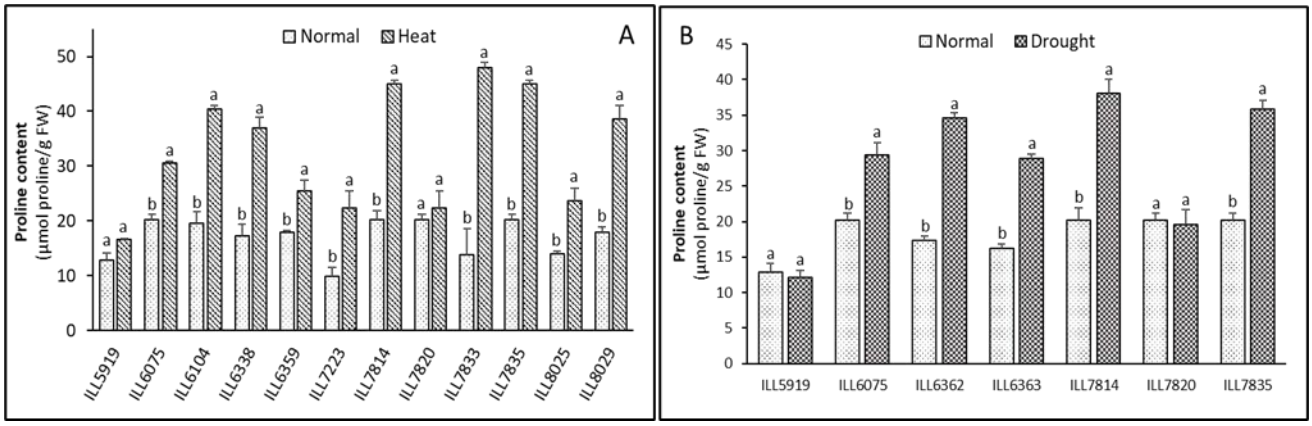

Figure IV.2*. Proline content response to heat stress (A) and drought stress (B) in lentil accessions. Different letters on the bars indicate significant differences between means using LSD test.*

IV.3.3. Total Flavonoids Content Response to Heat and Drought

Total flavonoids content (TFC) increased significantly ($p<0.05$) for five (ILL6075, ILL6104, ILL6338, ILL7833and ILL7835) accessions due to heat stress, while all the accessions except ILL5919 enhanced TFC in response to drought (Figure IV3A and B). ILL6338 and ILL7835 had the maximum TFC under heat stress conditions with 0.64 and 0.77 mg g^{-1} FW, respectively. Under heat stress, the highest accumulation of TFC was reached by the accessions ILL6362, ILL6363 and ILL7814 with up 0.22 mg g^{-1} FW.

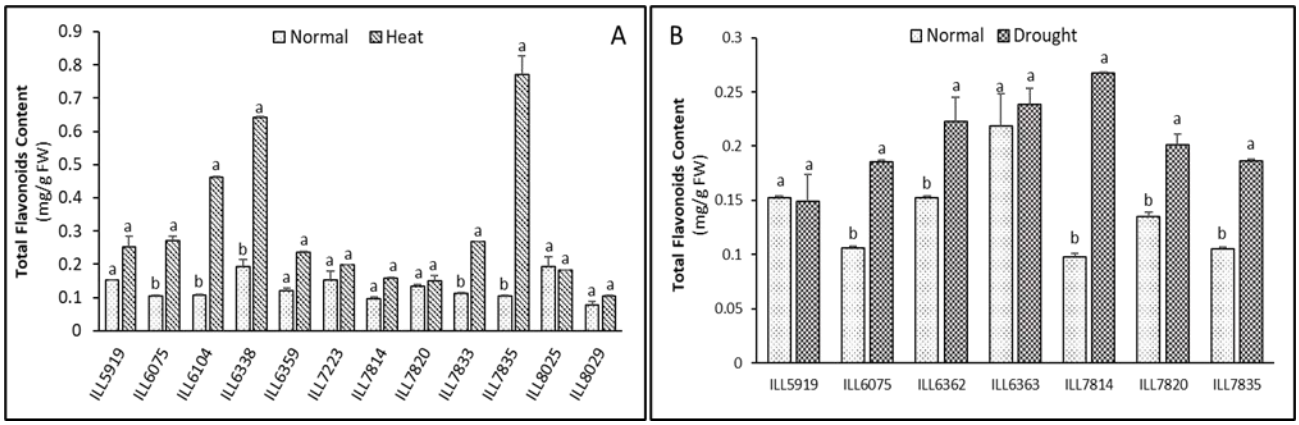

Figure IV.3*. Total flavonoids content in response to heat stress (A) and drought stress (B) in lentil accessions. Different letters on the bars indicate significant differences between means using LSD test.*

IV.3.4. Total Phenolics Content Response to Heat and Drought

Total phenolics content (TPC) increased significantly for all the accessions except ILL5919 in response to heat stress, as compared to normal conditions (Figure IV.4A). The maximum accumulation of TPC under heat stress was achieved by ILL6104, ILL6338, ILL7814, ILL7835 and ILL8029 with 7.50 to 8.79 mg g^{-1} FW. However, only two accessions (ILL7814 and ILL7835) had significantly enhanced TPC in response to drought stress (Figure IV.4B). The average of TPC of ILL7814 and ILL7835 were 7.67 and 8.79 mg g^{-1} FW under drought conditions, respectively.

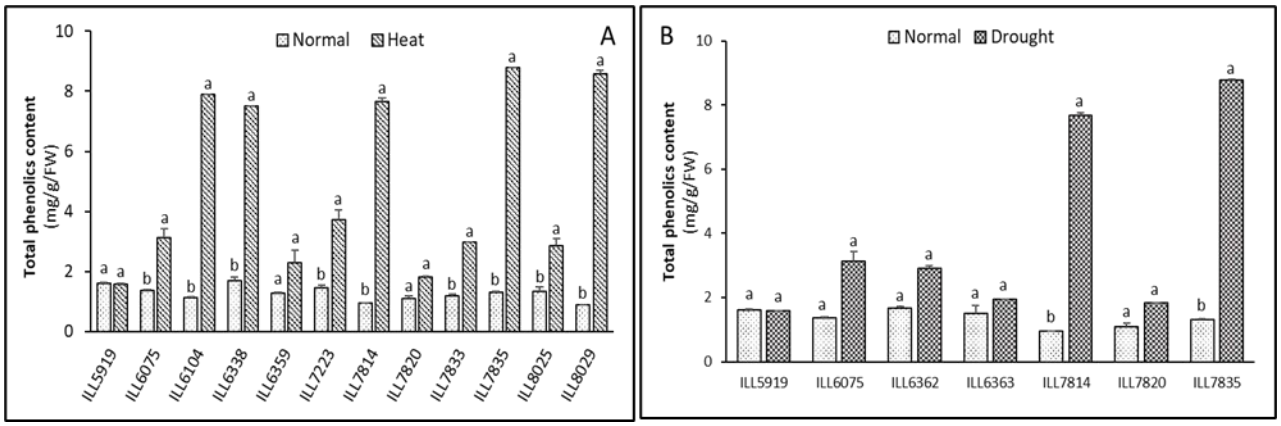

Figure IV.4. Total phenolic content in response to heat stress (A) and drought stress (B) in lentil accessions. Different letters on the bars indicate significant differences between means using LSD test.

IV.3.5. Tannin Content Response to Heat and Drought

As compared to control, tannin content (TC) increased significantly ($p<0.05$) because of heat stress for all the accessions, excepting ILL5919 (Figure IV.5A). The accessions ILL6104, ILL7814, ILL7835 and ILL8029 had the highest accumulation of TC under heat stress with up 20 μgEag g^{-1}

FW. In contrast, drought stress reduced significantly the TC for the accessions ILL5919, ILL6075 and ILL6362, while ILL7814 and ILL7835 increased TC with an average of 5.07 and 8.37 µgEag g^{-1} FW, respectively (Figure IV.5B).

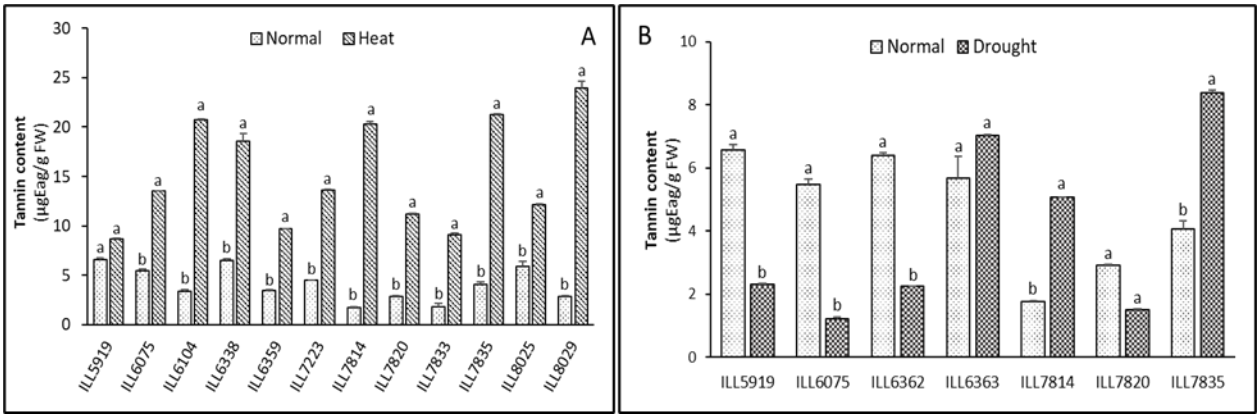

Figure IV.5. *Tannin content in response to heat stress (A) and drought stress (B) in lentil accessions. Different letters on the bars indicate significant differences between means using LSD test.*

IV.3.6. Total Soluble Sugars and Reducing Sugars under Heat and Drought

Total soluble sugars (TSS) increased significantly ($p<0.05$) for eight accessions in heat stress a compared to normal conditions, with the highest accumulation recorded by the accessions ILL7814 and ILL6338 with 71.57 and 74.24 mg g^{-1}, respectively (Figure IV.6A). Further, the accumulation of TSS was increased significantly for three accessions (ILL5919, ILL6075 and 7835) because of drought stress (Figure IV.6B). ILL7835 had the maximum TSS with an average of 125 mg g^{-1} in drought conditions.

Reducing sugars (RS) enhanced significantly ($p<0.05$) in response to heat stress for seven accessions, as compared to normal conditions (Figure IV.6C). The accessions ILL8029, ILL6104 and ILL7814 accumulated the highest RS in heat stress with 0.62, 0.72 and 0.79 mg g^{-1}, respectively. For drought stress, the accessions ILL6075, ILL6363 and ILL7814 increased significantly RS, while ILL5919, ILL6362, ILL7820 and ILL7835 had similar RS compared to normal conditions (Figure IV.6D). ILL6075, ILL6363 and ILL6362 had the maximum RS with 0.54, 0.56 and 0.66 mg g^{-1} under drought stress, respectively, whereas ILL5919 and ILL7820 recorded the lowest value of RS with 0.20 and 0.27 mg g^{-1}, respectively.

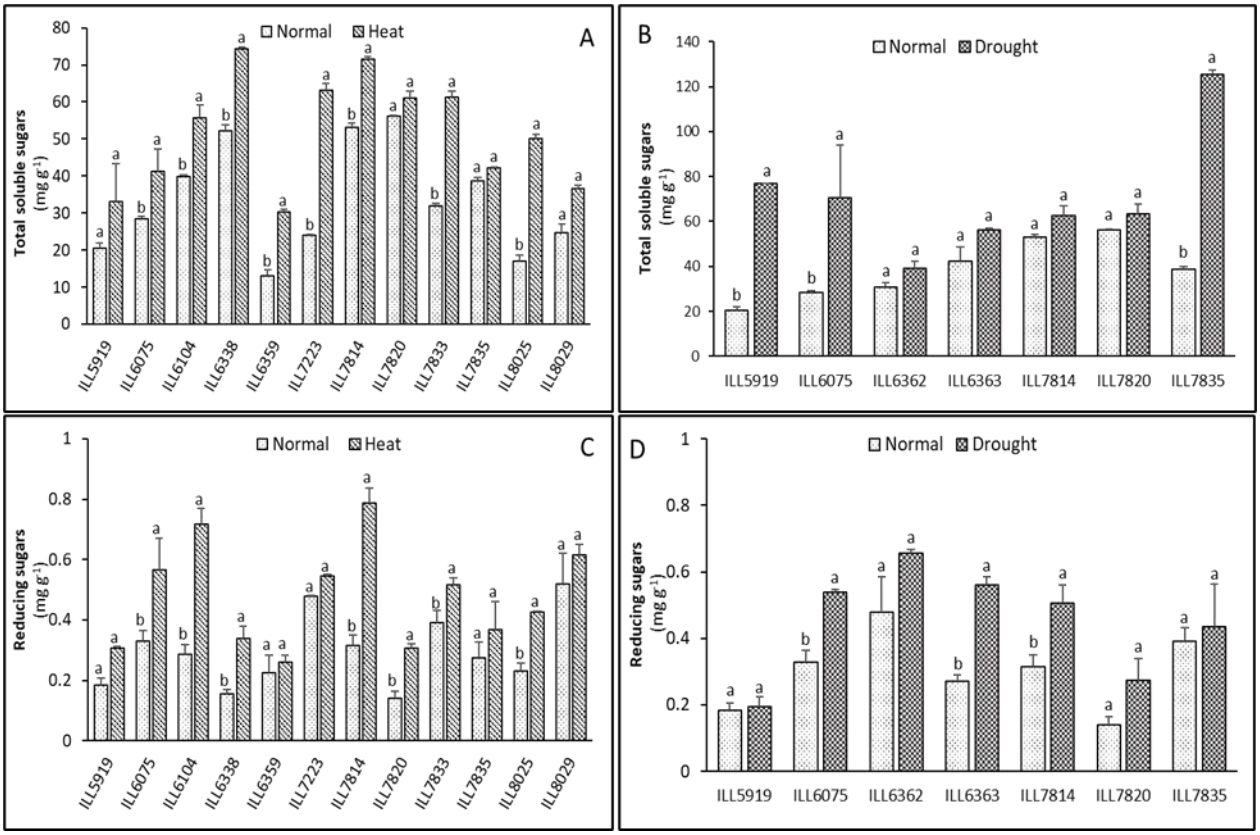

Figure IV.6. Total soluble sugars and reducing sugars in response to heat stress and drought stress in lentil accessions. Different letters on the bars indicate significant differences between means using LSD test.

IV.3.7. Total Antioxidant Activity Response to Heat and Drought

Measurement of total antioxidant activity (TAA) at high-temperature stress in all twelve accessions revealed that seven accessions had significantly ($p<0.05$) higher TAA than that of the control, with ILL7835 and ILL8029 reaching the highest TAA (up 70 %) (Figure IV.7A). However, the accessions ILL7814, ILL6363 and ILL7835 increased significantly their TAA in response to drought stress, with 60.05, 62.79 and 65.82 %, respectively (Figure IV.7B). ILL5919 and ILL7820 accumulated the lowest TAA in both heat and drought stress.

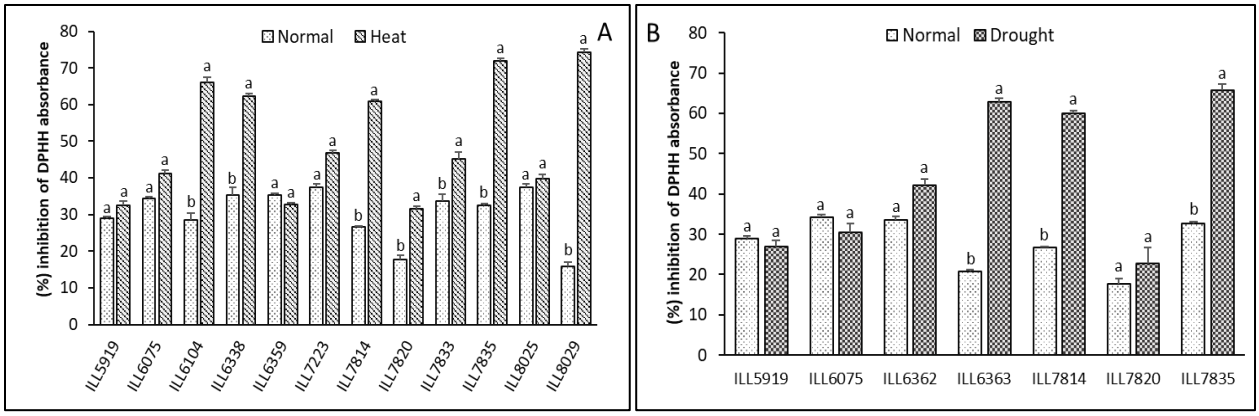

Figure IV.7. Total antioxidant activity expressed as percentage of inhibition of DPPH in response to heat stress (A) and drought stress (B) in lentil accessions. Different letters on the bars indicate significant differences between means using LSD test.

IV.3.8. Genotypic Correlation under Normal, Heat and Drought Stress

Under normal conditions, TFC had significant positive correlation ($p<0.01$) with TC and TPC, while negative correlation at $p<0.05$ was obtained between TPC and SOD ($r=-0.57$) (Figure IV.8A). Further, highly positive correlation ($p<0.001$) was found between TPC and TC ($r=0.88$). CAT, AOP, SOD and RS were clustered together in the same group, whereas TSS and PC were identified in the second cluster. TC, TPC, TFC and TAA grouped together under normal conditions. Further, genotypic correlation revealed the existence of three groups (Figure IV.8B).

Under heat stress, SOD had significant positive correlation with TAA, TC, PC, and RS, while CAT was positively correlated ($p<0.01$) with AOP ($r=0.72$) (Figure IV.8C). Highly positive correlation ($p<0.001$) was identified between TPC, TAA and TC under heat stress. CAT and AOP grouped together under heat stress, while TFC cluster alone in the second group. However, the remaining variables were clustered in the third group (Figure IV.8D). Genotypic correlation showed that ILL8029, ILL6104, ILL7814, ILL6338 and ILL7835 were clustered together in the same group, whereas ILL6075, ILL7833, ILL7223, ILL7820 and ILL8025 were identified a separate group. The third group composed of two accessions (ILL5919 and ILL6339).

SOD had significant positive correlation with CAT, TAA and TC under drought stress (Figure IV.8E). AOP was positively correlated with CAT, PC ($p<0.05$) and TPC ($p<0.01$). Further, TAA was positively correlated at $p<0.001$ with TC, while PC had a positive correlation with RS ($r=0.83$). Correlation analysis revealed that CAT, SOD, AOP, TPC, TAA and TC were clustered together in the same group, however the second group composed of RS, PC and TFC. TSS was clustered alone in the third group (Figure IV.8F). In addition, the accessions ILL7835, ILL6363

and ILL7814 were grouped in the same cluster, while ILL7820 and ILL5919 was identified in the second group. The third group contained the accessions ILL6075 and ILL6362.

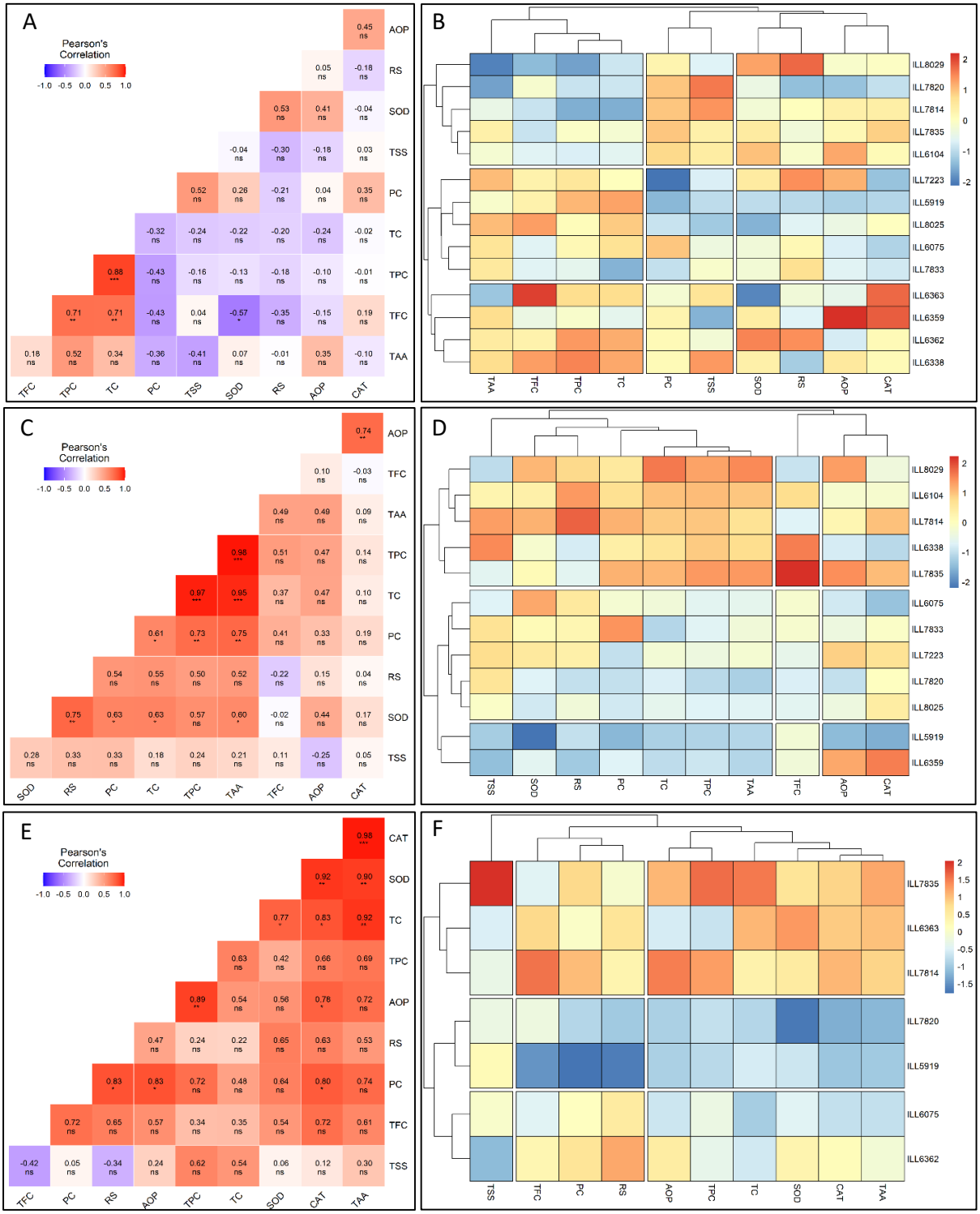

Figure IV.8. Pearson's correlation coefficients among various traits for no-stress (A), high-temperature (C) and drought stress (E) and dendrogram along with heatmap for non-stress (B), high-temperature (D), and drought stress (F).

IV.4. Discussion

In the current study, twelve lentil accessions were exposed to heat stress during the reproductive stage under controlled conditions, while seven accessions were subjected to drought stress by reducing field capacity (75% field capacity). Our findings demonstrated that high-temperature and drought stress caused considerable changes in biochemical processes. The accessions were selected depending on their tolerance to high-temperature and drought stress in our previous studies (El Haddad et al., 2022, 2020). The two accessions ILL5919 and ILL7820 were selected used as susceptible to both stress. This study showed that almost all tolerant accessions to heat stress accumulated significantly higher antioxidant activity of catalase, ascorbate peroxidase and superoxide dismutase. Under drought stress, the tolerant lines ILL7814 and ILL7835 showed important enhancement in CAT and SOD activity, no-significant difference was found in APX for all the accessions. The moderately line ILL6363 recorded the highest accumulation of SOD under drought stress, the tolerant accessions ILL6075 and ILL6362 had significant in SOD activity. Further, the susceptible lines ILL5919 and ILL7820 had the lowest antioxidant activity under both heat and drought stress. In addition, the moderately heat tolerant accessions (ILL6075, ILL6359, ILL7223 and ILL8025) had a significant increase in CAT, APX and SOD under high-temperature stress, as compared to normal conditions. Similarly, the drought moderately tolerant accession ILL6363 had significant improvement only SOD activity.

Both drought and heat stress can cause over production in ROS, which deteriorates many metabolic function such in plants as photosynthetic components. Antioxidant enzymes are reported as the first line of defense used by plants to scavenge superoxide radicals into H_2O_2 and O_2 (Singh et al., 2019). In our study, enhanced antioxidant enzyme contents due to high-temperature and drought stress were observed for almost all the tolerant and moderately tolerant lentil lines. Under unfavorable environmental conditions, the complex antioxidant enzyme activities plays an important role in overcoming uncontrolled ROS generation and protecting plants from oxidative damage (H. A. Hussain et al., 2019; Mittler et al., 2022). Our investigation showed a marked effect of CAT, APX and SOD activities in both tolerant and sensitive accessions, with higher antioxidant enzyme activities in tolerant lines compared to susceptible one. Similar findings were reported of Chakraborty and Pradhan (2011) in lentil who observed significant increase of CAT, APX and SOD in response to high-temperatures stress, although they were greater in tolerant accessions. However, we found no significant increase in APX activity for all the accessions

subjected to drought stress, white its activity decreased not significantly for the four sensitive accessions ILL5919 and ILL7820, ILL6363, and ILL6075, which had also no significant increase in CAT, suggesting that they had lowed level of oxidative stress because of induced antioxidant enzymes activities and detoxified ROS as described by Singh et al. (2019). Still, most of the drought tolerant accessions exhibited much greater CAT and APX compared to the sensitive one. Further, our results revealed higher contribution of SOD activity in tolerant accessions compared to sensitive lines under drought stress, which agreed to findings of (Sarker and Oba, 2018).

Proline is one most important osmoprotectant in plants and it can be used as a solute to adjust alterations in cell's water environment, thereby minimizing the effects of high-temperatures and drought stress on plants. After stress relief, proline generated under stress conditions can also function as a repository for energy and ammonia sources directly engaging in plant metabolism (HHussain et al., 2019). In the current study, concentrations of proline has significantly enhanced due to high-temperature and drought stress for all the accessions, except the sensitive accessions ILL5919 and ILL7820 which had no significant increase in both stress conditions. Similar findings are consistent with Singh et al (2019) who report also a higher proline concentration in heat tolerant lentil accessions as compared to sensitive ones. Further, Hayat et al. (2012) reported that the high accumulation of proline led to minimize photo damage to chloroplast thylakoid membranes by scavenging and/or reducing production of ROS. Furthermore, exogenous proline application modulates drought stress by stimulating plant growth, which is accomplished by inducing the antioxidant mechanism, alleviating oxidative damage, improving compatible solute synthesis, and accelerating proline accumulation, resulting in improved photosynthesis and yield attributes (Anjum et al., 2011). El-Beltagi et al. (2020) observed that the sequence combination of antioxidant and proline resulted enhancement in Chickpea plants growth under drought stress by up-regulating the antioxidant defense system and osmolyte synthesis. Exogenous proline can interact with enzymes to preserve the aggregation and thermal denaturation of proteins (Ignatova and Gierasch, 2006).

The soluble sugars and reducing sugars are also among the main osmotic regulators in plants that might serve various cellular functions such energy storage, osmoprotectant and signals for adapting to abiotic stresses. In our study, concentrations of soluble sugars were increased for almost all the accessions in response to high-temperature and drought stress. Our results are consistent with the investigation of (Shah et al., 2021), who observed a significant increase in

soluble sugar content and proline content of lentil cultivar exposed to water deficit stress condition. The same results were recorded by Sehgal et al (2019) in lentil, indicating a marked increase of soluble sugars under heat and drought stress. Further, Sita et al. (2018) found that heat tolerant lentil accessions accumulated more reducing sugars than sensitive ones, which was explained by increased acid invertase activity (hydrolysis of sucrose into glucose and fructose) of the tolerant accessions.

Our findings demonstrated that tolerant accessions exhibited higher concentrations of total flavonoids, total phenolics, and tannins than sensitive accessions under both high-temperature and drought stress. These second metabolites act as an osmoprotectant and play a key role in scavenging of free radicals against abiotic stress induced oxidative damage (Bueno and Lopes, 2020). Hassan et al. (2020) reported that total phenolic and flavonoids contents are strong antioxidants and their accumulation in plants have a high association with drought stress tolerance. Total antioxidant activity measured by free radical scavenging, increased in both heat and drought stress. Our results showed also that the tolerant accessions accumulated greater concentration of the total antioxidant activity compared to the susceptible accessions. ILL6104, ILL6338, ILL7814 and ILL8029 recorded the highest concentrations of total antioxidant activity under heat stress, whereas ILL6363, ILL7814 and ILL7835 were the top under drought stress, with up 60% inhibition of DPPH. The enhanced antioxidant potential in tolerant accessions under high-temperature and drought may reduce oxidative damage to membranes and proteins, maintaining organelle functioning and the whole plant performance (Ahanger and Ahmad, 2019).

In our study, our results revealed that TPC had significant correlation with TAA under heat stress, whereas AOP had a positive correlation CAT and TPC under drought stress conditions. The accumulation of TPC in plants has been shown to have a string association with stress resistance. (Hussain et al., 2019). Proline content had also a high correlation with SOD, TPC and TAA under high-temperature stress, as well with CAT and AOP under drought stress. Our results are in agreement with the finding made by (Singh et al., 2019). Similarly, TC was positively correlated with TAA, CAT and SOD under drought stress and with TAA, SOD, PCA and PC under heat stress. Tannins were reported to have important antioxidant activity in plants for removing as well as preventing of ROS formation (Grela et al., 2017). Reducing sugars had also positive correlation with SOD under high-temperature and with proline content under drought stress. Hussain et al.

(2020) revealed that reducing sugars played significant part in in osmoregulation protecting plants against environmental conditions.

IV.5. Conclusion

From the current chapter, it was concluded that tolerant lentil accessions had better response to high-temperature and drought stress conditions compared to the sensitive accessions, and produced high activities for almost all the components. Our findings validate the categorization of these accessions into tolerant or sensitive to heat or drought stress. We noticed also moderate response of the moderately tolerant accessions to the two stresses. One of the aims of having differentially sensitive accessions was to probe the mechanisms associated to tolerance to high-temperature and drought stress. Our observations revealed that tolerant accessions accumulated higher concentrations of catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), whereas susceptible accessions had low enzymatic responses. Proline content (PC) increased significantly with high temperature and drought stress in tolerant accessions, but not in susceptible accessions. Because of high temperature and drought stress, the concentrations of total antioxidant activity (TAA), total phenolic content (TPC), tannins (TC), and total flavonoids (TFC) increased significantly in tolerant accessions compared to susceptible accessions. Furthermore, when tolerant accessions were stressed, there were significant increases in total soluble sugars (TSS) and reducing sugars (RS) compared to normal conditions. Consequently, those accessions might be able to sustain physiological processes and generate high yield potential in the regions severely affected by the increasing temperatures and drought events. However, studies are needed to explore the underlying mechanism and identify genes associated with heat or drought tolerance in lentil.

Chapter V: General Discussion

Global climate change is defined as a process that affects all life forms and ecosystems on a global scale and it is reported as the most pressing issue of the twenty-first century (Koç, 2021). Morocco, one of the most affected countries by this phenomenon, is listed as one of the "risk countries" that will be menaced by more high extreme temperatures and decrease in the total annual precipitation amounts (Driouech et al., 2021). High temperature and drought stress and their combination during the reproductive stage all had major effects on the growth and yield of lentil. In this study, a FIGS set containing 162 international germplasm accessions of lentil originated from ICARDA gene bank were initially screened under heat and drought stress in field conditions. For that, late planting approach was used to synchronize the reproductive stage to high temperatures. Till now, this methodology was adapted to evaluate heat tolerance responses in many species such as chickpea (Gaur et al., 2019), lentil (Bhandari et al., 2016), wheat (Rehman et al., 2021), barley (Bahrami et al., 2019) and maize (Iqbal et al., 2020). Our results revealed that higher temperature strongly affect plant height, number of primary, secondary and tertiary branches, number of pods, biological biomass and grain yield. However, the combined heat-drought stress had more deteriorated effects compared to heat stress due to water used efficiency's reduction. In addition, both stresses accelerated the rate of plant growth and development, markedly reducing the flowering and podding periods. Our study revealed that lentil accessions responded differently to heat stress and the combined heat-drought stress in phenology, plant height, biomass, seed's weight and yield. Tolerant accessions under both stresses produced significantly higher yields than the other accessions, which associated positively with pod and seed numbers. Tolerant accessions were distinguished by early flowering, which enabled them to avoid heat and drought stress and shorter crop cycles. These variations allowed us to select potential sources of heat tolerance at the two tested environments (Marchouch and Tessaout) using heat tolerance index (HTI). The results revealed that four accessions (ILL7833, ILL6338, ILL7835 and ILL6104) had good performance under heat stress at Marchouch and three accessions (ILL7835, ILL7814 and ILL8029) were highly heat tolerant at Tessaout. The accession named ILL7835 emerged as heat tolerant at both the locations, showing its stable performance under heat stress. These accessions were characterized by a short phenological cycle with 53.5 days to achieve 50% flowering and about 86 days to 95% maturity at Marchouch, however, the duration was less at Tessaout with 45 days to

50% of flowering and 82 days to 95% maturity. Furthermore, 14 and 26 accessions were categorized as heat tolerant at Marchouch and Tessaout, respectively. The moderately-heat tolerant group comprised of 26 accessions at Marchouch and 60 accessions at Tessaout. In this study, heat sensitive and highly heat-sensitive clusters were comprised of 76 and 42 accessions, respectively, at Marchouch, while it was 39 and 34 accessions at Tessaout.

Under the combined heat-drought stress, the selection was performed using STI and GMP, which were used largely in screening many species such as chickpea (Erdemci, 2018), lentil (Mishra et al., 2016), barley (Zare, 2012), maize (Papathanasiou et al., 2015), and bread wheat (Grzesiak et al., 2019) against abiotic stresses. A greater tolerance for heat-drought stress is indicated by higher GMP and STI values (Farshadfar and Elyasi, 2012). Our outcomes showed that the most tolerant lines to the combined heat-drought stress were ILL7835, ILL6075 and ILL6362 (STI >1) at Marchouch and ILL7814, ILL7835 and 7804 (STI >1) at Tessaout. Altogether, ILL7835 was identified as a good source of tolerance under heat stress and combined heat-drought stress at Tessaout, as well as at Marchouch, while ILL7814 was identified as promising accession under both stresses at Tessaout. These selected lines are characterized by early flowering, which helped them to escape from heat and drought stress and shorter crop cycles. The production of accessions with short duration is one of the main strategies utilized in breeding programs. Early flowering and maturity are typically the stress escape mechanisms that can allow lentil to perform well under stress situations. Furthermore, the identified lines had a higher number of filled pods per plant, making them an excellent source for the lentil breeding program. They were used in the crossing program at ICARDA to transfer heat and drought tolerance in a high yielding genetic background. According to our research, the FIGS method for identifying heat-tolerant germplasm in lentils is effective under heat and combination heat-drought stress. This supports previous findings that demonstrated the utility of the FIGS strategy in identification of desirable germplasm againt many biotic and abiotic constraints, such as stem rust (Bari et al., 2012; Endresen et al., 2012), powdery mildew (Vikas et al., 2020), Ascochyta lentils resistance (Dadu et al., 2019) and drought stress in Vicia baba (Khazaei et al., 2013). Our findings confirm Stenberg and Ortiz's (2021) assertion that FIGS has great potential to improve breeding efforts and, as a result, sustainable plant production with such refinement.

Afterwards, 20 lentil accessions were selected based on our preliminary field screening results as primary candidates for high-temperature and combined high-temperature stress studies. This time,

two separate experiments were used to examine the selected accessions. The first experiment was carried out in the field to investigate the impact of high temperature and drought stress on traits associated with phenology, grain yield, nutritional quality, and canopy temperature, whereas the second experiment was carried out in controlled environments to investigate the possible genotypic variation in lentil for transpiration response to high VPD conditions. The outcomes showed that all the studied parameters were influenced by high-temperatures including pod number, biomass, seed weight, grain yield and nutritional quality, however, combined temperature-drought stress was more detrimental than temperature stress alone. These findings were consistent with our previous studies (Choukri et al., 2020; El Haddad et al., 2020). The PCA analysis showed almost all tolerant accessions were clustered together under high-temperature stress, recording the highest grain yield and protein content, and medium Zn and Fe concentrations. Similarly, tolerant accessions to combined temperature-drought stress were assembled in the same group and had the highest grain yield and moderate concentrations of Zn and Fe. Our findings support the outcomes of our earlier field screening where almost all tolerant accessions showed a high adaptation to high temperatures and combined temperature-drought stress under field conditions. In this study, accessions such as ILL7835, ILL7814, and Bakria (ILL4605) combine excellent nutritional quality with moderate to high yield under both heat and combined heat-drought stress, which is very appealing to lentil breeding programs.

In controlled conditions, our findings revealed considerable genetic variation among lentil accessions in transpiration response to rising VPD. In the greenhouse, the plants were subjected to VPD ranging from 1.18 to 4.5 kPa. Over the whole VPD range studied, almost all of the tolerant accessions had lower TRlim than the sensitive lentil lines, which had a continuously increasing TR with increasing VPD. These outcomes validate our field observations, and They are in accord with Belko et al. (2013) conclusion in cowpea. Accessions with TRlim may have a significant capacity to save more water in the soil, indicating a more effective way to manage TR when water is available. According to a number of findings, accessions with a conservative water behavior in late-season water deficit conditions have the opportunity to use the conserved soil water to maintain physiological activity during the grain filling period and produce more grain than accessions without the TRlim trait (Ghanem et al., 2017; Messina et al., 2015; Sinclair et al., 2017). In our investigation, two tolerant accessions, ILL7833 and ILL7835, limited their TR at early VPD (2.8 kPa), the lowest BP of all lentil VPD studies that have been previously published. Overall, the

lowest BP for lentil was reported by Guiguitant et al. (2017) at 3.31 KPa. Fascinatingly, the moderately drought and heat tolerant check variety Bakria (ILL4605) showed the lowest TR among all examined lines under rising VPD, and most likely had the lowest conductivity among the studies accessions. Our findings found potential accessions such as ILL7835, ILL7814, and ILL4605 that might be useful in breeding for high yields, protein, and micronutrient contents under high-temperature and drought stress. In the future, the parameters of the tested lentil accessions in response to high temperatures, water deficit, and greater VPD would aid in the formulation of novel experimental methodologies to shed light on the molecular and genetic basis of heat and drought resistance mechanisms. As a result, integrating crop physiology with molecular approaches might be a potential way to improving lentils under conditions of high temperatures, water scarcity, and greater VPD.

After that, ten lentil accessions were selected for high-temperature stress and five accessions were identified for drought stress, with two common susceptible lines (ILL5919 and ILL7820) to both stress. The accessions were chosen based on their tolerance to high-temperature and drought stress as identified in our previous studies (El Haddad et al., 2022, 2020). During the reproductive stage, high temperature stress was applied by increasing temperature above 32 C, whereas drought stress was imposed at 70% field capacity. After stress treatment, whole plants were collected and used for measuring different biochemical analysis. Our investigation showed that almost all tolerant accessions to heat stress accumulated significantly higher antioxidant activity of catalase, ascorbate peroxidase and superoxide dismutase. ILL6359, ILL7223, ILL7814, ILL7835 and ILL8029 generated the highest accumulation of CAT, APX and SOD under high-temperature conditions. Under drought stress, the tolerant lines ILL7814 and ILL7835 showed significant enhancement in CAT and SOD activity, no-significant difference was found in APX for all the accessions even through tolerant lines increased the accumulation of APX. The moderately line ILL6363 had the top accumulation of SOD under drought stress, the tolerant accessions ILL6075 and ILL6362 had significant in SOD activity. Further, the susceptible lines ILL5919 and ILL7820 had the lowest antioxidant activity under both high-temperature and drought stress. In addition, the moderately heat tolerant accessions (ILL6075, ILL6359, ILL7223 and ILL8025) had a significant increase in CAT, APX and SOD under high-temperature stress, as compared to normal conditions. Similarly, the drought moderately tolerant accession ILL6363 had significant improvement only SOD activity.

In our study, improved antioxidant enzyme contents because of high-temperature and drought stress were observed for almost all the tolerant and moderately tolerant lentil lines. Under challenging conditions, the complex antioxidant enzyme activities play a critical role in overcoming uncontrolled ROS generation and protecting plants from oxidative damage (Mittler et al., 2022). Our investigation showed a marked effect of CAT, APX and SOD activities in both tolerant and sensitive accessions, with higher antioxidant enzyme activities in tolerant lines compared to susceptible one. Similar findings were reported of Chakraborty and Pradhan (2011) in lentil who observed significant increase of CAT, APX and SOD in response to high-temperatures stress, although they were greater in tolerant accessions. However, our study did not reveal no significant increase in APX activity for all the accessions subjected to drought stress, white its activity decreased not significantly for the four sensitive accessions ILL5919 and ILL7820, ILL6363, and ILL6075, which had also no significant increase in CAT, suggesting that they had lowed level of oxidative stress because of induced antioxidant enzymes activities and detoxified ROS as described by Singh et al. (2019). Still, totality of drought tolerant accessions exhibited much greater CAT and APX compared to the sensitive one. Further, our results revealed higher contribution of SOD activity in tolerant accessions compared to sensitive lines under drought stress.

In the current study, we observed also that tolerant accessions enhanced significantly with the increase of temperature and drought stress, however, the susceptible accessions (ILL5919 and ILL7820) had no significant increase in both stress conditions. Proline is an important osmoprotectant in plants and can be used as a solute to adjust changes in the water environment of cells, thereby minimizing the effects of high temperatures and drought stress on plants. This osmoprotectant component produced under stress conditions can also serve as a storage site for energy and ammonia sources that are directly involved in plant metabolism. Further, it was reported also that the high accumulation of proline led to minimize photo damage to chloroplast thylakoid membranes by scavenging and/or reducing production of ROS (Hayat et al., 2012). El-Beltagi et al. (2020) observed that up-regulating the antioxidant defense system and osmolyte synthesis in the sequence combination of antioxidant and proline enhanced the growth of chickpea plants under drought stress. Exogenous proline can interact with enzymes to maintain protein aggregation and thermal denaturation. (Ignatova and Gierasch, 2006).

In our study, concentrations of soluble sugars was increased for almost all the accessions in response to increase temperature and drought stress. Our findings are consistent with the investigation of Shah et al. (2021), who discovered a significant increase in the soluble sugar and proline content of lentil cultivars subjected to water deficit stress. The same results were recorded by Sehgal et al (2019) in lentil, indicating a marked increase of soluble sugars under heat and drought stress. Sita et al. (2018) found that heat tolerant lentil accessions accumulated more reducing sugars than sensitive ones, which was explained by increased acid invertase activity (hydrolysis of sucrose into glucose and fructose) of the tolerant accessions. The soluble sugars and reducing sugars are among the main osmotic regulators in plants that might serve various cellular functions such energy storage, osmoprotectant and signals for adapting to abiotic stresses. Accessions with higher accumulation of TSS and RS at stress environmental conditions have more chance to generate more yields compared to susceptible accessions.

Furthermore, we identified that tolerant accessions had higher concentrations of total flavonoids, total phenolics, and tannins than sensitive accessions under both high-temperature and drought stress. These second metabolites act as an osmoprotectant and play an important role in free radical scavenging against abiotic stress-induced oxidative damage (Bueno and Lopes, 2020). Total phenolic and flavonoids contents are strong antioxidants and their accumulation in plants have a high association with stress tolerance (Hassan et al., 2020). Total antioxidant activity measured by free radical scavenging, improved with high temperature and drought stress. We observed that the tolerant accessions accumulated greater concentration of the total antioxidant activity compared to the susceptible accessions. ILL6104, ILL6338, ILL7814 and ILL8029 generated the highest concentrations of total antioxidant activity under heat stress, whereas ILL6363, ILL7814 and ILL7835 were the top under drought stress, with up 60% inhibition of DPPH. Under high-temperature and drought conditions, the enhanced antioxidant potential in tolerant accessions may reduce oxidative damage to membranes and proteins, preserving organelle function and overall plant performance, leading to maintain high productivity (Ahanger and Ahmad, 2019).

General conclusions and perspectives

Morocco and many other Mediterranean countries are currently facing a serious climate change challenge. Temperature rises and frequent droughts had a strong impact on the productivity of major stable crops. Lentil is one of most affected species by increasing temperatures and limited water conditions. The reproductive phase was reported to be the most sensitive to changes in the external environment, and exposure to heat and drought stress during this stage reduces crop productivity significantly. The assessment of 162 lentil accessions under high temperature and drought stress (chapter II) showed that heat, as well as combined heat-drought stress, severely a effects flower production and pod set, leading to a substantial loss in grain yield in lentil. The adaptation by selecting the robust accessions can be a strong approach to mitigating the impact of climate change. Our findings indicate that the FIGS approach to identifying heat tolerant germplasm in lentil is effective under heat and combined heat-drought stress. This research identified a group of highly heat tolerant accessions using HTI based on flowering time, and grain yield under stress and non-stress conditions. Our findings confirmed that STI, GMP and MP are the most suitable criteria, not only for selecting the high yielding accessions under individual heat and drought stresses, but also in combined heat-drought stress.

The evaluation of selected accessions at field and controlled conditions (chapter III) revealed that almost all tolerant accessions showed a high adaptation to high temperatures and combined temperature-drought stress under field conditions and exhibited a fairly clear TRlim at high VPD under controlled conditions. Two tolerant accessions (ILL 7833 and ILL 7835) exhibited the lowest breakpoint found in lentil. Several accessions such as ILL 7835, ILL 7814 and Bakria (ILL 4605) were identified as moderate to high yield under both heat and combined heat-drought stress with good nutritional quality (e.g., micronutrient, protein content).

Assessing the biochemical response of lentil accessions against high temperatures and drought stress (chapter IV) revealed that tolerant accessions accumulated higher enzymatic activities compared to the sensitive ones. In addition, tolerant accessions had maintained higher antioxidant activity, osmolytes and phenolics metabolism under high temperature and drought stress conditions.

Still, identifying molecular markers linked to tolerance mechanisms is an urgent need in order to improve lentil adaptation to high temperatures, water stress, and higher VPD.

Annex

Annex II.1. *Details of FIGS subset used in this study*

Accession	IG number	Origin	site_code	Longitude	Latitude
ILL 206	206	ETH	ETH50/51::11	41.86667	9.58333
ILL 221	221	IND	IND-S297	77.23333	28.65
ILL 247	247	ETH	ETH61::2	41.86667	9.58333
ILL 504	504	RUS	RUS::E11	47.1	42.13333
ILL 729	729	IRN	IRN-S240	57.8	28.68333
ILL 1683	1683	ETH	ETH-S937	39.91667	9.81667
ILL 1734	1734	ETH	ETH-S964	43.58333	9.18333
ILL 1861	1861	SDN	SDN-S6	32.74972	15.41472
ILL 2524	2524	IND	IND::4	85.93333	26
ILL 3484	3484	NPL	NPL-S275	86.08333	26.85
ILL 3635	3635	IND	IND::11	77.36667	23.23333
ILL 4605	4605	MAR	Check	-	-
ILL 4743	4743	YEM	YEM80-1:5	44.08333	14.25
ILL 4758	4758	YEM	YEM80-1:105	44.7	13.86667
ILL 4772	4772	YEM	YEM80-1:138	44	15
ILL 4902	4902	IRN	IRN-S321	58.11667	27.9
ILL 4910	4910	RUS	RUS::E38	46.03333	51.53333
ILL 5505	5505	SDN	SDN-S7	30.45	19.16667
ILL 5918	69527	ETH	ETH-S998	39.53333	5.31667
ILL 5919	69528	ETH	ETH-S999	39.9	6.21667
ILL 5929	69538	ETH	ETH-S1007	37.46667	5.63333
ILL 5940	69549	ETH	ETH-S1012	39.83333	12.55
ILL 5943	69552	ETH	ETH-S1015	39.65	11.66667
ILL 5955	69564	ETH	ETH-S1023	35.96667	4.81667
ILL 5957	69566	ETH	ETH-S1024	38.21667	10.05
ILL 5958	69567	ETH	ETH-S1025	38.21667	10.05
ILL 5964	69573	ETH	ETH-S1030	41.16667	7.5
ILL 6053	70108	PAK	PAK85:1032	67.86667	24.55
ILL 6054	70109	PAK	PAK85:1033	68.08333	24.58333
ILL 6055	70110	PAK	PAK85:1034	68.18333	24.66667
ILL 6057	70112	PAK	PAK85:1035	68.2	24.58333
ILL 6058	70113	PAK	PAK85:1036	68.63333	24.63333
ILL 6059	70114	PAK	PAK85:1037	68.83333	24.65
ILL 6060	70115	PAK	PAK85:1038	68.93333	24.81667
ILL 6072	70127	PAK	PAK85:1043	69.01667	25.53333
ILL 6074	70129	PAK	PAK85:1044	69.26667	25.73333
ILL 6075	70130	PAK	PAK85:1045	69.41667	25.83333
ILL 6077	70132	PAK	PAK85:1046	68.85	26.03333

ILL 6079	70134	PAK	PAK85:1047	68.68333	25.91667
ILL 6080	70135	PAK	PAK85:1049	68.48333	25.73333
ILL 6081	70136	PAK	PAK85:1050	68.41667	25.81667
ILL 6086	70141	PAK	PAK85:1071	68.21667	27.56667
ILL 6087	70142	PAK	PAK85:1078	68.48333	28.16667
ILL 6088	70143	PAK	PAK85:1085	69.21667	27.96667
ILL 6089	70144	PAK	PAK85:1087	69.73333	28.06667
ILL 6091	70146	PAK	PAK85:1089	69.68333	28.18333
ILL 6092	70147	PAK	PAK85:1091	70.31667	28.43333
ILL 6094	70149	PAK	PAK85:1094	70.56667	28.85
ILL 6095	70150	PAK	PAK85:1098	70.9	29.66667
ILL 6096	70151	PAK	PAK85:1099	71.48333	30.18333
ILL 6099	70154	PAK	PAK85:1102	71.3	30.66667
ILL 6100	70155	PAK	PAK85:1104	71.26667	30.4
ILL 6101	70156	PAK	PAK85:1105	71.45	30.38333
ILL 6102	70157	PAK	PAK85:1106	71.48333	30.45
ILL 6104	70159	PAK	PAK85:1107	71.55	30.53333
ILL 6105	70160	PAK	PAK85:1109	71.23333	30.56667
ILL 6107	70162	PAK	PAK85:1138	71.53333	32.58333
ILL 6319	71251	PAK	PAK83:872	68.7	27.96667
ILL 6320	71252	PAK	PAK83:873	68.7	27.96667
ILL 6322	71254	PAK	PAK83:876	71.25	30.06667
ILL 6325	71257	PAK	PAK83:884	68.7	27.96667
ILL 6332	71264	PAK	PAK83:894	73.16667	30.68333
ILL 6337	71269	PAK	PAK83:891	73.51667	30.81667
ILL 6338	71270	PAK	PAK83:895	72.68194	30.52778
ILL 6346	71278	PAK	PAK83:898	72.45	30.4
ILL 6356	71288	PAK	PAK83:903	71.25	30.06667
ILL 6359	71291	PAK	PAK83:905	70.98333	29.46667
ILL 6360	71292	PAK	PAK83:906	70.66667	28.63333
ILL 6361	71293	PAK	PAK83:907	70.98333	29.46667
ILL 6362	71294	PAK	PAK83:908	71.25	30.06667
ILL 6363	71295	PAK	PAK83:909	68.56667	27.28333
ILL 6364	71296	PAK	PAK83:910	68.56667	27.28333
ILL 6385	71317	PAK	PAK85-2:1173	63.06667	25.98333
ILL 7223	75942	NPL	NPL-S278	86.21667	26.66667
ILL 7232	75951	NPL	NPL-S285	86.2	26.66667
ILL 7238	75957	NPL	NPL-S291	86.08333	26.85
ILL 7250	75969	NPL	NPL-S302	86.88333	26.6
ILL 7264	75983	NPL	NPL-S312	86.85	26.51667
ILL 7266	75985	NPL	NPL-S314	86.61667	26.66667
ILL 7286	76005	NPL	NPL88:1	88.08333	26.53333
ILL 7289	76008	NPL	NPL88:3	87.65	26.56667

ILL 7290	76009	NPL	NPL88:4	87.6	26.56667
ILL 7295	76014	NPL	NPL88:8	87.26667	26.56667
ILL 7300	76019	NPL	NPL88:12	87.26667	26.6
ILL 7301	76020	NPL	NPL88:13	87.31667	26.36667
ILL 7303	76022	NPL	NPL88:15	87.16667	26.48333
ILL 7304	76023	NPL	NPL88:16	87.08333	26.75
ILL 7305	76024	NPL	NPL88:17	87.9	26.55
ILL 7306	76025	NPL	NPL88:18	86.75	26.48333
ILL 7307	76026	NPL	NPL88:19	86.6	26.5
ILL 7308	76027	NPL	NPL88:20	86.51667	26.61667
ILL 7309	76028	NPL	NPL88:21	86.18333	26.83333
ILL 7310	76029	NPL	NPL88:22	86.25	26.76667
ILL 7311	76030	NPL	NPL88:23	86.31667	26.75
ILL 7312	76031	NPL	NPL88:24	86.41667	26.7
ILL 7313	76032	NPL	NPL88:25	86.11667	26.83333
ILL 7314	76033	NPL	NPL88:26	86.06667	26.83333
ILL 7316	76035	NPL	NPL88:28	85.96667	26.78333
ILL 7317	76036	NPL	NPL88:29	85.5	27.06667
ILL 7325	76044	NPL	NPL88:37	85	27.16667
ILL 7326	76045	NPL	NPL88:38	84.98333	27.01667
ILL 7327	76046	NPL	NPL88:39	84.96667	27.03333
ILL 7328	76047	NPL	NPL88:40	84.96667	27.03333
ILL 7339	76058	NPL	NPL-S328	84.9	27.06667
ILL 7344	76063	NPL	NPL-S332	86.21667	26.65
ILL 7380	76099	NPL	NPL89:8	83.36667	27.36667
ILL 7383	76102	NPL	NPL89:9	83.31667	27.35
ILL 7389	76108	NPL	NPL89:13	83.36667	27.36667
ILL 7795	114851	NPL	NPL95E:5	84.91083	27.03278
ILL 7796	114855	NPL	NPL95E:6	84.96417	27.02778
ILL 7797	114862	NPL	NPL95E:8	85.00611	26.95694
ILL 7798	114864	NPL	NPL95E:9	85.18722	26.87444
ILL 7799	114868	NPL	NPL95E:10	85.27139	26.77972
ILL 7800	114873	NPL	NPL95E:14	85.31833	26.95167
ILL 7801	114875	NPL	NPL95E:15	85.33639	27.03278
ILL 7804	114892	NPL	NPL95E:19	85.55389	26.94333
ILL 7806	114905	NPL	NPL95E:23	85.89583	26.7075
ILL 7807	114907	NPL	NPL95E:24	85.83861	26.67556
ILL 7808	114912	NPL	NPL95E:25	85.7975	26.65583
ILL 7812	114927	NPL	NPL95E:29	86.18861	26.86722
ILL 7813	114929	NPL	NPL95E:30	86.1975	26.85917
ILL 7814	114931	NPL	NPL95E:31	86.27583	26.76667
ILL 7815	114937	NPL	NPL95E:32	86.21944	26.65111
ILL 7816	114939	NPL	NPL95E:33	86.25694	26.64111

ILL 7817	114942	NPL	NPL95E:34	86.2875	26.63056
ILL 7818	114945	NPL	NPL95E:35	86.36528	26.70722
ILL 7819	114948	NPL	NPL95E:36	86.43083	26.73056
ILL 7820	114951	NPL	NPL95E:38	87.27722	26.48583
ILL 7824	114967	NPL	NPL95E:42	87.17444	26.63583
ILL 7826	114978	NPL	NPL95E:44	87.31694	26.4625
ILL 7827	114979	NPL	NPL95E:45	87.41861	26.47333
ILL 7829	114991	NPL	NPL95E:49	87.54917	26.45556
ILL 7830	114995	NPL	NPL95E:50	87.57083	26.45083
ILL 7831	114999	NPL	NPL95E:51	87.65667	26.45472
ILL 7832	115003	NPL	NPL95E:53	87.71194	26.47167
ILL 7833	115006	NPL	NPL95E:55	87.70778	26.6075
ILL 7835	115010	NPL	NPL95E:57	86.74917	26.51472
ILL 7836	115012	NPL	NPL95E:58	86.7775	26.45611
ILL 7837	115015	NPL	NPL95E:59	86.66083	26.63556
ILL 7838	115020	NPL	NPL95E:60	86.56389	26.66583
ILL 8012	117702	PAK	PAK96:7	72.44139	29.67722
ILL 8013	117705	PAK	PAK96:8	72.44667	29.68139
ILL 8014	117706	PAK	PAK96:9	72.56528	29.68556
ILL 8015	117707	PAK	PAK96:10	72.57139	29.66944
ILL 8016	117709	PAK	PAK96:12	72.64306	29.59444
ILL 8017	117710	PAK	PAK96:13	72.80722	29.54583
ILL 8018	117711	PAK	PAK96:14	72.83917	29.55417
ILL 8019	117712	PAK	PAK96:15	72.79556	29.61806
ILL 8020	117713	PAK	PAK96:16	72.66361	29.68889
ILL 8021	117715	PAK	PAK96:17	72.01056	29.49389
ILL 8022	117720	PAK	PAK96:19	72.91056	29.69472
ILL 8023	117723	PAK	PAK96:20	72.92333	29.62444
ILL 8024	117724	PAK	PAK96:21	72.94028	29.49583
ILL 8025	117726	PAK	PAK96:22	72.93139	29.45694
ILL 8026	117727	PAK	PAK96:23	72.85	29.41444
ILL 8028	117730	PAK	PAK96:25	71.95417	30.30194
ILL 8029	117734	PAK	PAK96:26	72.70361	30.52278
ILL 8054	117785	PAK	PAK96:51	73.24417	34.42306
ILL 8056	117788	PAK	PAK96:54	72.46333	34.92417
ILL 8061	117800	PAK	PAK96:61	71.94278	34.58056
ILL 8280	123531	RUS	RUS-S139	46.78333	50.95
ILL 8437	123688	IND	IND::22	80.91667	26.85

Annex II.2. *Day to 50% flowering (DF), days to 95% maturity (DM), seed yield under stress condition (Yp), seed yield under normal condition (Yp) and heat tolerant index (HTI) of heat tolerant accessions of lentil.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
Tessoaut Location						**Marchouch Location**					
ILL 6359	47.00	89.00	1.96	2.96	0.94	ILL 6363	54.00	85.00	2.38	3.91	0.97
ILL 7295	46.00	88.50	1.91	2.71	0.94	ILL 8025	54.50	86.50	1.91	1.70	0.87
ILL 2524	43.00	87.00	1.95	2.91	0.94	ILL 7819	51.50	84.50	2.00	2.43	0.85
ILL 6053	42.50	88.50	1.68	1.66	0.90	ILL 7223	51.50	88.00	2.05	3.80	0.75
ILL 7389	46.00	88.00	2.00	3.57	0.88	ILL 6361	53.00	85.50	2.10	4.21	0.74
ILL 8019	43.00	83.50	1.93	3.14	0.88	ILL 6053	52.50	85.00	1.91	3.13	0.72
ILL 8018	45.50	88.50	1.80	2.60	0.86	ILL 6075	55.00	86.00	1.71	2.15	0.69
ILL 6094	45.00	95.00	1.93	3.41	0.84	ILL 4902	68.00	90.00	1.78	3.70	0.64
ILL 8025	44.50	87.00	1.88	3.16	0.84	ILL 8061	61.50	93.00	1.42	1.25	0.61
ILL 6095	45.00	90.00	1.46	1.02	0.82	ILL 6362	57.00	88.00	2.01	5.30	0.59
ILL 4605	50.84	84.59	1.95	3.81	0.81	ILL 7806	55.50	88.00	1.59	2.65	0.57
ILL 7932	44.00	84.50	1.21	0.76	0.64	ILL 6359	51.00	86.50	1.27	0.90	0.51
ILL 6364	44.50	86.50	1.74	3.64	0.62	ILL 880	70.50	100.00	1.53	3.66	0.48
ILL 5919	50.00	81.00	1.32	1.77	0.59	ILL 504	67.00	106.00	1.38	2.80	0.45
ILL 7250	44.00	90.00	1.39	2.16	0.56						
ILL 7796	48.50	88.00	1.64	3.65	0.55						
ILL 7817	43.00	85.00	1.75	4.12	0.55						
ILL 6055	47.00	86.50	1.42	2.65	0.51						
ILL 8437	46.50	97.00	1.27	1.93	0.50						
ILL 6080	45.50	84.00	1.08	0.99	0.48						
ILL 8023	44.50	91.00	1.35	2.46	0.47						
ILL 7795	44.00	88.00	1.27	2.16	0.45						
ILL 6107	44.50	89.50	1.46	3.26	0.43						
ILL 7808	44.00	85.00	1.35	2.82	0.41						
ILL 7301	46.00	85.00	1.25	2.32	0.41						
ILL 7327	44.50	90.00	0.96	1.00	0.37						

Annex II.3. *Day to 50% flowering (DF), days to 95% maturity (DM), seed yield in stress condition (Yp), seed yield in normal condition (Yp) and heat tolerant index (HTI) of moderately heat tolerant, heat sensitive and highly heat sensitive accessions cluster of lentil at Tessaout and at Marchouch.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
Marchouch Station						**Tessaout Station**					
Moderately heat tolerant						**Moderately heat tolerant**					
ILL 7308	53.00	86.00	1.52	2.82	0.49	ILL 206	44.50	92.00	1.21	3.68	0.14
ILL 8029	55.00	85.00	1.43	2.76	0.45	ILL 221	62.50	98.00	1.31	4.68	0.12
ILL 3635	54.00	85.50	1.31	2.12	0.43	ILL 1734	50.50	96.50	0.93	1.59	0.26
ILL 7807	52.50	92.00	1.36	2.99	0.37	ILL 1861	42.00	95.00	0.85	2.15	0.06
ILL 7301	54.00	85.00	1.25	2.47	0.35	ILL 3484	46.50	94.50	1.10	2.11	0.31
ILL 7798	53.50	86.00	0.99	0.91	0.33	ILL 3635	45.00	83.50	1.30	3.64	0.23
ILL 7295	53.00	86.00	1.15	2.13	0.32	ILL 4743	46.50	86.00	0.77	2.71	-0.09
ILL 6325	50.50	90.00	1.16	2.28	0.30	ILL 4772	43.50	89.50	0.70	2.01	-0.04
ILL 6095	53.50	84.50	0.87	0.55	0.28	ILL 4902	65.00	101.00	0.99	2.76	0.17
ILL 729	66.50	106.00	1.24	3.78	0.26	ILL 5918	46.50	81.50	0.68	1.72	0.00
ILL 6086	53.00	87.00	1.07	2.22	0.25	ILL 5929	51.00	90.00	1.04	2.77	0.16
ILL 6364	51.00	81.00	1.20	3.16	0.24	ILL 5958	45.00	89.50	0.69	1.53	0.04
ILL 7286	53.50	85.50	0.85	0.90	0.24	ILL 6059	51.00	88.50	0.81	2.48	0.00
ILL 6337	51.00	87.00	1.30	3.89	0.24	ILL 6074	46.00	88.00	0.61	1.13	0.04
ILL 6094	53.50	85.00	1.01	2.03	0.23	ILL 6075	43.00	89.00	1.22	4.40	0.02
ILL 7830	54.50	85.50	0.90	1.38	0.23	ILL 6077	48.50	88.50	0.79	1.21	0.19
ILL 5958	53.50	84.50	0.92	1.75	0.20	ILL 6079	45.00	84.50	0.78	2.41	-0.03
ILL 8019	53.00	85.50	1.08	2.87	0.20	ILL 6088	43.50	85.00	0.93	1.94	0.18
ILL 7344	54.00	87.00	1.12	3.33	0.18	ILL 6092	46.00	86.50	0.81	1.03	0.23
ILL 7309	52.00	85.00	0.71	0.70	0.16	ILL 6096	44.50	90.50	1.09	3.11	0.13
ILL 8017	54.50	87.00	1.20	4.14	0.16	ILL 6101	45.50	88.50	1.23	3.11	0.25
ILL 7250	53.00	87.50	0.87	2.05	0.14	ILL 6102	45.00	90.50	1.05	2.96	0.11
ILL 7238	53.50	86.00	0.80	1.60	0.14	ILL 6325	44.50	94.00	0.74	1.83	0.03
ILL 7300	57.50	88.00	0.82	2.00	0.13	ILL 6337	43.50	88.00	0.75	1.71	0.05
ILL 1861	52.50	100.00	0.56	0.50	0.08	ILL 6346	46.00	90.50	0.90	2.38	0.09
ILL 7380	53.00	86.00	0.75	2.15	0.05	ILL 6356	45.50	87.50	1.17	3.26	0.17
Heat sensitive						ILL 6361	45.00	86.50	1.01	2.25	0.20
ILL 7290	51.00	83.50	0.71	1.14	0.11	ILL 6363	47.00	85.00	0.80	2.56	-0.03
ILL 8056	62.00	93.00	0.68	1.43	0.10	ILL 6385	60.50	91.00	0.94	2.24	0.20
ILL 7264	53.00	87.00	0.60	0.67	0.09	ILL 7223	45.00	89.00	1.16	2.40	0.31
ILL 7312	51.00	85.50	0.70	1.35	0.09	ILL 7290	45.50	88.50	0.87	2.96	-0.05
ILL 6057	52.00	88.00	0.63	0.95	0.08	ILL 7303	54.00	98.00	0.88	3.22	-0.05
ILL 7305	52.00	87.00	0.78	2.00	0.08	ILL 7304	44.50	86.50	0.70	2.12	-0.06
ILL 7824	53.50	85.50	1.10	4.24	0.08	ILL 7306	43.50	92.00	1.10	2.10	0.31
ILL 6091	55.00	86.00	0.68	1.46	0.08	ILL 7308	44.50	83.50	0.74	1.91	0.02
ILL 4743	49.50	86.00	0.71	1.47	0.08	ILL 7309	46.50	84.00	0.92	2.00	0.17
ILL 7310	54.00	85.00	0.60	0.90	0.07	ILL 7312	44.00	84.50	0.68	2.06	-0.07
ILL 6088	51.50	85.00	0.80	2.45	0.05	ILL 7313	44.50	87.00	0.80	1.72	0.10
ILL 7831	53.00	86.00	0.82	2.62	0.05	ILL 7316	44.50	85.00	1.31	4.55	0.08
ILL 8016	53.00	85.00	0.62	1.30	0.04	ILL 7326	44.50	87.00	0.93	2.45	0.09
ILL 8012	54.00	85.00	0.75	2.24	0.04	ILL 7380	44.50	91.50	0.76	2.51	-0.07
ILL 6322	50.00	84.00	0.94	3.50	0.03	ILL 7383	46.50	87.00	1.04	3.31	0.05

ILL 7316	53.00	87.00	0.72	2.10	0.03
ILL 6087	51.00	85.00	0.65	1.63	0.02
ILL 8022	53.50	86.00	0.62	1.55	0.02
ILL 206	54.00	86.00	0.69	2.11	0.02
ILL 6077	54.50	85.00	0.79	2.99	0.00
ILL 7826	58.00	90.00	0.56	1.59	-0.01
ILL 6079	58.00	87.00	0.49	1.25	-0.02
ILL 7796	54.00	88.00	0.44	0.77	-0.02
ILL 8021	54.50	85.00	0.40	0.67	-0.04
ILL 8026	52.50	85.00	0.85	3.69	-0.04
ILL 7303	58.50	104.50	0.92	4.40	-0.04
ILL 4758	47.50	83.00	0.78	3.10	-0.05
ILL 7795	55.00	86.00	0.37	0.60	-0.05
ILL 221	55.00	88.00	0.65	2.52	-0.05
ILL 7339	54.00	87.50	0.52	1.73	-0.06
ILL 8015	53.50	85.00	0.46	1.34	-0.06
ILL 7836	52.00	84.00	0.70	2.95	-0.07
ILL 7389	54.50	84.50	0.92	4.55	-0.07
ILL 6074	53.00	86.00	0.40	1.00	-0.07
ILL 7306	54.00	86.00	0.70	3.13	-0.08
ILL 1734	54.50	94.50	0.56	2.23	-0.08
ILL 5918	51.00	83.00	0.38	0.95	-0.09
ILL 7827	53.00	85.00	0.66	2.96	-0.09
ILL 7325	51.00	81.00	0.38	1.10	-0.10
ILL 6059	65.00	98.00	0.32	1.35	-0.12
ILL 6099	66.50	95.00	0.17	0.39	-0.12
ILL 5940	54.00	87.00	0.37	1.30	-0.12
ILL 8437	52.00	102.50	0.76	3.93	-0.12
ILL 6058	65.00	98.00	0.23	0.80	-0.12
ILL 6319	50.00	82.50	0.80	4.23	-0.13
ILL 7818	54.50	86.00	0.49	2.31	-0.14
ILL 6096	55.00	84.00	0.35	1.38	-0.14
ILL 5957	48.00	81.00	0.51	2.24	-0.14
ILL 4605	49.34	82.65	0.77	4.07	-0.14
ILL 3484	51.50	85.00	0.57	2.82	-0.14
ILL 7289	56.00	89.00	0.33	1.40	-0.15
ILL 7817	55.00	85.00	0.21	0.59	-0.15
ILL 6100	52.00	84.00	0.50	2.45	-0.15
ILL 7304	53.50	85.00	0.37	1.70	-0.16
ILL 7327	53.00	85.00	0.45	2.25	-0.17
ILL 7307	54.00	85.00	0.41	2.07	-0.17
ILL 247	54.50	88.00	0.26	1.10	-0.17
ILL 7812	53.00	85.00	0.50	2.68	-0.17
ILL 8013	54.00	95.50	0.81	4.85	-0.17
ILL 6356	51.50	84.50	0.86	5.10	-0.17
ILL 6332	73.00	98.00	0.19	1.31	-0.17
ILL 5955	56.00	87.00	0.20	0.80	-0.18
ILL 7838	50.50	83.50	0.32	1.39	-0.18
ILL 8028	58.00	90.00	0.52	3.08	-0.18
ILL 7798	45.50	91.50	1.05	2.81	0.14
ILL 7799	43.00	88.00	1.43	4.00	0.27
ILL 7801	45.00	85.50	0.43	0.86	-0.09
ILL 7806	44.50	90.50	0.90	0.93	0.33
ILL 7807	45.00	86.50	1.20	4.86	-0.07
ILL 7818	43.50	87.00	1.08	2.95	0.14
ILL 7826	49.50	87.50	0.81	1.87	0.10
ILL 7827	43.50	88.50	0.93	2.73	0.05
ILL 7831	45.00	85.50	0.85	2.84	-0.04
ILL 8013	46.50	90.00	1.30	4.18	0.14
ILL 8014	48.00	93.50	0.96	1.90	0.23
ILL 8016	44.00	83.50	1.04	2.13	0.24
ILL 8020	45.00	82.00	0.95	2.16	0.17
ILL 8024	44.50	85.00	1.06	2.83	0.15
ILL 8026	45.00	85.50	0.97	3.43	-0.04
ILL 8028	43.50	87.00	1.11	3.51	0.07
ILL 8056	47.50	95.00	0.94	3.01	0.02
ILL 8061	60.50	93.00	1.34	3.76	0.30
Heat sensitive					
ILL 247	43.00	88.50	0.58	2.01	-0.15
ILL 5505	49.50	85.00	0.64	2.46	-0.15
ILL 5957	47.00	80.00	0.28	0.58	-0.17
ILL 5964	43.50	85.00	0.32	0.94	-0.20
ILL 6057	44.00	88.50	0.13	1.01	-0.39
ILL 6058	42.50	86.50	0.16	1.05	-0.37
ILL 6060	46.00	87.00	0.50	2.23	-0.25
ILL 6086	45.00	87.00	0.59	2.97	-0.30
ILL 6087	45.00	83.50	0.55	2.76	-0.30
ILL 6099	46.00	89.00	0.42	1.58	-0.22
ILL 6104	44.00	87.50	0.43	1.92	-0.27
ILL 6105	45.50	85.00	0.84	3.41	-0.15
ILL 6320	43.00	85.50	0.75	3.26	-0.22
ILL 6332	51.00	92.00	0.37	2.31	-0.37
ILL 6338	47.00	91.00	0.59	1.96	-0.12
ILL 6360	44.50	87.50	0.71	3.37	-0.27
ILL 7238	46.00	96.50	0.96	4.91	-0.30
ILL 7264	45.00	87.00	0.84	3.56	-0.18
ILL 7286	44.00	88.00	0.69	3.16	-0.25
ILL 7310	43.00	86.50	0.40	2.49	-0.40
ILL 7311	50.00	89.50	0.40	2.00	-0.29
ILL 7314	44.00	85.00	0.47	2.03	-0.26
ILL 7317	45.50	90.00	0.66	2.94	-0.23
ILL 7325	44.50	90.00	0.53	1.66	-0.13
ILL 7328	54.50	95.00	1.08	4.69	-0.12
ILL 7344	44.00	87.00	0.63	3.17	-0.31
ILL 7813	49.50	84.50	0.55	2.81	-0.29
ILL 7815	48.00	93.00	0.61	2.43	-0.18
ILL 7816	44.00	92.50	0.75	3.03	-0.17
ILL 7819	43.50	86.00	0.41	1.19	-0.16

ILL 7328	65.00	90.00	0.24	1.42	-0.18
ILL 6055	50.50	84.00	0.56	3.10	-0.18
ILL 6092	58.00	89.00	0.41	2.38	-0.18
ILL 6360	51.00	83.00	0.42	2.20	-0.18
ILL 8018	52.00	82.00	0.44	2.55	-0.20
ILL 7311	57.00	86.00	0.25	1.48	-0.21
ILL 6102	54.00	88.00	0.22	1.17	-0.21
ILL 7313	53.00	87.00	0.30	1.70	-0.21
ILL 8023	51.50	85.00	0.61	3.85	-0.22
ILL 8054	52.50	82.50	0.24	1.41	-0.22
ILL 6385	70.00	89.00	0.21	1.94	-0.23
ILL 6080	54.00	84.00	0.35	2.36	-0.24
Highly heat sensitive					
ILL 5919	51.50	83.00	0.52	3.61	-0.26
ILL 5929	55.00	88.00	0.18	1.60	-0.27
ILL 8014	61.00	88.50	0.30	2.70	-0.28
ILL 5964	55.00	85.00	0.27	2.28	-0.28
ILL 7837	54.50	86.50	0.61	4.58	-0.28
ILL 7317	55.50	89.00	0.17	1.71	-0.29
ILL 7266	56.50	89.00	0.23	2.20	-0.29
ILL 8020	54.00	86.00	0.40	3.27	-0.29
ILL 7808	52.50	84.00	0.27	2.30	-0.29
ILL 7832	51.00	84.00	0.38	3.05	-0.29
ILL 6105	57.00	89.00	0.26	2.50	-0.30
ILL 7314	54.00	86.00	0.29	2.62	-0.30
ILL 6101	55.00	84.00	0.21	2.25	-0.31
ILL 7326	54.00	86.00	0.46	3.95	-0.32
ILL 7813	50.00	84.00	0.55	4.45	-0.32
ILL 7800	52.50	87.00	0.41	3.75	-0.34
ILL 7232	57.00	88.00	0.25	2.85	-0.34
ILL 7797	55.00	87.00	0.31	3.20	-0.34
ILL 7815	51.00	84.00	0.47	4.16	-0.35
ILL 7801	55.00	83.00	0.21	2.60	-0.35
ILL 7820	54.50	84.00	0.29	3.16	-0.35
ILL 6346	54.00	86.00	0.37	3.65	-0.35
ILL 7383	55.00	83.50	0.46	4.30	-0.35
ILL 4910	55.00	86.00	0.21	2.70	-0.36
ILL 6089	55.50	86.00	0.22	2.80	-0.36
ILL 8024	53.00	88.00	0.23	2.83	-0.37
ILL 6060	53.00	88.00	0.20	2.69	-0.37
ILL 6072	54.00	88.00	0.48	4.67	-0.38
ILL 7814	54.00	85.00	0.20	2.78	-0.38
ILL 6107	53.00	87.00	0.50	4.80	-0.38
ILL 8280	55.00	85.00	0.20	2.85	-0.38
ILL 2524	54.00	83.00	0.21	2.97	-0.39
ILL 5943	53.00	85.00	0.19	3.09	-0.42
ILL 7804	58.00	88.00	0.20	3.35	-0.42
ILL 7829	55.00	84.00	0.28	3.80	-0.42
ILL 4772	53.00	84.00	0.24	3.54	-0.43
ILL 7820	44.50	87.50	0.80	3.03	-0.12
ILL 7830	44.50	86.00	0.67	3.78	-0.37
ILL 7836	45.50	84.50	0.26	0.54	-0.19
ILL 7837	42.50	82.50	0.66	2.71	-0.20
ILL 8015	48.00	84.50	0.68	4.01	-0.39
ILL 8017	45.00	84.00	0.55	2.71	-0.30
ILL 8021	45.00	87.00	0.60	2.97	-0.30
ILL 8054	44.50	88.00	0.61	3.49	-0.38
ILL 8280	50.00	91.00	0.22	1.66	-0.40
Highly heat sensitive					
ILL 504	66.50	93.50	0.18	2.22	-0.47
ILL 729	63.50	98.50	0.66	4.83	-0.49
ILL 880	45.50	86.50	0.23	3.11	-0.65
ILL 4758	42.00	89.00	0.31	2.91	-0.56
ILL 4910	43.50	95.00	0.29	3.36	-0.65
ILL 5940	47.00	93.00	0.42	3.56	-0.55
ILL 5943	46.00	88.50	0.31	2.96	-0.55
ILL 5955	43.50	85.50	0.19	1.81	-0.47
ILL 6054	44.50	86.50	0.22	2.48	-0.56
ILL 6072	46.00	88.00	0.33	3.68	-0.66
ILL 6081	43.50	86.50	0.23	1.87	-0.45
ILL 6089	45.00	88.50	0.27	2.40	-0.50
ILL 6091	45.50	85.50	0.55	4.09	-0.53
ILL 6100	44.50	96.50	0.28	3.20	-0.62
ILL 6319	45.00	90.50	0.32	2.56	-0.48
ILL 6322	44.00	91.00	0.26	2.41	-0.51
ILL 6362	43.50	90.50	0.55	3.85	-0.50
ILL 7232	43.50	89.50	0.53	3.43	-0.44
ILL 7266	44.50	87.50	0.36	3.41	-0.59
ILL 7289	44.50	83.00	0.20	3.48	-0.75
ILL 7300	43.50	87.00	0.49	3.61	-0.51
ILL 7305	44.50	85.00	0.13	2.10	-0.57
ILL 7307	45.00	87.00	0.32	2.51	-0.47
ILL 7339	44.00	86.50	0.35	4.07	-0.71
ILL 7797	47.50	92.00	0.24	3.52	-0.71
ILL 7800	44.50	84.50	0.26	2.41	-0.51
ILL 7804	43.00	83.50	0.53	3.37	-0.44
ILL 7812	43.50	85.00	0.53	3.54	-0.46
ILL 7824	45.50	85.00	0.74	4.74	-0.47
ILL 7829	44.00	84.00	0.43	3.61	-0.57
ILL 7833	45.00	85.00	0.23	3.28	-0.68
ILL 7838	44.00	83.00	0.43	2.95	-0.45
ILL 8012	43.00	84.50	0.41	2.84	-0.45
ILL 8022	47.50	93.00	0.46	4.21	-0.62

ILL 6081	54.00	86.00	0.30	4.17	-0.45
ILL 7799	55.00	82.00	0.22	3.80	-0.46
ILL 7816	61.50	89.50	0.21	4.95	-0.56
ILL 6054	52.00	86.00	0.25	4.85	-0.56
ILL 5505	58.50	109.00	0.17	5.08	-0.61
ILL 6320	54.00	88.50	0.17	7.20	-0.83

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Screening the FIGS Set of Lentil (*Lens culinaris* Medikus) Germplasm for Tolerance to Terminal Heat and Combined Drought-Heat Stress

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Abstract: Lentil (*Lens culinaris* Medikus) is one of the most important cool season food legume crops grown in many countries. Seeds are typically rich in protein, fiber, prebiotic carbohydrates and minerals, such as iron and zinc. With changing climate and variability, the lentil crop faces frequent droughts and heat stress of varying intensity in its major production zones. In the present study, a set of 162 lentil accessions selected through the Focused Identification of Germplasm Strategy (FIGS) were screened for tolerance to heat stress and combined heat-drought stresses under field conditions at two contrasting locations, namely Marchouch and Tessaout in Morocco. The results showed a significant genotypic variation for heat tolerance and combined heat-drought tolerance among the accessions at both locations. Based on the heat tolerance index (HTI), accessions, namely ILL 7833, ILL 6338 and ILL 6104, were selected as potential sources of heat tolerance at Marchouch, and ILL 7814 and ILL 8029 at Tessaout. Using the stress tolerance index (STI), ILL 7835, ILL 6075 and ILL 6362 were identified as the most tolerant lines (STI > 1) at Marchouch, and ILL 7814, ILL 7835 and ILL 7804 (STI > 1) at Tessaout, under the combined heat-drought stress conditions. Accession ILL 7835 was identified as a good source of stable tolerance to heat stress and combined heat-drought stress at both locations.

Keywords: lentil; heat stress; combined heat-drought stress; heat tolerance index; stress tolerance index

1. Introduction

Lentil (*Lens culinaris* Medikus) is an annual, diploid (2n = 14) and self-pollinated crop. Its seeds are rich in protein (22–35%), fiber, prebiotic carbohydrates and minerals, such as iron and zinc [1]. It plays a major role in alleviating malnutrition and micronutrient deficiencies of people living in Central

and West Asia and North Africa (CWANA), South Asia, East Africa, and North America [2]. Being a legume crop, it enhances nitrogen in the soil through symbiotic nitrogen fixation and, hence, plays a crucial role in the diversification and intensification of cereal-based cropping systems, worldwide [3]. In 2018, the total world area under lentil production was 6.1 million hectares, with a production of 6.3 million tons; in the African continent, Morocco ranked second in lentil production after Ethiopia. Nevertheless, the average productivity of lentil in Morocco was recorded as 798 kg/ha, which is still very low, compared to the world average of 1038 kg/ha [4].

Lentil has been mainly cultivated under rainfed conditions in the marginal areas where abiotic stresses, such as drought and heat, significantly reduced crop yield and productivity [5]. During 2007–2008 season, a severe drought struck the Mediterranean region, and caused huge yield reduction of lentil in Morocco, France, the Russian Republic, Spain, the Syrian Arab Republic and Turkey [4]. Next, there was a steep fall in lentil production and productivity in Morocco during 2016, which was declared as the warmest year of this century, and eventually the severe drought stress damaged the total cropped area of about 9581 ha [6]. It is predicted that the global mean surface temperature during mid and late 21st century will increase by 2 °C, leading to an extreme variation in precipitation events and creating more heat waves that menace crop cultivation [7].

In lentil, the reproductive phase is very sensitive to changes in the external environment, and exposure to heat and drought stress during this stage reduces crop productivity significantly [8,9]. Lentil performs well when its reproductive stage coincides with the average day/night temperatures of 15–25 °C/8–10 °C [10]. However, heat waves (temperatures >32 °C) during the flowering and pod-filling stages cause damage to reproductive organs, leading to flower drop, pollen sterility, pod abortion and reducing the total number of seeds in lentil [11–14]. On the other hand, the terminal drought stress caused by irregular and deficient precipitation during the reproductive phase shortens the duration of the seed filling phase, by accelerating the process of senescence and maturity and reducing the seed size in lentil [15]. As the combined stress reduces both total number of seeds and seed size, and cause more yield reductions than the individual stresses, the interaction between heat and drought stress is considered the most serious challenge, which has a more significant negative impact on crop yield and productivity than each stress individually [16–18]. Seed development is a crucial growth period under heat or drought stress in all grain crops; however, their combination affects adversely seed filling by suppressing the transfer of the assimilates needed, leading to low grain yields and poor grain quality [19]. The combined effects of heat and drought have been studied in some crops such as groundnut (*Arachis hypogaea L.*) [20], chickpea (*Cicer arietinum L.*) [21–23], barley (*Hordeum vulgare L.*) [24,25] and wheat (*Triticum aestivum L.*) [26,27], but only to a limited extent in lentil [18].

Commonly, traits including early flowering, early maturity and yield under stress conditions were employed as the key traits to identify heat and drought tolerant germplasm in many crops, such as lentil [28] and chickpea [29]. For instance, a heat tolerance index (HTI), based on the yield under heat stress, yield potential and flowering time, has been used effectively to evaluate the heat response in chickpea under heat conditions [30,31]. Likewise, several quantitative drought tolerance indices, such as the stress tolerance index (STI), geometric mean productivity (GMP), mean productivity (MP), harmonic mean (HARM) and stress tolerance (TOL) have been used widely to assess genotypes with better drought stress tolerance in many crops [32–34]. Siahsar et al. [35] reported that STI, GMP and HARM were the best indices for the selection of lentil lines under drought stress. Additionally, these tolerance indices have been used in selecting superior genotypes under heat stress conditions [36,37]. The adaptation of a genotype to terminal drought and heat stress is a sought-after strategy to minimize the economic impact of climate change on agriculture [38–40]. The development of new heat and drought tolerant lentil cultivars would improve yield stability and facilitate an increase in area under sustainable cropping systems [41]. Nevertheless, it requires extensive screening of the germplasm being conserved in gene banks which demands huge investment and time. To facilitate this process, the 'Focused Identification of Germplasm Strategy (FIGS) approach was developed by the International

Center for Agricultural Research in the Dry Areas (ICARDA). FIGS creates 'best-bet' trait-specific subsets of germplasm, by passing accession-level information, especially agro-climatic site information, through a series of filters, which increase the chances of finding the adaptive trait of interest [42]. This approach is based on the premise that the environment highly influences the natural selection process and consequently, the geographical distribution of crop species [43]. Previous studies confirm the effectiveness of the FIGS approach in the identification of desirable germplasm in wheat (*Triticum ssp.*) for major biotic stresses, such as Russian wheat aphid (*Diuraphis noxia*) [44], stem rust (*Puccinia graminis Pers.*) [45,46] and Sunn pest (*Eurygaster intergriceps Put.*) [47], as well for drought stress in faba bean (*Vicia faba L.*) [42]. The present study aimed to assess genetic variability for heat and drought tolerance in the ICARDA lentil germplasm, using the FIGS approach. The objectives of the study were: (i) to investigate the individual and combined effects of terminal heat and drought stress during the reproductive phase; and (ii) to use indices and identify promising accessions with tolerance to heat stress and combined heat-drought stress for future breeding.

2. Materials and Methods

2.1. Plant Material and Study Area

A FIGS set comprising 162 germplasm accessions of lentil developed by the ICARDA gene bank in 2013 was evaluated. These accessions were originated from Pakistan (66), Nepal (68), Ethiopia (13), India (4), Yemen (3), Russia (3), Sudan (2) and Iran (2). The FIGS set, along with the Moroccan cultivar, Bakria as a local check, was evaluated in an alpha lattice design, with two replicates at two locations: Tessaout (31.42° N, 6.47° W, 68 m altitude) in the 2013–2014 cropping season, and Marchouch (33.56° N, 6.63° W, 392 m altitude) in the 2014–2015 cropping season in Morocco. At both stations, each germplasm was grown in a 2-row plot of 1 m length, with a spacing of 30 cm between rows. In each row, seeds were sown by hand at a 2 cm depth maintaining a 10 cm space between plants, and the total plot size maintained was 0.6 m^2. In general, the Tessaout research station represents a typical Mediterranean semi-arid environment, characterized by a hot dry summer with an annual rainfall of 266 mm [48]. The Marchouch station also represents a Mediterranean semi-arid environment, but with higher annual precipitation (400 mm) [49]. The experimental site in Tessaout is silty-clay soil, whereas in Marchouch, it is vertisol.

2.2. Treatments

At each of the two locations, three experiments involving the same set of FIGS germplasm were conducted by manipulating the planting date and water supply, in order to impose heat and water stress at the reproductive phase of the plant growth. These three experiments were considered to represent three treatments, namely the normal date of planting (treatment A), late planting with irrigation at field capacity throughout the crop period (treatment B) and late planting without irrigation during the reproductive phase (treatment C), which were referred to as treatments. Treatment A resulted in optimal growing conditions (>150 mm well-distributed rainfall and below 27 °C temperature) without any heat and water stress to the plants. Treatment B (planted 50 days after normal planting date with irrigation at field capacity throughout the crop duration) imposed heat stress, as the plants were exposed under field conditions to a temperature above 32 °C during the reproductive phase (Table A1), while regular irrigation at field capacity avoided any water stress to the plants. Treatment C (planted 50 days after normal planting date without irrigation during the reproductive phase) imposed a combined heat and water stress. Treatment A was sown on 20 December, and no irrigation was applied during the crop period as the crop received well distributed enough rainfall at Tessaout (157.9 mm) and Marchouch (167.8 mm). Treatments B and C were planted on 8 February. Irrigation was applied to maintain water supply at field capacity using sprinkler system throughout the crop duration in treatment B, whereas irrigation was stopped from the flowering initiation stage onward in treatment C, to impose water stress in addition to the heat stress. All the three treatments were kept weed-free

throughout the growing season. A wide temperature variation was recorded between normal and late planted treatments at the Tessaout and Marchouch research stations. During the reproductive stage, the averages of the maximum and minimum temperatures were 26.33 °C and 12.72 °C in Treatment A. However, the maximum temperatures during the flowering stage reached the threshold level of 42 °C in treatments B and C at Tessaout and 34 °C at Marchouch (Figure 1). Therefore, late planting with irrigation at field capacity was successful in imposing heat stress during the reproductive phase of test genotypes in treatment B, and late planting without irrigation in imposing drought and heat stress in treatment C.

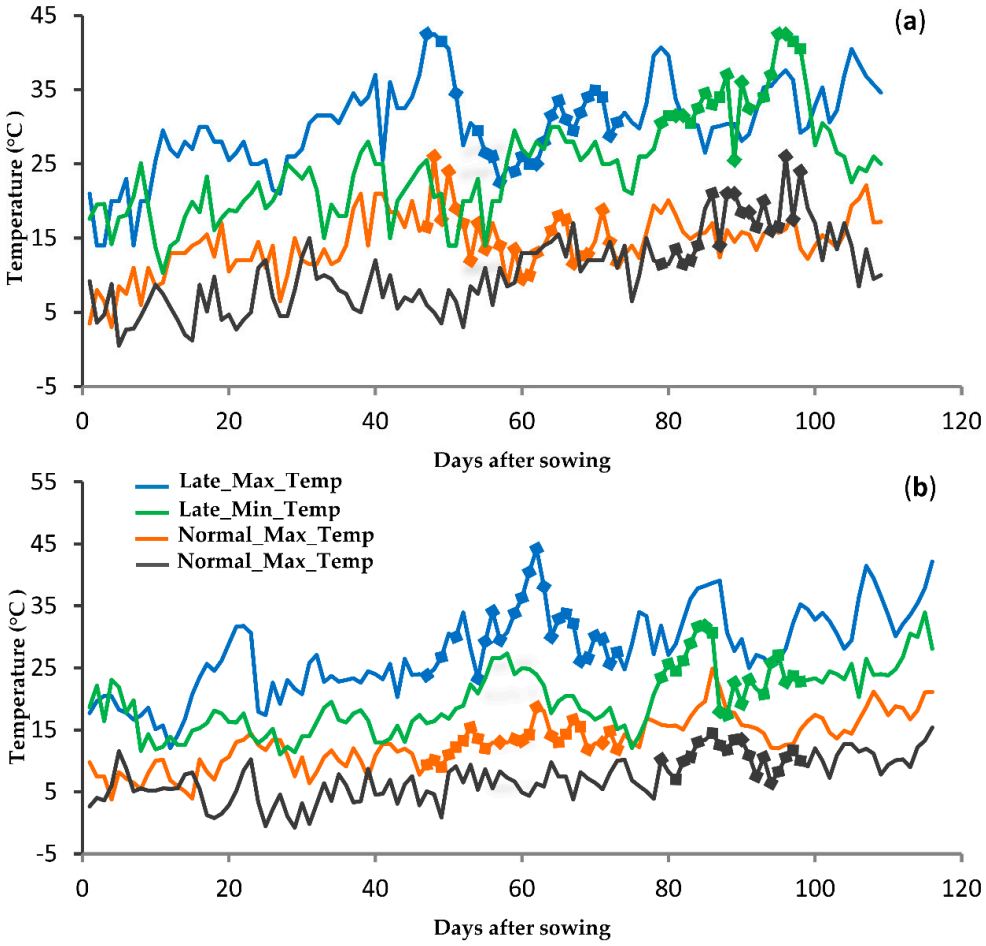

Figure 1. Averages of the maximum and minimum temperatures in normal and late planting at Tessaout (**a**) and Marchouch (**b**) during the crop season. The square icons indicate the flowering period for both normal and late sown crops.

2.3. Investigation and Calculation of Agronomic Traits

Data were recorded for the phenological traits (days to 50% flowering and maturity) on a plot basis, whereas five plants were selected randomly from each plot for the assessment of the morphological (numbers of primary, secondary and tertiary branches, and plant height) and yield (total numbers of filled and unfilled pods, biological yield, grain yield and 100-seed weight) traits, following the lentil ontology [50]. The heat tolerance index (HTI) was calculated for each genotype, following the multiple regression approach, as suggested by Bidinger et al. [51], and as used in chickpea [52]. Grain yield under stressed and non-stressed conditions was used to assess the tolerance of genotypes against the stress. This approach considers grain yield under heat stress conditions (Ys) to be a function of yield potential (Yp), days to 50% flowering (F) and heat tolerance index (HTI), such that the yield of a genotype can be expressed as Ysi = a + bYp + cFi + HTIi + E, where E is the random error with

zero mean and variance σ, and a, b, c are regression parameters estimated by least square methods. The heat tolerance index (HTI) was calculated for each accession as the difference between the estimated late-season grain yield and the estimated optimal-season grain yield plus standardized residuals from regression.

On the basis of grain yield under stress (YS) and normal conditions (YP), the following quantitative tolerance indices were estimated to assess the combined effect of drought and heat stresses: stress tolerance index STI $= \frac{Y_{pi} * Y_{si}}{Y_p^2}$ [53]; tolerance index TOL $= Y_{pi} - Y_{si}$ [54]; geometric mean productivity GMP $= \sqrt{Y_{pi} * Y_{si}}$ [53]; mean productivity MP $= \frac{Y_{pi} + Y_{si}}{2}$ [54]; and harmonic mean HARM $= \frac{2(Y_{pi} * Y_{si})}{Y_{pi} + Y_{si}}$ [55] where, Ysi = yield of a genotype under stress condition, Ypi = yield of a genotype under normal sown condition, Ys = overall genotypic mean under stress condition, and Yp = overall genotypic mean under normal condition.

2.4. Statistical Analysis

Analysis of variance was performed using the general linear model (GLM) using IBM SPSS statistics 23. Treatment means were compared by least significant difference (LSD). Correlation coefficients (Pearson's) were calculated by multivariate analysis for heat stress condition, while Spearman's correlation coefficient was used for the combined heat-drought stress. Hierarchical cluster analysis using Ward's squared Euclidean distance method was performed for genotype grouping.

3. Results

3.1. Effects of Heat Stress on Morphological, Phenological, and Yield Contributing Traits

The analysis of variance (ANOVA) revealed significant differences among genotypes for all traits under stressed and non-stressed conditions at both the locations, Tessaout (Table 1) and Marchouch (Table 2). Furthermore, a highly significant variation ($p < 0.001\%$) was observed for all traits among treatments in Tessaout, as well as at Marchouch. The analysis also showed significant genotype x treatment interactions for all the traits at both locations, except for plant height, number of primary branches per plant, number of tertiary branches per plant and biomass yield per plant at Tessaout.

There existed a wide range of variability among lentil genotypes for phenological traits at both Tessaout (Table 1) and Marchouch (Table 2). Days to 50% flowering in normal planting conditions ranged from 61 to 78 days at Tessaout, and from 79 to 98 days at Marchouch. The range of days to 95% maturity varied from 100 to 119 days at Tessaout, and from 113 to 126 days at Marchouch under normal planting conditions. Heat stress decreased the crop duration by 23% at Tessaout, and 26% at Marchouch, while the combined heat-drought stress caused 28% reduction in crop duration at Tessaout and 27% at Marchouch. At each location, days to 50% of flowering was almost similar under both stress conditions: 46 days at Tessaout and 55 days at Marchouch (Table A1).

Under normal planting, plant height ranged from 17 to 37 cm, with the overall mean of 25 cm at Tessaout and from 14 to 52 cm, with a mean of 31.51 cm at Marchouch. Heat stress reduced plant height by 18% at Tessaout and by 31% at Marchouch. However, the combined heat-drought stress reduced plant height more than the heat stress: 29% at Tessaout and 35% at Marchouch (Table A1).

Heat stress and combined heat-drought stress reduced the number of primary, secondary and tertiary branches per plant significantly. The reduction was more pronounced under combined stress conditions at both locations, particularly at Marchouch. The number of primary branches per plant reduced by 24% at Tessaout and 30% at Marchouch. Likewise, the number of secondary and tertiary branches per plant were reduced by 38% and 63% at Tessaout, whereas in Marchouch, there was an 80% reduction in the number of secondary branches per plant, and a 91% reduction in the number of tertiary branches per plant (Table A1).

Under normal planting, the number of total pods per plant ranged from six to 160 at Tessaout, which decreased by 47% under heat stress and 62% under combined heat-drought stress conditions.

In contrast, the number of total pods per plant ranged from three to 230 at Marchouch but declined by 72% due to heat stress and 91% as a result of combined heat-drought stress. Likewise, heat stress and combined heat-drought stresses reduced number of filled pods by 58% and 65% at Tessaout, while it was 69% and 91% at Marchouch, compared to normal planting. The mean numbers of total pods and filled pods per plant were higher under stress conditions at Tessaout than at Marchouch (Table A1).

Biomass per plant ranged from 1.4 to 39.6 g at Tessaout and from 1.07 to 36.2 g at Marchouch under normal planting conditions. The heat stress reduced biomass by 69% at Tessaout and 77% at Marchouch. However, the reduction in biomass was higher under combined stress at both locations: it was recorded 71% at Tessaout and 85% at Marchouch. Similarly, the combined heat-drought stress decreased the seed yield by 76% at Tessaout and 90% at Marchouch, more than the heat stress alone (68% at Tessaout; 71% at Marchouch). The mean grain yields under heat stress and combined heat-drought stress were more at Tessaout than at Marchouch. Under the normal planting, the hundred-seed weight ranged from 0.50 to 3.70 g at Tessaout and 0.50 to 3.85 g at Marchouch. The combined heat-drought stress increased the hundred-seed weight by 21% at Tessaout and 10% at Marchouch. However, the increase was higher under heat stress by 38% at Tessaout and 27% at Marchouch (Table A1).

Table 1. Analysis of variance (ANOVA) expressed in mean square for different traits among 162 lentil accessions at Tessaout during 2013–2014.

Source	df	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP	HSW
Accession (A)	161	3179.93 **	9020.56 **	7926.79 **	85.73 *	2809.87 **	3116.53 **	134,681.09 **	17,979.63 **	97,275.47 **	6602.61 **	240.04 **	128.57 **
Treatment (T)	2	8801.50 **	81,401.01 **	194,468.02 **	87.07 **	4447.67 **	7474.59 **	221,759.77 **	6956.78 **	169,051.73 **	17,534.56 **	847.36 **	153.11 **
A × T	322	1373.34 ns	3564.90 **	9245.98 **	109.86 ns	2796.58 *	2834.41 ns	158,553.22 *	30,640.38 **	101,053.29 *	9055.03 ns	235.05 **	84.96 **
Error	486	2734.02	1675.88	4595.96	181.53	3506.01	3745.33	180,293.52	31,008.66	125,776.68	11,795.35	258.51	53.9
R^2		0.83 **	0.98 **	0.98 **	0.61 **	0.74 **	0.78 **	0.74 **	0.66 **	0.75 **	0.74 **	0.84 **	0.87 **

PH, plant height; DF, days to 50% flowering; DM, days to 95% maturity; PBPP, number of primary branches per plant; SBPP, number of secondary branches per plant; TBPP, number of tertiary branches per plant; NTPP, number of total pods per plant; NUPP, number of unfilled pods per plant; NFPP, number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight; df, degrees of freedom. Significant difference at: * $p < 0.01$, ** $p < 0.001$, ns denotes a non-significant difference.

Table 2. Analysis of variance (ANOVA) expressed in mean square for different traits among 162 lentil accessions at Marchouch during 2014–2015.

Source	df	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP	HSW
(A)	161	5998.87 **	12,577.23 **	8763.72 **	179.10 **	4794.79 **	2163.08 **	217,688.81 **	16,449.68 **	164,517.17 **	4042.86 **	297.13 **	168.53 **
(T)	2	23,422.11 **	235,449.92 **	223,732.48 **	103.03 **	46,726.49 **	33,818.93 **	638,843.72 **	45,673.66 **	344,672.12 **	18,250.69 **	976.43 **	67.07 **
A × T	322	6986.42 **	6242.97 **	5907.75 **	222.12 **	7827.42 **	4388.39 **	324,520.74 **	35,015.62 **	238,902.17 **	6559.96 **	382.04 **	86.57 **
Error	486	4714.99	503.09	952.59	122.45	4694.98	2275.94	160,769.5	14,911.52	121,599.93	2839.61	217.94	74.55
R^2		0.88 **	0.99 **	0.99 **	0.8 **	0.93 **	0.95 **	0.88 **	0.87 **	0.86 **	0.91 **	0.88 **	0.81 **

PH, plant height; DF, days to 50% flowering; DM, days to 95% maturity; PBPP, number of primary branches per plant; SBPP, number of secondary branches per plant; TBPP, number of tertiary branches per plant; NTPP, number of total pods per plant; NUPP, number of unfilled pods per plant; NFPP, number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight; df, degrees of freedom. Significant difference at: * $p < 0.01$, ** $p < 0.001$.

3.2. Correlations among the Traits under Heat Stress and Combined Heat-Drought Stress

The correlations between the variables under normal planting showed highly significant positive correlation at 0.01 level of grain yield with the number of the secondary and tertiary branches per plant and number of filled pods per plant at Tessaout and Marchouch (Tables A2 and A3). A highly positive correlation ($p < 0.01\%$) was also noticed between the grain yield and biomass at Marchouch. However, grain yield was negatively correlated with days to 50% flowering at Marchouch. Days to 50% flowering were positively correlated with plant height, days to 95% maturity, number of secondary and tertiary branches and hundred-seed weight at Tessaout (Table A2). Nevertheless, it was positively correlated only with plant height and days of 95% of maturity at Marchouch under normal planting (Table A3).

Under heat stress, grain yield was positively correlated ($p < 0.01\%$) with the number of the secondary and tertiary branches per plant, the number of total pods and biomass at both stations, Tessaout and Marchouch. Furthermore, grain yield had a positive correlation with days to 95% maturity at only Tessaout. A highly positive correlation of 0.01% was identified among plant height, days to 50% flowering, days to 95% maturity, biomass and hundred-seed weight in heat stress conditions at both stations. In combined heat-drought stress conditions, grain yield was positively correlated with all variables at both stations, except with days to 50% flowering at Marchouch. Additionally, positive associations ($p < 0.01\%$) existed among days to 50% flowering, days to 95% maturity and hundred-seed weight at Tessaout and Marchouch (Tables A2 and A3).

3.3. Classification of Genotypes Based on Heat Tolerance Index

Germplasm accessions were classified into representative groups based on the heat tolerance index (HTI). Hierarchical cluster analysis using Ward's incremental squared Euclidean distance method resulted in five clusters. These genotypic clusters differed significantly in HTI and defined as highly heat tolerant (HTI >1), heat tolerant (HTI means 0.68 in Marchouch and 0.66 in Tessaout), moderately heat tolerant (0.25 and 0.10), heat sensitive (−0.08 and −0.25) and highly heat sensitive (−0.37 and −0.55).

Based on the HTI, four accessions (ILL 7833, ILL 6338, ILL 7835 and ILL 6104) showed good performance under heat stress at Marchouch and three accessions (ILL 7835, ILL 7814 and ILL 8029) as highly heat tolerant at Tessaout. ILL 7835 emerged as heat tolerant at both the locations, showing its stable performance under heat stress. These accessions were characterized by a short phenological cycle with 53.5 days to achieve 50% flowering and about 86 days to 95% maturity at Marchouch, however, the duration was less at Tessaout with 45 days to 50% of flowering and 82 days to 95% maturity (Table 3). Additionally, 14 and 26 accessions were categorized as heat tolerant at Marchouch and Tessaout (Table A4), respectively. The moderately-heat tolerant group comprised of 26 accessions at Marchouch and 60 accessions at Tessaout. In this study, heat sensitive and highly heat-sensitive clusters were comprised of 76 and 42 accessions, respectively, at Marchouch. Whereas, it was 39 and 34 accessions at Tessaout (Table A5).

3.4. Response to Combined Heat-Drought Stress

Based on the stress tolerance index (STI) and the geometric mean productivity (GMP), genotype ILL 7835 was identified as tolerant to heat and drought at Marchouch and three accessions, ILL 7814, ILL 7835 and ILL 7804 at Tessaout. Genotype ILL 7835 showed tolerance to drought and heat stresses at both locations. The three highly tolerant genotypes at Tessaout achieved 50% flowering in around 43 days, while 50% flowering was achieved in about 60 days for the ILL 7835 at Marchouch. Furthermore, these genotypes reached 95% maturity in 83 days at Tessaout and in 90 days at Marchouch (Table 4). Using Spearman's correlation coefficient, the GMP was strongly correlated to STI ($r = 1$; $p < 0.01$) in Tessaout and Marchouch. Furthermore, highly positive correlation ($p < 0.01$) was recorded between yield potential (Yp) and yield under stress condition (Ys) ($p < 0.05$) at both stations. The Ys was positively correlated with the Yp, STI, GMP, MP and HARM indices, and negatively correlated with

stress tolerance (TOL) at Tessaout and Marchouch (Table 5). Additionally, a non-significant correlation was observed between Ys and TOL under combined heat-drought stress in Marchouch (Table 5).

Table 3. Day to 50% flowering, days to 95% maturity, grain yield per plant and heat tolerant index of highly heat tolerant accessions of lentil at Marchouch and Tessaout.

		Marchouch				Tessaout			
Location	**Accession**	**DF**	**DM**	**GYP**	**HTI**	**DF**	**DM**	**GYP**	**HTI**
Marchouch	ILL 7833	53	85.00	4.33	1.97	45	80.00	0.23	−0.68
	ILL 6338	54	86.00	3.16	1.52	47	83.00	0.59	−0.12
	ILL 7835	54	86.00	3.02	1.39	43.5	81.00	3.52	1.95
	ILL 6104	53	85.00	2.52	1.11	44	82.20	0.43	−0.27
	Mean	53.5	85.5	3.26	1.49	44.87	81.55	1.19	0.22
	SD	0.5	0.5	0.66	0.31	1.34	1.14	1.34	1.02
Tessaout	ILL 7835	54	86.00	3.02	1.39	43.5	81.00	3.52	1.95
	ILL 7814	54	85.00	0.2	−0.38	43.5	82.50	3.26	1.83
	ILL 8029	55	85.00	1.43	0.44	47.5	83.50	2.65	1.36
	Mean	54.33	85.33	1.55	0.48	44.83	82.33	3.14	1.71
	SD	0.47	0.47	1.15	0.72	1.88	1.02	0.36	0.25

DF, days to 50% flowering; DM, days to 95% maturity; GYP, grain yield per plant; HTI, heat tolerance index.

Table 4. Days to 50% flowering, days to 95% maturity, grain yield and stress tolerance indices of the ten best tolerant accessions of lentil grown under normal planting and combined heat-drought stress at Marchouch and Tessaout.

Location	**Accession**	**DF**	**DM**	**Ys**	**Yp**	**STI**	**GMP**	**MP**	**TOL**	**HARM**
Marchouch	ILL 7835	59.50	89.50	1.88	3.98	1.12	2.73	2.93	2.10	2.55
	ILL 6075	58.00	87.00	1.99	2.15	0.64	2.07	2.07	0.16	2.07
	ILL 6362	59.00	87.50	0.77	5.30	0.61	2.02	3.04	4.53	1.34
	ILL 7819	55.00	87.50	1.54	2.43	0.56	1.93	1.98	0.89	1.88
	ILL 7266	56.00	88.00	1.20	2.20	0.40	1.62	1.70	1.00	1.55
	ILL 6361	59.00	89.00	0.60	4.21	0.38	1.59	2.40	3.61	1.05
	ILL 880	64.00	98.00	0.67	3.66	0.37	1.56	2.16	2.99	1.13
	ILL 4605	49.00	83.30	0.59	4.07	0.36	1.55	2.33	3.48	1.03
	ILL 6088	57.50	87.50	0.90	2.45	0.33	1.48	1.67	1.55	1.31
	ILL 7815	58.50	85.50	0.48	4.16	0.30	1.41	2.32	3.69	0.85
	Mean	57.55	88.28	1.062	3.461	0.507	1.796	2.26	2.4	1.476
	SD	3.64	3.64	0.53	1.02	0.23	0.38	0.43	1.38	0.51
Tessaout	ILL 7814	42.50	83.00	2.71	4.63	1.68	3.54	3.67	1.92	3.42
	ILL 7835	42.50	81.50	2.28	5.30	1.61	3.47	3.79	3.02	3.19
	ILL 7804	43.00	82.50	2.53	3.37	1.14	2.91	2.95	0.84	2.89
	ILL 6101	45.00	86.00	2.20	3.11	0.91	2.62	2.66	0.91	2.58
	ILL 6100	45.00	83.50	2.22	3.20	0.95	2.67	2.71	0.98	2.62
	ILL 7807	45.50	83.50	1.40	4.86	0.91	2.61	3.13	3.46	2.18
	ILL 6091	47.00	82.50	1.23	4.09	0.67	2.24	2.66	2.86	1.88
	ILL 8029	46.50	83.50	1.11	4.25	0.63	2.17	2.68	3.14	1.76
	ILL 4605	51.58	81.07	1.15	3.81	0.59	2.09	2.48	2.66	1.77
	ILL 8061	66.50	97.00	1.16	3.76	0.58	2.08	2.46	2.60	1.77
	Mean	47.51	84.41	1.79	4.04	0.97	2.64	2.92	2.24	2.41
	SD	6.82	4.39	0.61	0.69	0.38	0.51	0.45	0.95	0.59

DF, days to 50% flowering; DM, days to 95% maturity; Ys, seed yield in combined heat-drought condition; Yp, seed yield in normal condition; STI, stress tolerance index; GMP, geometric mean productivity; MP, mean productivity; TOL, tolerance index; and HARM, harmonic mean.

Table 5. Spearman's correlation coefficients between stress tolerance parameters in lentil under stressed and non-stressed environments at Tessaout and Marchouch.

Location	Stress Parameter	Ys	Yp	STI	GMP	MP	TOL	HARM
Tessaout	Yp	0.192 *	1					
	STI	0.862 **	0.613 **	1				
	GMP	0.862 **	0.613 **	1.00 **	1			
	MP	0.471 **	0.941 **	0.817 **	0.817 **	1		
	TOL	−0.157 *	0.905 **	0.293 **	0.293 **	0.726 **	1	
	HARM	0.979 **	0.334 **	0.936 **	0.230 **	0.595 **	0.051 ns	1
Marchouch	Yp	0.137 *	1					
	STI	0.544 **	0.831 **	1				
	GMP	0.544 **	0.831 **	1.00 **	1			
	MP	0.241 **	0.978 **	0.907 **	0.907 **	1		
	TOL	0.30 ns	0.977 **	0.728 **	0.728 **	0.916 **	1	
	HARM	0.950 **	0.373 **	0.728 **	0.728 **	0.468 **	0.265 **	1

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level, ns denotes non-significant difference. Ys, seed yield in combined heat-drought condition; Yp, seed yield in normal condition; STI, stress tolerance index; GMP, geometric mean productivity; MP, mean productivity; TOL, tolerance index; and HARM, harmonic mean.

4. Discussion

The present study demonstrated delayed sowing as an effective approach to screening lentil germplasm for heat tolerance. A similar methodology was used to assess heat tolerance responses in chickpea [30,31], lentil [19,56] and mung bean [57,58] successfully. High temperature and low vapor pressure deficits (VPD) decrease soil moisture and increase transpiration rate, resulting in combined heat and drought stress [59]. Hence, frequent irrigation must be provided to remove the confounding effects of drought stress, to assess the effect of heat stress. Even the photoperiod may change during late planting, and previous studies by Summerfield et al. [60] and Erskine et al. [61] reported that temperature had a much bigger effect on the flowering time than the photoperiod in lentil.

Our findings showed that heat stress adversely affected plant height, number of branches and pods, grain yield and biomass, which agrees with several investigations in chickpea [62,63], lentil [12] and faba bean [64]. However, the influence of the combined heat-drought stress was more severe when compared to heat stress only, due to the reduction in water use efficiency. Heat and combined heat-drought stresses shortened vegetative and reproductive periods by accelerating the rate of plant growth and development. Similar findings were reported in previous studies [65,66]. The combined heat-drought stress largely decreased the number of pods more than the individual heat stress, leading to severe yield losses and biomass. This is in agreement with the previous research of Sehgal et al. [18,19], which has also shown the impact of combined heat-drought stress in lentil.

The days to 50% flowering under stressed conditions have shown quite similar results in both locations: approximately 46 days at Tessaout and 54 days at Marchouch. Lentil responded in the same way to heat stress and combined heat-drought stress by forcing early flowering (Figure 1) in both locations. Awasthi et al. [22] reported a very similar response in days to 50% flowering eventually, accelerated markedly in chickpea genotypes under heat and combined heat-drought stresses environment.

Overall, grain yield decreased under stress conditions, with a more pronounced effect under the combined heat-drought stress condition. High temperature stress led to yield loss by altering pollen and stigma development, pollination and pod set, while drought directly influenced the seed filling stage [19,67,68]. Moreover, yield is always influenced by photosynthetic ability and the performance of reproductive organs under stressed conditions [69]. In this study, the number of primary, secondary and tertiary branches were significantly positively correlated with seed yield ($p < 0.01$) under stressed environments, at both stations, except heat stress of Tessaout, which was not correlated with seed yield (Tables A2 and A3). Previous studies agreed with our findings and demonstrated a positive direct effect of primary branches on seed yield in lentil [70,71]. Recently, Ahmadi et al. [72] suggested that if lentil genotypes have a greater number of branches—mainly secondary branches—the final potential would increase the plant yield under stress conditions. It appears that plant biomass was low, mostly due to combined heat-drought stress that demands high water use efficiency. Further, heat stress, in combination with drought, increases leaf temperature and decreased net photosynthetic rate and stomatal conductance, resulting in a more damaging impact under the combined stress condition [73]. Overall, biomass reduction largely affected the number of total pods and seed yield per plant [74]. It was also positively correlated with number of total pods per plant, the numbers of primary, secondary and tertiary branches, and seed yield under heat stress and combined heat-drought stress at Tessaout and Marchouch (Table 5). The present study showed increased 100-seed weight under heat stress and the combined heat-drought stress condition, due to the reduction of seed numbers per plant. Similar results were reported by Chakherchaman et al. [75] in lentil under drought stress.

In the present study, three accessions, namely, ILL7833, ILL6338 and ILL6104, were selected as potential sources of heat tolerance at Marchouch and two accessions (ILL7814 an ILL8029) at Tessaout. Tolerant accessions produced significantly more pods under heat stress, as compared to heat sensitive genotypes. Under the combined stress of heat and drought, the most tolerant lines were ILL 7835, ILL 6075 and ILL6362 (STI >1) at Marchouch and ILL7814, ILL7835 and 7804 (STI >1) at Tessaout. Altogether, ILL7835 was identified as a good source of tolerance under heat stress and combined

heat-drought stress at Tessaout, as well as at Marchouch, while ILL7814 was identified as promising accession under both stresses at Tessaout. These selected lines are characterized by early flowering, which helped them to escape from heat and drought stress and shorter crop cycles (Tables 3 and 4). Usually, early flowering and maturity are the stress escape mechanism that can help lentil to perform well under stress conditions, and the development of genotypes with short duration is one of the major strategies used in breeding programs [76]. Although, early duration is considered to be the best adapted trait for chickpea genotypes to Mediterranean (spring sown) and south Indian environments [77,78]. Furthermore, the identified lines had a greater number of filled pods per plant, representing an excellent source for the lentil breeding program, and can be directly used in the crossing program to transfer heat and drought tolerance in a high yielding genetic background.

The selection of superior germplasm against combined stress was carried out by using a stress tolerance index (STI) and geometric mean productivity (GMP). STI and GMP have been used earlier for screening genotypes of chickpea [37,79,80], lentil [81,82], bread wheat [83], barley [84,85], fenugreek [86], oat [87] and maize [88,89] against abiotic stresses. Higher GMP and STI values indicate more tolerance to drought stress [90]. The stress tolerance index (STI) showed a highly positive correlation with both yield under stress conditions (Ys) and yield potential (Yp), GMP, MP and HARM at both locations. These results are in conformity with those of Ganjali et al. [91] in chickpea under stress and non-stress conditions. However, stress tolerance (TOL) was negatively correlated with Ys at Tessaout and non-significantly correlated at Marchouch, while it was positively correlated with potential yield at both locations. Similar results were reported also by Chakherchaman et al. [75] and Rad et al. [81] for lentil under drought stress conditions. Majidi et al. [92] also reported non-significant correlation between TOL and Ys, and a highly significant correlation between TOL and Yp, confirming that selection based on TOL should decrease yield in the moisture stress environment and increase grain yield under normal conditions. The limitations of using the TOL index have been described previously in several studies [35,93]. In general, our findings confirmed the effectiveness of using STI and GMP as reliable selection criteria for terminal heat or drought tolerance, as reported earlier by Fernandez [53], Farshadfar et al. [94] and Talebi et al. [95].

5. Conclusions

The present study shows that heat, as well as combined heat-drought stress, severely affects flower production and pod set, leading to a substantial loss in grain yield in lentil. The field screening technique has been demonstrated as an effective way for evaluating and selecting promising lines under heat or drought stress. The adaptation by selecting the robust genotypes can be a strong approach to mitigating the impact of climate change. Our study suggests that the FIGS approach in identifying heat tolerant germplasm in lentil is a successful approach under heat and combined heat-drought stress. This research identified a group of highly heat tolerant genotypes using HTI based on flowering time, and grain yield under stress and non-stress conditions. Our findings confirmed that STI, GMP and MP are the most suitable criteria, not only for selecting the high yielding genotypes under individual heat and drought stresses, but also in combined heat-drought stress. These lines would be an excellent source of stress tolerance to increase the adaptation of lentil under climate change, a major issue that currently affects agriculture.

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Appendix A

Table A1. Minimum, maximum and mean (±SD) values of traits of 162 lentil accessions under normal planting, heat stress and combined heat-drought stress in the field experiments at Tessaout during 2013–2014 and Marchouch during 2014–2015.

Trait	Heat Stress Conditions				Heat-Drought Conditions				Normal Conditions			
	Tessaout		Marchouch		Tessaout		Marchouch		Tessaout		Marchouch	
	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD	Min–Max	Mean ± SD
PH	15.00–28.0	20.32 ± 2.40	15.00–34.0	21.79 ± 3.13	12.75–28.90	17.67 ± 2.91	15.00–30.0	20.52 ± 2.83	17.00–37.0	24.95 ± 2.87	14.00–52	31.51 ± 6.09
DF	41.00–67.0	45.98 ± 4.28	47.00–73.0	54.58 ± 4.15	40.00–67.0	45.92 ± 4.70	48.50–73.0	54.62 ± 4.11	61.00–78.0	65.37 ± 1.90	79.00–98	87.62 ± 5.07
DM	80.00–103	88.21 ± 4.70	81.00–109	87.09 ± 4.82	77.00–101.0	82.97 ± 4.26	72.00–109.0	86.22 ± 4.39	100.00–119.0	115.63 ± 2.66	113.00–126	118.83 ± 2.48
PBPP	1.00–3.67	2.53 ± 0.58	1.00–4.67	2.35 ± 0.71	1.00–3.67	2.29 ± 0.59	1.00–5.00	1.81 ± 0.69	2.00–4.00	3.01 ± 0.68	1.00–5.00	2.59 ± 0.80
SBPP	2.00–17.10	9.42 ± 3.30	1.00–20.00	6.30 ± 3.31	2.00–16.50	8.02 ± 3.39	1.00–17.00	3.82 ± 2.62	4.00–25.00	13.10 ± 0.68	5.33–34.70	19.61 ± 5.98
TBPP	0.15–16.50	6.22 ± 3.43	0.20–8.90	2.54 ± 1.45	0.10–12.30	3.88 ± 3.22	0.00–9.00	1.22 ± 0.94	3.00–21.00	10.57 ± 2.79	1.00–30.70	14.34 ± 4.95
NTPP	2.29–126.0	29.88 ± 19.94	2.00–140.0	18.50 ± 15.89	1.00–70.75	21.22 ± 14.08	1.00–36.00	6.09 ± 3.79	6.00–160.45	56.68 ± 24.08	3.00–230.00	65.60 ± 40.69
NUPP	0.00–83.50	10.83 ± 9.13	0.00–38.0	3.44 ± 2.63	0.00–33.50	5.55 ± 3.47	0.00–14.00	1.82 ± 1.63	0.00–57.20	11.54 ± 7.65	0.00–82.67	17.11 ± 11.31
NFPP	0.50–96.20	19.06 ± 15.19	1.00–102.0	15.06 ± 12.11	0.00–62.75	15.66 ± 11.03	1.00–30.50	4.26 ± 2.13	2.00–129.45	45.14 ± 20.61	1.00–195.33	48.50 ± 35.15
BPP	0.25–15.80	3.99 ± 1.59	0.45–11.89	2.55 ± 1.92	0.50–32.21	3.72 ± 2.34	0.50–11.30	1.63 ± 1.03	1.40–39.60	12.86 ± 6.19	1.07–36.20	11.25 ± 4.08
GYP	0.13–3.94	0.88 ± 0.69	0.16–4.89	0.74 ± 0.68	0.12–2.78	0.66 ± 0.55	0.13–2.49	0.27 ± 0.20	0.15–7.71	2.74 ± 1.22	0.39–7.33	2.59 ± 1.49
HSW	0.90–5.00	2.50 ± 0.58	1.10–5.00	2.36 ± 0.59	0.70–4.20	1.94 ± 0.46	0.90–4.30	1.93 ± 0.52	0.50–3.70	1.54 ± 0.51	0.53–3.85	1.73 ± 0.62

PH, Plant height; DF, Days to 50% flowering; DM, Days to 95% maturity; PBPP, Number of primary branches per plant; SBPP, Number of secondary branches per plant; TBPP, Number of tertiary branches per plant; NTPP, Number of total pods per plant; NUPP, Number of unfilled pods per plant; NFPP, Number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight.

Table A2. Pearson's correlation coefficients among various traits based on162 accessions of lentil under normal planting (A), heat stress (B) and combined heat-drought stress (C) at Tessaout.

Trait	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP
A. Under normal planting											
DF	0.156 **										
DM	0.020ns	0.141 *									
PBPP	−0.070ns	−0.067ns	−0.050ns								
SBPP	0.242 **	0.152 **	0.015ns	−0.058ns							
TBPP	0.164 **	0.148 **	0.020ns	−0.060ns	0.570 **						
NTPP	0.080ns	−0.010ns	−0.055ns	0.062ns	0.428 **	0.372 **					
NUPP	0.090ns	−0.050ns	−0.132 *	0.070ns	0.199 **	0.163 **	0.521 **				
NFPP	0.058ns	0.010ns	−0.010ns	0.040ns	0.409 **	0.360 **	0.931 **	0.174 **			
BPP	−0.008ns	0.103ns	0.079ns	−0.089ns	0.086ns	0.095ns	0.131 *	0.144 **	0.090ns		
GYP	0.056ns	0.058ns	0.011ns	0.033ns	0.400 **	0.396 **	0.873 **	0.148 **	0.944 **	0.112 *	
HSW	0.119 *	0.123 *	−0.120ns	−0.027ns	0.084ns	0.043ns	0.061ns	0.026ns	0.059ns	−0.057ns	0.070ns

Table A2. *Cont.*

Trait	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP
B. Under heat stress condition											
DF	0.234 **										
DM	0.146 **	0.363 **									
PBPP	0.070ns	0.110ns	0.090ns								
SBPP	0.050ns	0.05ns	0.124 *	0.147 **							
TBPP	0.115 *	0.030ns	0.110ns	0.226 **	0.657 **						
NTPP	0.020ns	0.010ns	0.110ns	0.129 *	0.515 **	0.520 **					
NUPP	0.040ns	0.040ns	0.122 *	0.127 *	0.295 **	0.281 **	0.694 **				
NFPP	0.03ns	−0.020ns	0.050ns	0.090ns	0.496 **	0.512 **	0.885 **	0.278 **			
BPP	0.179 **	0.216 **	0.249 **	0.208 **	0.438 **	0.374 **	0.468 **	0.344 **	0.402 **		
GYP	0.070ns	0.020ns	0.116 *	0.110ns	0.491 **	0.522 **	0.854 **	0.372 **	0.898 **	0.476 **	
HSW	0.182 **	0.139 *	0.090ns	−0.010ns	−0.110ns	−0.030ns	−0.020ns	−0.060ns	0.010ns	0.040ns	0.020ns
Trait	**PH**	**DF**	**DM**	**PBPP**	**SBPP**	**TBPP**	**NTPP**	**NUPP**	**NFPP**	**BPP**	**GYP**
C. Under heat-drought conditions											
DF	0.121 *										
DM	0.191 **	0.432 **									
PBPP	0.080ns	0.010ns	0.148 **								
SBPP	0.311 **	0.090ns	0.203 **	0.135 *							
TBPP	0.210 **	−0.080ns	0.110ns	0.060ns	0.694 **						
NTPP	0.272 **	0.145 **	0.224 **	0.174 **	0.665 **	0.645 **					
NUPP	0.184 **	0.230 **	0.145 **	0.040ns	0.337 **	0.238 **	0.522 **				
NFPP	0.241 **	0.080ns	0.200 **	0.185 **	0.631 **	0.647 **	0.943 **	0.209 **			
BPP	0.356 **	0.391 **	0.344 **	0.182 **	0.460 **	0.294 **	0.435 **	0.378 **	0.352 **		
GYP	0.278 **	0.121 *	0.238 **	0.186 **	0.598 **	0.589 **	0.902 **	0.223 **	0.948 **	0.391 **	
HSW	0.086ns	0.159 **	0.144 **	0.019ns	−0.154 **	−0.135 *	0.020ns	0.111 *	−0.023ns	0.073ns	0.014ns

*. Correlation is significant at the 0.05 level, **. Correlation is significant at the 0.01 level, ns denotes non significant difference. PH, Plant height; DF, Days to 50% flowering; DM, Days to 95% maturity; PBPP, Number of primary branches per plant; SBPP, Number of secondary branches per plant; TBPP, Number of tertiary branches per plant; NTPP, Number of total pods per plant; NUPP, Number of unfilled pods per plant; NFPP, Number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight.

Table A3. Pearson's correlation coefficients among various traits based on 162 accessions of lentil under normal planting (A), heat stress (B) and combined heat-drought stress (C) at Marchouch.

	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP
A. Under normal planting											
DF	0.205 **										
DM	0.321 **	0.462 **									
PBPP	0.043ns	−0.010ns	−0.008ns								
SBPP	0.053ns	0.022ns	0.034ns	0.032ns							
TBPP	0.142 *	−0.015ns	0.057ns	0.034ns	0.670 **						
NTPP	−0.012ns	−0.086ns	0.068ns	0.073ns	0.272 **	0.222 **					
NUPP	−0.087ns	−0.064ns	−0.005ns	−0.052ns	0.295 **	0.287 **	0.545 **				
NFPP	0.018ns	−0.076ns	0.080ns	0.110ns	0.207 **	0.152 **	0.954 **	0.268 **			
BPP	0.143 *	0.001ns	0.074ns	0.114 *	0.517 **	0.472 **	0.406 **	0.348 **	0.342 **		
GYP	0.017ns	−0.126 *	0.060ns	0.090ns	0.177 **	0.110ns	0.861 **	0.228 **	0.908 **	0.302 **	
HSW	0.221 **	−0.050ns	0.030ns	0.144 **	0.020ns	0.050ns	−0.110ns	−0.129 *	−0.078ns	0.146 **	−0.060ns
B. Under heat stress condition											
DF	0.163 **										
DM	0.358 **	0.619 **									
PBPP	0.030ns	0.040ns	0.150 **								
SBPP	0.148 **	0.054ns	0.148 **	0.405 **							
TBPP	0.069ns	0.071ns	0.073ns	0.277 **	0.674 **						
NTPP	0.223 **	0.030ns	0.115 *	0.357 **	0.531 **	0.431 **					
NUPP	0.118 *	0.139 *	0.257 **	0.287 **	0.407 **	0.307 **	0.684 **				
NFPP	0.228 **	−0.010ns	0.060ns	0.335 **	0.504 **	0.417 **	0.975 **	0.504 **			
BPP	0.293 **	0.251 **	0.341 **	0.360 **	0.535 **	0.445 **	0.648 **	0.616 **	0.579 **		
GYP	0.248 **	−0.023ns	0.040ns	0.314 **	0.511 **	0.422 **	0.931 **	0.511 **	0.946 **	0.584 **	
HSW	0.208 **	0.190 **	0.269 **	0.227 **	0.114 *	0.103ns	0.092ns	0.145 **	0.065ns	0.284 **	0.110ns

Table A3. *Cont.*

Trait	PH	DF	DM	PBPP	SBPP	TBPP	NTPP	NUPP	NFPP	BPP	GYP
C. Under heat-drought condition											
DF	0.092ns										
DM	0.125 *	0.561 **									
PBPP	0.224 **	−0.044ns	0.118*								
SBPP	0.180 **	0.041ns	0.198 **	0.586 **							
TBPP	0.115*	−0.010ns	0.115*	0.357 **	0.537 **						
NTPP	0.188 **	−0.010ns	0.153 **	0.535 **	0.463 **	0.419 **					
NUPP	0.139*	−0.042ns	0.060ns	0.489 **	0.426 **	0.441 **	0.769 **				
NFPP	0.175 **	0.013ns	0.177 **	0.439 **	0.379 **	0.307 **	0.913 **	0.443 **			
BPP	0.237 **	0.070ns	0.223 **	0.335 **	0.424 **	0.464 **	0.436 **	0.350 **	0.389 **		
GYP	0.190 **	0.020ns	0.168 **	0.417 **	0.402 **	0.371 **	0.890 **	0.482 **	0.942 **	0.408 **	
HSW	0.194 **	0.152 **	0.169 **	0.158 **	0.204 **	0.179 **	0.139 *	0.139 *	0.107ns	0.228 **	0.123 *

*. Correlation is significant at the 0.05 level, **. Correlation is significant at the 0.01 level, ns denotes non significant difference. PH, Plant height; DF, Days to 50% flowering; DM, Days to 95% maturity; PBPP, Number of primary branches per plant; SBPP, Number of secondary branches per plant; TBPP, Number of tertiary branches per plant; NTPP, Number of total pods per plant; NUPP, Number of unfilled pods per plant; NFPP, Number of filled pods per plant; BPP, biomass per plant; GYP, grain yield per plant; HSW, hundred-seed weight.

Table A4. Day to 50% flowering (DF), days to 95% maturity (DM), seed yield under stress condition (Yp), seed yield under normal condition (Yp) and heat tolerant index (HTI) of heat tolerant accessions of lentil.

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
	Tessoaut Location						**Marchouch Location**				
ILL 6359	47.00	89.00	1.96	2.96	0.94	ILL 6363	54.00	85.00	2.38	3.91	0.97
ILL 7295	46.00	88.50	1.91	2.71	0.94	ILL 8025	54.50	86.50	1.91	1.70	0.87
ILL 2524	43.00	87.00	1.95	2.91	0.94	ILL 7819	51.50	84.50	2.00	2.43	0.85
ILL 6053	42.50	88.50	1.68	1.66	0.90	ILL 7223	51.50	88.00	2.05	3.80	0.75
ILL 7389	46.00	88.00	2.00	3.57	0.88	ILL 6361	53.00	85.50	2.10	4.21	0.74
ILL 8019	43.00	83.50	1.93	3.14	0.88	ILL 6053	52.50	85.00	1.91	3.13	0.72
ILL 8018	45.50	88.50	1.80	2.60	0.86	ILL 6075	55.00	86.00	1.71	2.15	0.69
ILL 6094	45.00	95.00	1.93	3.41	0.84	ILL 4902	68.00	90.00	1.78	3.70	0.64
ILL 8025	44.50	87.00	1.88	3.16	0.84	ILL 8061	61.50	93.00	1.42	1.25	0.61

Table A4. *Cont.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
	Tessoaut Location						**Marchouch Location**				
ILL 6095	45.00	90.00	1.46	1.02	0.82	ILL 6362	57.00	88.00	2.01	5.30	0.59
ILL 4605	50.84	84.59	1.95	3.81	0.81	ILL 7806	55.50	88.00	1.59	2.65	0.57
ILL 7932	44.00	84.50	1.21	0.76	0.64	ILL 6359	51.00	86.50	1.27	0.90	0.51
ILL 6364	44.50	86.50	1.74	3.64	0.62	ILL 880	70.50	100.00	1.53	3.66	0.48
ILL 5919	50.00	81.00	1.32	1.77	0.59	ILL 504	67.00	106.00	1.38	2.80	0.45
ILL 7250	44.00	90.00	1.39	2.16	0.56						
ILL 7796	48.50	88.00	1.64	3.65	0.55						
ILL 7817	43.00	85.00	1.75	4.12	0.55						
ILL 6055	47.00	86.50	1.42	2.65	0.51						
ILL 8437	46.50	97.00	1.27	1.93	0.50						
ILL 6080	45.50	84.00	1.08	0.99	0.48						
ILL 8023	44.50	91.00	1.35	2.46	0.47						
ILL 7795	44.00	88.00	1.27	2.16	0.45						
ILL 6107	44.50	89.50	1.46	3.26	0.43						
ILL 7808	44.00	85.00	1.35	2.82	0.41						
ILL 7301	46.00	85.00	1.25	2.32	0.41						
ILL 7327	44.50	90.00	0.96	1.00	0.37						

Table A5. Day to 50% flowering (DF), days to 95% maturity (DM), seed yield in stress condition (Yp), seed yield in normal condition (Yp) and heat tolerant index (HTI) of moderately heat tolerant, heat sensitive and highly heat sensitive accessions cluster of lentil at Tessaout and at Marchouch.

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
		Marchouch Station						**Tessaout Station**			
	Moderately heat tolerant						Moderately heat tolerant				
ILL 7308	53.00	86.00	1.52	2.82	0.49	ILL 206	44.50	92.00	1.21	3.68	0.14
ILL 8029	55.00	85.00	1.43	2.76	0.45	ILL 221	62.50	98.00	1.31	4.68	0.12
ILL 3635	54.00	85.50	1.31	2.12	0.43	ILL 1734	50.50	96.50	0.93	1.59	0.26
ILL 7807	52.50	92.00	1.36	2.99	0.37	ILL 1861	42.00	95.00	0.85	2.15	0.06
ILL 7301	54.00	85.00	1.25	2.47	0.35	ILL 3484	46.50	94.50	1.10	2.11	0.31
ILL 7798	53.50	86.00	0.99	0.91	0.33	ILL 3635	45.00	83.50	1.30	3.64	0.23
ILL 7295	53.00	86.00	1.15	2.13	0.32	ILL 4743	46.50	86.00	0.77	2.71	−0.09
ILL 6325	50.50	90.00	1.16	2.28	0.30	ILL 4772	43.50	89.50	0.70	2.01	−0.04

Table A5. *Cont.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
		Marchouch Station						**Tessaout Station**			
ILL 6095	53.50	84.50	0.87	0.55	0.28	ILL 4902	65.00	101.00	0.99	2.76	0.17
ILL 729	66.50	106.00	1.24	3.78	0.26	ILL 5918	46.50	81.50	0.68	1.72	0.00
ILL 6086	53.00	87.00	1.07	2.22	0.25	ILL 5929	51.00	90.00	1.04	2.77	0.16
ILL 6364	51.00	81.00	1.20	3.16	0.24	ILL 5958	45.00	89.50	0.69	1.53	0.04
ILL 7286	53.50	85.50	0.85	0.90	0.24	ILL 6059	51.00	88.50	0.81	2.48	0.00
ILL 6337	51.00	87.00	1.30	3.89	0.24	ILL 6074	46.00	88.00	0.61	1.13	0.04
ILL 6094	53.50	85.00	1.01	2.03	0.23	ILL 6075	43.00	89.00	1.22	4.40	0.02
ILL 7830	54.50	85.50	0.90	1.38	0.23	ILL 6077	48.50	88.50	0.79	1.21	0.19
ILL 5958	53.50	84.50	0.92	1.75	0.20	ILL 6079	45.00	84.50	0.78	2.41	−0.03
ILL 8019	53.00	85.50	1.08	2.87	0.20	ILL 6088	43.50	85.00	0.93	1.94	0.18
ILL 7344	54.00	87.00	1.12	3.33	0.18	ILL 6092	46.00	86.50	0.81	1.03	0.23
ILL 7309	52.00	85.00	0.71	0.70	0.16	ILL 6096	44.50	90.50	1.09	3.11	0.13
ILL 8017	54.50	87.00	1.20	4.14	0.16	ILL 6101	45.50	88.50	1.23	3.11	0.25
ILL 7250	53.00	87.50	0.87	2.05	0.14	ILL 6102	45.00	90.50	1.05	2.96	0.11
ILL 7238	53.50	86.00	0.80	1.60	0.14	ILL 6325	44.50	94.00	0.74	1.83	0.03
ILL 7300	57.50	88.00	0.82	2.00	0.13	ILL 6337	43.50	88.00	0.75	1.71	0.05
ILL 1861	52.50	100.00	0.56	0.50	0.08	ILL 6346	46.00	90.50	0.90	2.38	0.09
ILL 7380	53.00	86.00	0.75	2.15	0.05	ILL 6356	45.50	87.50	1.17	3.26	0.17
	Heat sensitive					ILL 6361	45.00	86.50	1.01	2.25	0.20
ILL 7290	51.00	83.50	0.71	1.14	0.11	ILL 6363	47.00	85.00	0.80	2.56	−0.03
ILL 8056	62.00	93.00	0.68	1.43	0.10	ILL 6385	60.50	91.00	0.94	2.24	0.20
ILL 7264	53.00	87.00	0.60	0.67	0.09	ILL 7223	45.00	89.00	1.16	2.40	0.31
ILL 7312	51.00	85.50	0.70	1.35	0.09	ILL 7290	45.50	88.50	0.87	2.96	−0.05
ILL 6057	52.00	88.00	0.63	0.95	0.08	ILL 7303	54.00	98.00	0.88	3.22	−0.05
ILL 7305	52.00	87.00	0.78	2.00	0.08	ILL 7304	44.50	86.50	0.70	2.12	−0.06
ILL 7824	53.50	85.50	1.10	4.24	0.08	ILL 7306	43.50	92.00	1.10	2.10	0.31
ILL 6091	55.00	86.00	0.68	1.46	0.08	ILL 7308	44.50	83.50	0.74	1.91	0.02
ILL 4743	49.50	86.00	0.71	1.47	0.08	ILL 7309	46.50	84.00	0.92	2.00	0.17
ILL 7310	54.00	85.00	0.60	0.90	0.07	ILL 7312	44.00	84.50	0.68	2.06	−0.07

Table A5. *Cont.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
		Marchouch Station						**Tessaout Station**			
ILL 6088	51.50	85.00	0.80	2.45	0.05	ILL 7313	44.50	87.00	0.80	1.72	0.10
ILL 7831	53.00	86.00	0.82	2.62	0.05	ILL 7316	44.50	85.00	1.31	4.55	0.08
ILL 8016	53.00	85.00	0.62	1.30	0.04	ILL 7326	44.50	87.00	0.93	2.45	0.09
ILL 8012	54.00	85.00	0.75	2.24	0.04	ILL 7380	44.50	91.50	0.76	2.51	−0.07
ILL 6322	50.00	84.00	0.94	3.50	0.03	ILL 7383	46.50	87.00	1.04	3.31	0.05
ILL 7316	53.00	87.00	0.72	2.10	0.03	ILL 7798	45.50	91.50	1.05	2.81	0.14
ILL 6087	51.00	85.00	0.65	1.63	0.02	ILL 7799	43.00	88.00	1.43	4.00	0.27
ILL 8022	53.50	86.00	0.62	1.55	0.02	ILL 7801	45.00	85.50	0.43	0.86	−0.09
ILL 206	54.00	86.00	0.69	2.11	0.02	ILL 7806	44.50	90.50	0.90	0.93	0.33
ILL 6077	54.50	85.00	0.79	2.99	0.00	ILL 7807	45.00	86.50	1.20	4.86	−0.07
ILL 7826	58.00	90.00	0.56	1.59	−0.01	ILL 7818	43.50	87.00	1.08	2.95	0.14
ILL 6079	58.00	87.00	0.49	1.25	−0.02	ILL 7826	49.50	87.50	0.81	1.87	0.10
ILL 7796	54.00	88.00	0.44	0.77	−0.02	ILL 7827	43.50	88.50	0.93	2.73	0.05
ILL 8021	54.50	85.00	0.40	0.67	−0.04	ILL 7831	45.00	85.50	0.85	2.84	−0.04
ILL 8026	52.50	85.00	0.85	3.69	−0.04	ILL 8013	46.50	90.00	1.30	4.18	0.14
ILL 7303	58.50	104.50	0.92	4.40	−0.04	ILL 8014	48.00	93.50	0.96	1.90	0.23
ILL 4758	47.50	83.00	0.78	3.10	−0.05	ILL 8016	44.00	83.50	1.04	2.13	0.24
ILL 7795	55.00	86.00	0.37	0.60	−0.05	ILL 8020	45.00	82.00	0.95	2.16	0.17
ILL 221	55.00	88.00	0.65	2.52	−0.05	ILL 8024	44.50	85.00	1.06	2.83	0.15
ILL 7339	54.00	87.50	0.52	1.73	−0.06	ILL 8026	45.00	85.50	0.97	3.43	−0.04
ILL 8015	53.50	85.00	0.46	1.34	−0.06	ILL 8028	43.50	87.00	1.11	3.51	0.07
ILL 7836	52.00	84.00	0.70	2.95	−0.07	ILL 8056	47.50	95.00	0.94	3.01	0.02
ILL 7389	54.50	84.50	0.92	4.55	−0.07	ILL 8061	60.50	93.00	1.34	3.76	0.30
ILL 6074	53.00	86.00	0.40	1.00	−0.07			Heat sensitive			
ILL 7306	54.00	86.00	0.70	3.13	−0.08	ILL 247	43.00	88.50	0.58	2.01	−0.15
ILL 1734	54.50	94.50	0.56	2.23	−0.08	ILL 5505	49.50	85.00	0.64	2.46	−0.15
ILL 5918	51.00	83.00	0.38	0.95	−0.09	ILL 5957	47.00	80.00	0.28	0.58	−0.17
ILL 7827	53.00	85.00	0.66	2.96	−0.09	ILL 5964	43.50	85.00	0.32	0.94	−0.20
ILL 7325	51.00	81.00	0.38	1.10	−0.10	ILL 6057	44.00	88.50	0.13	1.01	−0.39
ILL 6059	65.00	98.00	0.32	1.35	−0.12	ILL 6058	42.50	86.50	0.16	1.05	−0.37

Table A5. *Cont.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
		Marchouch Station						**Tessaout Station**			
ILL 6099	66.50	95.00	0.17	0.39	−0.12	ILL 6060	46.00	87.00	0.50	2.23	−0.25
ILL 5940	54.00	87.00	0.37	1.30	−0.12	ILL 6086	45.00	87.00	0.59	2.97	−0.30
ILL 8437	52.00	102.50	0.76	3.93	−0.12	ILL 6087	45.00	83.50	0.55	2.76	−0.30
ILL 6058	65.00	98.00	0.23	0.80	−0.12	ILL 6099	46.00	89.00	0.42	1.58	−0.22
ILL 6319	50.00	82.50	0.80	4.23	−0.13	ILL 6104	44.00	87.50	0.43	1.92	−0.27
ILL 7818	54.50	86.00	0.49	2.31	−0.14	ILL 6105	45.50	85.00	0.84	3.41	−0.15
ILL 6096	55.00	84.00	0.35	1.38	−0.14	ILL 6320	43.00	85.50	0.75	3.26	−0.22
ILL 5957	48.00	81.00	0.51	2.24	−0.14	ILL 6332	51.00	92.00	0.37	2.31	−0.37
ILL 4605	49.34	82.65	0.77	4.07	−0.14	ILL 6338	47.00	91.00	0.59	1.96	−0.12
ILL 3484	51.50	85.00	0.57	2.82	−0.14	ILL 6360	44.50	87.50	0.71	3.37	−0.27
ILL 7289	56.00	89.00	0.33	1.40	−0.15	ILL 7238	46.00	96.50	0.96	4.91	−0.30
ILL 7817	55.00	85.00	0.21	0.59	−0.15	ILL 7264	45.00	87.00	0.84	3.56	−0.18
ILL 6100	52.00	84.00	0.50	2.45	−0.15	ILL 7286	44.00	88.00	0.69	3.16	−0.25
ILL 7304	53.50	85.00	0.37	1.70	−0.16	ILL 7310	43.00	86.50	0.40	2.49	−0.40
ILL 7327	53.00	85.00	0.45	2.25	−0.17	ILL 7311	50.00	89.50	0.40	2.00	−0.29
ILL 7307	54.00	85.00	0.41	2.07	−0.17	ILL 7314	44.00	85.00	0.47	2.03	−0.26
ILL 247	54.50	88.00	0.26	1.10	−0.17	ILL 7317	45.50	90.00	0.66	2.94	−0.23
ILL 7812	53.00	85.00	0.50	2.68	−0.17	ILL 7325	44.50	90.00	0.53	1.66	−0.13
ILL 8013	54.00	95.50	0.81	4.85	−0.17	ILL 7328	54.50	95.00	1.08	4.69	−0.12
ILL 6356	51.50	84.50	0.86	5.10	−0.17	ILL 7344	44.00	87.00	0.63	3.17	−0.31
ILL 6332	73.00	98.00	0.19	1.31	−0.17	ILL 7813	49.50	84.50	0.55	2.81	−0.29
ILL 5955	56.00	87.00	0.20	0.80	−0.18	ILL 7815	48.00	93.00	0.61	2.43	−0.18
ILL 7838	50.50	83.50	0.32	1.39	−0.18	ILL 7816	44.00	92.50	0.75	3.03	−0.17
ILL 8028	58.00	90.00	0.52	3.08	−0.18	ILL 7819	43.50	86.00	0.41	1.19	−0.16
ILL 7328	65.00	90.00	0.24	1.42	−0.18	ILL 7820	44.50	87.50	0.80	3.03	−0.12
ILL 6055	50.50	84.00	0.56	3.10	−0.18	ILL 7830	44.50	86.00	0.67	3.78	−0.37
ILL 6092	58.00	89.00	0.41	2.38	−0.18	ILL 7836	45.50	84.50	0.26	0.54	−0.19
ILL 6360	51.00	83.00	0.42	2.20	−0.18	ILL 7837	42.50	82.50	0.66	2.71	−0.20

Table A5. *Cont.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
		Marchouch Station						**Tessaout Station**			
ILL 8018	52.00	82.00	0.44	2.55	−0.20	ILL 8015	48.00	84.50	0.68	4.01	−0.39
ILL 7311	57.00	86.00	0.25	1.48	−0.21	ILL 8017	45.00	84.00	0.55	2.71	−0.30
ILL 6102	54.00	88.00	0.22	1.17	−0.21	ILL 8021	45.00	87.00	0.60	2.97	−0.30
ILL 7313	53.00	87.00	0.30	1.70	−0.21	ILL 8054	44.50	88.00	0.61	3.49	−0.38
ILL 8023	51.50	85.00	0.61	3.85	−0.22	ILL 8280	50.00	91.00	0.22	1.66	−0.40
ILL 8054	52.50	82.50	0.24	1.41	−0.22		Highly heat sensitive				
ILL 6385	70.00	89.00	0.21	1.94	−0.23	ILL 504	66.50	93.50	0.18	2.22	−0.47
ILL 6080	54.00	84.00	0.35	2.36	−0.24	ILL 729	63.50	98.50	0.66	4.83	−0.49
	Highly heat sensitive					ILL 880	45.50	86.50	0.23	3.11	−0.65
ILL 5919	51.50	83.00	0.52	3.61	−0.26	ILL 4758	42.00	89.00	0.31	2.91	−0.56
ILL 5929	55.00	88.00	0.18	1.60	−0.27	ILL 4910	43.50	95.00	0.29	3.36	−0.65
ILL 8014	61.00	88.50	0.30	2.70	−0.28	ILL 5940	47.00	93.00	0.42	3.56	−0.55
ILL 5964	55.00	85.00	0.27	2.28	−0.28	ILL 5943	46.00	88.50	0.31	2.96	−0.55
ILL 7837	54.50	86.50	0.61	4.58	−0.28	ILL 5955	43.50	85.50	0.19	1.81	−0.47
ILL 7317	55.50	89.00	0.17	1.71	−0.29	ILL 6054	44.50	86.50	0.22	2.48	−0.56
ILL 7266	56.50	89.00	0.23	2.20	−0.29	ILL 6072	46.00	88.00	0.33	3.68	−0.66
ILL 8020	54.00	86.00	0.40	3.27	−0.29	ILL 6081	43.50	86.50	0.23	1.87	−0.45
ILL 7808	52.50	84.00	0.27	2.30	−0.29	ILL 6089	45.00	88.50	0.27	2.40	−0.50
ILL 7832	51.00	84.00	0.38	3.05	−0.29	ILL 6091	45.50	85.50	0.55	4.09	−0.53
ILL 6105	57.00	89.00	0.26	2.50	−0.30	ILL 6100	44.50	96.50	0.28	3.20	−0.62
ILL 7314	54.00	86.00	0.29	2.62	−0.30	ILL 6319	45.00	90.50	0.32	2.56	−0.48
ILL 6101	55.00	84.00	0.21	2.25	−0.31	ILL 6322	44.00	91.00	0.26	2.41	−0.51
ILL 7326	54.00	86.00	0.46	3.95	−0.32	ILL 6362	43.50	90.50	0.55	3.85	−0.50
ILL 7813	50.00	84.00	0.55	4.45	−0.32	ILL 7232	43.50	89.50	0.53	3.43	−0.44
ILL 7800	52.50	87.00	0.41	3.75	−0.34	ILL 7266	44.50	87.50	0.36	3.41	−0.59
ILL 7232	57.00	88.00	0.25	2.85	−0.34	ILL 7289	44.50	83.00	0.20	3.48	−0.75
ILL 7797	55.00	87.00	0.31	3.20	−0.34	ILL 7300	43.50	87.00	0.49	3.61	−0.51
ILL 7815	51.00	84.00	0.47	4.16	−0.35	ILL 7305	44.50	85.00	0.13	2.10	−0.57
ILL 7801	55.00	83.00	0.21	2.60	−0.35	ILL 7307	45.00	87.00	0.32	2.51	−0.47
ILL 7820	54.50	84.00	0.29	3.16	−0.35	ILL 7339	44.00	86.50	0.35	4.07	−0.71

Table A5. *Cont.*

Accession	DF	DM	Ys	Yp	HTI	Accession	DF	DM	Ys	Yp	HTI
		Marchouch Station						**Tessaout Station**			
ILL 6346	54.00	86.00	0.37	3.65	−0.35	ILL 7797	47.50	92.00	0.24	3.52	−0.71
ILL 7383	55.00	83.50	0.46	4.30	−0.35	ILL 7800	44.50	84.50	0.26	2.41	−0.51
ILL 4910	55.00	86.00	0.21	2.70	−0.36	ILL 7804	43.00	83.50	0.53	3.37	−0.44
ILL 6089	55.50	86.00	0.22	2.80	−0.36	ILL 7812	43.50	85.00	0.53	3.54	−0.46
ILL 8024	53.00	88.00	0.23	2.83	−0.37	ILL 7824	45.50	85.00	0.74	4.74	−0.47
ILL 6060	53.00	88.00	0.20	2.69	−0.37	ILL 7829	44.00	84.00	0.43	3.61	−0.57
ILL 6072	54.00	88.00	0.48	4.67	−0.38	ILL 7833	45.00	85.00	0.23	3.28	−0.68
ILL 7814	54.00	85.00	0.20	2.78	−0.38	ILL 7838	44.00	83.00	0.43	2.95	−0.45
ILL 6107	53.00	87.00	0.50	4.80	−0.38	ILL 8012	43.00	84.50	0.41	2.84	−0.45
ILL 8280	55.00	85.00	0.20	2.85	−0.38	ILL 8022	47.50	93.00	0.46	4.21	−0.62
ILL 2524	54.00	83.00	0.21	2.97	−0.39						
ILL 5943	53.00	85.00	0.19	3.09	−0.42						
ILL 7804	58.00	88.00	0.20	3.35	−0.42						
ILL 7829	55.00	84.00	0.28	3.80	−0.42						
ILL 4772	53.00	84.00	0.24	3.54	−0.43						
ILL 6081	54.00	86.00	0.30	4.17	−0.45						
ILL 7799	55.00	82.00	0.22	3.80	−0.46						
ILL 7816	61.50	89.50	0.21	4.95	−0.56						
ILL 6054	52.00	86.00	0.25	4.85	−0.56						
ILL 5505	58.50	109.00	0.17	5.08	−0.61						
ILL 6320	54.00	88.50	0.17	7.20	−0.83						

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High-Temperature and Drought Stress Effects on Growth, Yield and Nutritional Quality with Transpiration Response to Vapor Pressure Deficit in Lentil

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Abstract: High temperature and water deficit are among the major limitations reducing lentil (*Lens culinaris* Medik.) yield in many growing regions. In addition, increasing atmospheric vapor pressure deficit (VPD) due to global warming causes a severe challenge by influencing the water balance of the plants, thus also affecting growth and yield. In the present study, we evaluated 20 lentil genotypes under field conditions and controlled environments with the following objectives: (i) to investigate the impact of temperature stress and combined temperature-drought stress on traits related to phenology, grain yield, nutritional quality, and canopy temperature under field conditions, and (ii) to examine the genotypic variability for limited transpiration (TR_{lim}) trait in response to increased VPD under controlled conditions. The field experiment results revealed that high-temperature stress significantly affected all parameters compared to normal conditions. The protein content ranged from 23.4 to 31.9%, while the range of grain zinc and iron content varied from 33.1 to 64.4 and 62.3 to 99.3 mg kg^{-1}, respectively, under normal conditions. The grain protein content, zinc and iron decreased significantly by 15, 14 and 15% under high-temperature stress, respectively. However, the impact was more severe under combined temperature-drought stress with a reduction of 53% in protein content, 18% in zinc and 20% in iron. Grain yield declined significantly by 43% in temperature stress and by 49% in the combined temperature-drought stress. The results from the controlled conditions showed a wide variation in TR among studied lentil genotypes. Nine genotypes displayed TR_{lim} at 2.76 to 3.51 kPa, with the genotypes ILL 7833 and ILL 7835 exhibiting the lowest breakpoint. Genotypes with low breakpoints had the ability to conserve water, allowing it to be used at later stages for increased yield. Our results identified promising genotypes including ILL 7835, ILL 7814 and ILL 4605 (Bakria) that could be of great interest in breeding for high yields, protein and micronutrient contents under high-temperature and drought stress. In addition, it was found that the TR_{lim} trait has the potential to select for increased lentil yields under field water-deficit environments.

Keywords: lentil; drought stress; temperature stress; grain yield; limited transpiration trait; vapor pressure deficit; crude protein; biofortification; canopy temperature; zinc and iron

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1. Introduction

Lentil (*Lens culinaris* Medik.) is an important cool-season food legume that plays a significant role in human and animal nutrition as well as in maintaining and improving soil fertility [1,2]. Lentil grains are highly digestible and nutritious, representing a true staple crop in the dryland of many developing countries with affordable levels of dietary proteins (22–35%), vitamins, fiber, carbohydrates and essential micronutrients such as zinc and iron [3]. As lentil is highly nutritious and can be cooked more quickly than other pulses, it is the pulse most preferred by poor rural households worldwide, particularly those living in developing nations such as India, Bangladesh, Pakistan and Ethiopia [4]. However, micronutrient deficiencies are currently becoming more serious due to the high consumption of carbohydrate-rich cereal-based diets, which are low in micronutrients and vitamins [5,6]. Micronutrient malnutrition, commonly known as hidden hunger, affects more than 2 billion people in developing countries, while all over the world, >3 billion people suffer from micronutrient deficiencies [7–11]. In the last decade, efforts have been made to reduce the risks of malnutrition using several approaches including nutrient supplementation, diet diversification and food fortification. However, genetic biofortification using conventional and transgenic breeding methods has been recognized as the most effective and sustainable method for developing new crop varieties to combat hidden hunger and provide essential micronutrients and vitamins to the poor population through daily diet [6,12,13]. Highly nutritional genotypes have been developed through genetic biofortification in lentil [14], chickpea [15] and wheat [16], helping to minimize micronutrient deficiencies substantially in recent decades. However, the increase in the world population to around 10 billion by 2050 will further escalate malnutrition [17,18], and more efforts will be required to meet the human population's nutritional needs for a healthy life. Enhancing the production of lentil would support food and nutritional security and improve the livelihoods of resource-poor farmers in developing nations. Still, lentil productivity in rainfed regions is expected to suffer from fluctuations in climate change, mainly due to the increased incidence of drought and higher temperatures [19].

High-temperature and drought stress have been reported as the major environmental factors that can markedly affect plant productivity and the quality of many cultivated crops [20]; however, their impact on cool-season food legumes appears to be more serious [21,22]. A temperature higher than 30 °C causes stress in most cool-season food legume crops. Both high-temperature and drought stress hamper plant growth by disturbing the normal physiology and morphology, thereby influencing an array of processes including growth, floral development, carbohydrates, protein content in grains, and micronutrient concentration (zinc and iron), which ultimately affect grain yield and nutritional quality [23,24]. Furthermore, the combined effect of high-temperature and drought stress on crops could be more severe than the individual stress impact. The reproductive stages of crops are more vulnerable to drought, high temperature and combined stress than the vegetative stages [14,25]. During anthesis and flowering periods, both stresses led to fertilization failures because of reduced pollen and ovule function and inhibited pollen development and sterility in legumes [26,27] and cereals [28,29]. While considering the current change in climate, it is expected that both the severity of high temperatures and the risk of drought will increase mainly in the subtropical region, impacting food security [30].

The impacts of high temperatures [31,32], water stress [33,34] and combined temperature-drought stress on lentil growth, yield and composition have been documented [20,25,35,36]. However, more information is needed on the physiological responses of lentil and their mechanisms to develop climate-resilient management strategies and new varieties. Since lentil is cultivated mainly in environments with high vapor pressure deficit (VPD) conditions (hot and dry areas) during the post-rainy season, where light rainfall is often noted, the study of the limited transpiration (TR_{lim}) trait could be crucial in water-deficit conditions [37]. VPD is defined as the difference between the amount of moisture in the air and the moisture the air can hold when saturated. Temperature and relative humidity are the two major factors controlling VPD variation [38]. The high VPD, which usually

occurs due to exacerbation of plant water stress in the middle of the day, affects plant photosynthesis, growth, yield and physiology [39]. The intensification of atmospheric water demand highly influences plant hydraulic status, and the key behind these impacts is plant stomatal behavior [40,41]. Regulation of stomatal conductance in high VPD conditions by limiting transpiration restricts the rate of water use and increases transpiration efficiency, helping to conserve water and support plant growth later in the season when drought develops [42,43]. TR_{lim} in response to high VPD has been reported in several crop species including wheat (*Triticum aestivum* L.) [41,44], maize (*Zea mays* L.) [45,46], sorghum (*sorghum bicolor* L.) [47,48], soybean (*Glycine max* L.) [49,50], peanut (*Arachis hypogaea* L.) [51,52], cowpea (*Vigna unguiculata* L.) [53,54], chickpea (*Cicer arietinum* L.) [55,56] and lentil [57]. Based on the available literature, the study by Guiguitant et al. [57] is the only investigation that examined lentil genotype differences in transpiration response to high VPD conditions. In the study by Guiguitant et al. [57], 17 lentil genotypes were tested for the expression of TR_{lim} and almost all tested lines showed a VPD breakpoint at approximately 3.4 kPa. In addition, Guiguitant et al. [57] reported a possible yield benefit of developing genotypes expressing the TR_{lim} trait, especially in regions with low rainfall, and that the impact of this trait on lentil productivity differs with geography and environments. Furthermore, several studies have shown increases in crop yield by 25 to 75% in water-limited areas based on crop simulation models [45,58–60]. Thus, developing and/or identifying drought- and heat-tolerant varieties is crucial in improving lentil yield under water-limited and high-temperature environments, which would directly contribute to food and nutritional security in the near future.

Based on the results from preliminary field screening [35], 20 lentil genotypes were selected as primary candidates for temperature and drought stress studies. Two independent experiments were conducted (i) to investigate the impact of high temperature and drought stress on traits associated with phenology, grain yield, nutritional quality and canopy temperature under field conditions, and (ii) to examine the possible genotypic variation in lentil for transpiration response to high VPD conditions over controlled environments. In the second experiment, transpiration was measured for plants subjected to different levels of VPD ranging from 1.20 to 4.50 kPa. The generated information may help to inform selection strategies and decisions within breeding programs and identify options to mitigate the impacts of high temperatures and water deficit on lentil productivity.

2. Results

2.1. Weather Data

Accumulated rainfall for normal planting was about 225 mm between December and April, while the late planting received around 26 mm mostly before the anthesis period. Under normal conditions, the minimum and maximum temperatures differed from −2.40 to 11.7 °C and 9.18 to 30.6 °C, respectively, during the vegetative stage, while they varied from 0.35 to 8.58 °C and 13.3 to 30.2 °C during the reproductive stage, respectively. Under the late planting, minimum and maximum temperatures were from 0.35 to 15.3 °C and from 13.3 to 37.5 °C respectively, before the flowering stage, and from 5.61 to 14.9 °C and from 22.6 to 36.9 °C, respectively, during the reproductive stage. Under normal planting, the RH ranged from 52 to 98% in the vegetative stage and 41 to 91% in the reproductive stage. It varied in late planting from 32 to 96% in the vegetative stage and from 42 to 79% during the reproductive stage. The maximum VPD registered in normal planting was 2.11 kPa and 3.25 kPa in late planting (Figure 1).

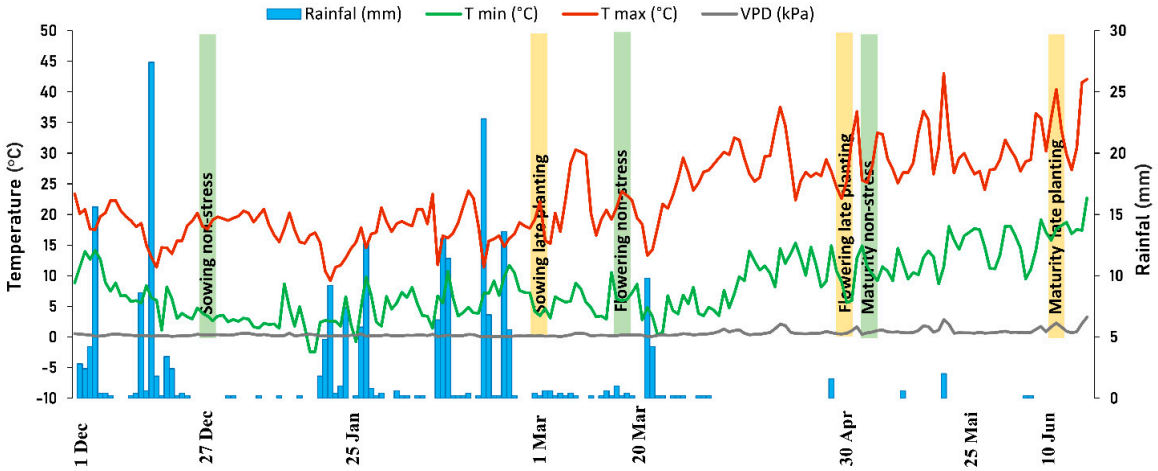

Figure 1. Rainfall, maximum and minimum temperatures, and vapor pressure deficit (VPD) during normal and late planting experiments in Marchouch, Morocco during 2016/17 season.

2.2. Combined Analysis of Variance

The combined analysis of variance indicated a significant difference among studied genotypes for all traits (Table S1). ANOVA showed a highly significant ($p < 0.001$) effect of temperature stress and combined temperature-drought stress on all measured characteristics. However, genotype × treatment interaction was significant for all traits except NUPP and HSW. Furthermore, the coefficient of variation (CV) among treatments was less than 13% for most traits, but 35% for both GYP and NFPP, with 25 and 49% for NTPP and NUPP, respectively.

2.3. Temperature and Combined Heat-Drought Impact on Plant Phenology

PLH declined significantly, by 19.2%, in temperature stress and by 29.6% in the combined temperature-drought stress compared to its level in normal conditions. EGV ranged from 17.0 to 30.0 cm with an overall mean of 23.1 cm under normal conditions, whereas high-temperature and combined temperature-drought stress reduced EGV by 26.2 and 22.9%, respectively. DFF varied between 82 and 89 days in normal conditions, and a reduction of 28 days was observed for both stressed treatments. Likewise, days to 50% flowering (D50F) declined significantly, by 27 days, under both temperature and combined temperature-drought stress conditions (Table 1).

Temperature stress alone and in combination with drought severely reduced the D50P by 26 and 27 days, respectively. Under normal conditions, plants achieved physiological maturity in about 127 days, while days to maturity decreased significantly by 19 and 20 days under temperature stress and combined temperature-drought stress, respectively. NFPP varied from 40.8 to 234.2 in normal conditions and was significantly reduced by 44 to 69% in temperature stress and by 48 to 71% in combined temperature-drought stress, respectively. Similarly, NUPP decreased substantially by 21–62% and 3–43% in temperature stress and combined temperature-drought stress, respectively. NTPP ranged from 56 to 240 pods in normal conditions with an average of 122.8, while it decreased significantly by 44–72% under temperature stress and 46–75% under combined temperature-drought stress conditions. GYP ranged from 1.59 to 8.86 g with an overall mean of 4.13 g under normal conditions. The late planting treatments showed a significant reduction in grain yield, by 43% under temperature stress and 49% under the combined stress. HSW varied from 1.93 and 4.97 g in normal conditions, with a mean of 2.64 g, whereas it was decreased significantly by 8% and 19% under the temperature stress and combined temperature-drought stress, respectively (Table 1).

Table 1. Minimum, maximum and mean morphological parameters of 20 lentil genotypes under normal, temperature stress and temperature-drought stress conditions.

Trait	Normal			Temperature Stress			Temperature-Drought Stress		
	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE
PLH	24.75	34.21	28.28 ^{a} ± 3.39	19.75	27.25	22.86 ^{b} ± 0.30	14.33	25.03	19.74 ^{c} ± 0.37
EGV	17.00	30.00	23.12 ^{a} ± 0.56	13.00	21.00	17.07 ^{b} ± 0.30	12.00	21.00	17.82 ^{c} ± 0.35
DFF	82.00	89.00	84.95 ^{a} ± 0.22	60.00	66.00	61.55 ^{b} ± 0.19	590.00	64.00	61.15 ^{b} ± 0.23
D50F	86.00	100.00	93.08 ^{a} ± 0.45	65.00	74.00	68.53 ^{b} ± 0.31	630.00	74.00	68.32 ^{b} ± 0.39
D50P	98.00	106.00	101.78 ^{a} ± 0.36	71.00	80.00	76.20 ^{b} ± 0.44	700.00	80.00	74.77 ^{c} ± 0.41
DM	124.00	131.00	127.20 ^{a} ± 0.32	101.00	105.00	103.53 ^{b} ± 0.17	100.00	105.00	101.70 ^{c} ± 0.17
NFPP	40.83	234.25	111.45 ^{a} ± 7.43	12.75	129.40	57.75 ^{b} ± 4.66	12.00	119.50	52.07 ^{b} ± 4.14
NUPP	3.50	25.25	11.34 ^{a} ± 0.88	1.33	20.00	6.15 ^{b} ± 0.51	2.00	24.50	7.89 ^{b} ± 0.79
NTPP	56.00	240.00	122.79 ^{a} ± 7.23	15.75	134.00	63.90 ^{b} ± 4.71	14.00	130.00	59.96 ^{b} ± 4.17
GYP	1.59	8.86	4.13 ^{a} ± 0.28	0.43	4.56	2.35 ^{b} ± 0.17	0.66	4.32	2.12 ^{b} ± 0.14
HSW	1.93	4.97	2.64 ^{a} ± 0.09	1.59	5.23	2.42 ^{b} ± 0.11	1.42	5.51	2.15 ^{c} ± 0.13
CP	23.40	31.90	28.91 ^{a} ± 0.35	22.00	27.00	24.36 ^{b} ± 0.20	11.00	14.90	13.34 ^{c} ± 0.16
Zn	33.10	64.40	47.86 ^{a} ± 1.52	27.40	59.10	41.12 ^{b} ± 1.41	30.40	50.90	39.28 ^{c} ± 0.80
Fe	62.30	99.30	78.19 ^{a} ± 1.44	51.30	81.60	66.71 ^{b} ± 1.42	52.40	73.50	62.86 ^{c} ± 0.81

Means for each variable followed by the same letters are not significantly different ($p < 0.05$). PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods plant^{-1}; NUPP, number of unfilled pods plant^{-1}; NTPP, number of total pods plant^{-1}; GYP, grain yield plant^{-1}; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content and Fe, iron content.

2.4. Influence of High-Temperature and Combined Temperature-Drought Stress on Fe, Zn and Protein Contents

Crude protein (CP) varied from 23.4 to 31.9% under normal conditions, with a mean of 28.9%. CP decreased significantly to 15% due to temperature stress and 53% under combined temperature-drought stress. Under normal conditions, the mean Zn concentration was 47.9 mg kg^{-1}, ranging from 33.1 to 64.4 mg kg^{-1}. High-temperature stress and combined temperature-drought stress significantly reduced Zn concentration by 14 and 18%, respectively. Similar results were observed for Fe with a reduction of 15% due to temperature stress and 20% under combined temperature-drought stress. Fe concentration varied from 62.3 to 99.3 mg kg^{-1} under normal conditions (Table 1).

2.5. Correlation Coefficient between Measured Traits

A significant positive correlation was observed between PLH and EGV (r = 0.38) under normal conditions. However, a highly significant negative correlation ($p < 0.01$) was observed among EGV, DFF and D50P. HSW had a negative correlation with DFF, D50F and D50P, and it had a positive correlation with EGV (r = 0.62, $p < 0.01$). GYP was positively correlated with NFPP and NTPP (r = 0.97 and r = 0.96 at $p < 0.01$, respectively). For quality traits, there were no correlations between CP and phenological and yield parameters under normal conditions. Positive correlations were observed between Fe and Zn (r = 0.68), D50P (r = 0.52) and DM (r = 0.38, $p < 0.05$), and negative correlations with EGV (r = −0.61, $p < 0.01$), HSW (r = −0.39, $p < 0.05$) and CP (r = −0.35, $p < 0.05$). In addition, a highly negative correlation was observed between Zn and CP (r = −0.47, $p < 0.01$) in normal conditions (Table S2).

A significant negative correlation was found between EGV and DFF, D50F, and D50P under both temperature stress and combined temperature-drought stress (Table S3). Similarly to normal conditions, GYP had a significant positive correlation with NTPP and NFPP (r = 0.90) under the two stressed conditions. Under temperature stress, Fe content showed a positive correlation with D50F (r = 0.37, $p < 0.05$), GYP (r = 0.34, $p < 0.05$) and Zn (r = 0.46, $p < 0.01$). A significant and positive correlation between Zn and Fe (r = 48, $p < 0.01$) was also observed in combined temperature-drought stress conditions. In addition, Zn was found to be positively correlated with HSW (r = 0.34, $p < 0.05$) and negatively correlated with CP under temperature stress (r = −0.36, $p < 0.05$) and combined temperature-drought

stress (r = −0.45, $p < 0.01$). Likewise, a positive correlation was observed between CP and DM (r = 0.36, $p < 0.05$) under temperature stress.

2.6. Principal Component Analysis for Stress and Normal Conditions

Principal component analysis (PCA) was performed to study the relationships between traits in the studied lentil genotypes. Under normal conditions, the first three PCAs (PC1: 31.3%, PC2: 22.6% and PC3: 16.5%) explained 70.4% of the total variability (Table 2). The results of PCA showed that EGV, D50P, HSW and Fe content were the most important traits contributing to PC1 of distinct origin. In PC2, which described 22.6% of the total variance, NFPP, GYP and D50F demonstrated large contributions, while Zn content and CP accounted for much of the total variance in PC3 (Table S4). These 20 genotypes were clustered into three groups based on hierarchical cluster analysis (Figure S1A). The check Bakria (ILL 4605) and three genotypes (ILL 5919, ILL 7813 and ILL 3484) were identified in group 1 and had the highest GYP (5.55 g) and HSW (3.27 g), and moderate Zn, Fe and CP. In group 2, ten genotypes were classified and recorded the highest Fe (85.1 mg kg^{-1}) and Zn (54.1 mg kg^{-1}), with medium GYP and HSW. Six genotypes were grouped in cluster 3 and exhibited the highest CP (29.9%), medium Fe (72.4 mg kg^{-1}) and HSW (2.5 g), and the lowest GYP (Table 3). The distribution of genotypes and measured traits along the first PC axes under normal conditions are shown in Figure 2.

Table 2. Eigenvalues and eigenvectors of the three PCA dimensions under normal conditions, temperature stress and the combined temperature-drought stress.

Traits	Normal			Temperature Stress			Temperature-Drought Stress		
	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
PLH	−0.52 *	0.21 ns	−0.07 ns	−0.31 ns	−0.02 ns	0.01 ns	−0.27 ns	−0.13 ns	0.19 ns
EGV	−0.78 **	−0.47 *	0.05 ns	−0.49 *	−0.42 ns	0.38 ns	−0.64 **	−0.05 ns	0.14 ns
DFF	0.50 *	0.40 ns	−0.50 *	0.86 **	0.30 ns	0.02 ns	0.64 **	−0.44 ns	0.18 ns
D50F	0.38 ns	0.62 **	−0.58 **	0.86 **	0.44 ns	0.01 ns	0.74 **	−0.44 *	0.44 ns
D50P	0.88 **	0.13 ns	0.06 ns	0.79 **	0.42 ns	−0.21 ns	0.77 **	−0.33 ns	0.30 ns
DM	0.46 *	0.16 ns	0.45 *	0.22 ns	0.41 ns	−0.70 **	0.89 **	−0.11 ns	0.19 ns
NFPP	−0.50 *	0.83 **	0.14 ns	−0.59 **	0.76 **	0.23 ns	0.55 *	0.81 **	0.16 ns
NTPP	−0.44 *	0.84 **	0.13 ns	−0.57 **	0.76 **	0.23 ns	0.59 **	0.78 **	0.09 ns
NUPP	0.48 *	−0.29 **	−0.08 ns	0.05 ns	0.20 ns	0.04 ns	0.22 ns	−0.20 ns	−0.13 ns
GYP	−0.49 *	0.81 **	0.11 ns	−0.44 ns	0.79 **	0.33 ns	0.49 *	0.76 **	0.19 ns
HSW	−0.71 **	−0.30 ns	0.42 ns	0.60 **	−0.09 ns	0.42 ns	0.13 ns	−0.50 *	0.35 ns
CP	−0.14 ns	−0.07 ns	−0.65 **	−0.34 ns	0.32 ns	−0.60 **	0.52 *	−0.30 ns	−0.39 ns
Zn	0.35 ns	0.18 ns	0.77 **	0.49 *	−0.08 ns	0.61 **	−0.61 **	0.14 ns	0.35 ns
Fe	0.74 *	0.24 ns	0.51 **	0.36 ns	0.47 *	0.44 ns	−0.64 **	0.05 ns	0.55 *
Eigenvalue	4.38	3.17	2.31	4.08	2.92	1.98	4.83	2.70	1.73
Variance explained (%)	31.29	22.65	16.49	34.00	24.32	16.52	34.51	19.30	12.42
Total variance (%)	31.29	53.29	70.43	34.00	58.32	74.85	34.51	53.81	66.23

** 0.01, * 0.05, ns not significant. PLH, plant height; EGV, early growth vigor; DFF, days to first flowering; D50F, days to 50% flowering; D50P, days to 50% podding, DM, days to 95% maturity; NFPP, number of filled pods $plant^{-1}$; NUPP, number of unfilled pods $plant^{-1}$; NTPP, number of total pods $plant^{-1}$; GYP, grain yield per plant; HSW, hundred-seed weight; CP, crude protein; Zn, zinc content; Fe, iron content.

For temperature stress, PC1 and PC2 (PC1: 34.0% and PC2: 24.3%) explained 58.3% of the total variation, while PC3 showed 16.5% of the total variation. PC1 was positively correlated with DFF, D50F, D50P, HSW and Zn, whereas PC2 was positively correlated with GYP, NTPP, NFPP and Fe content. Furthermore, negative correlations were observed between PC1 and EGV, NTPP and NFPP. PC3 had a significant positive correlation with Zn content and negative association with CP and DM (Table 2). Hierarchical cluster analysis was conducted, and the genotypes were grouped into three clusters (Figure S1B). Cluster 1 grouped only the check Bakria (ILL 4605), a moderate heat- and drought-tolerant variety, which had the highest HSW (4.95 g) and Zn and Fe contents with a mean of 56.6 and 73.8 mg kg^{-1}, respectively. Five heat-tolerant genotypes (ILL 7814, ILL 6104, ILL 7835, ILL 7833 and ILL 6338) and one moderately heat- and drought-tolerant genotype (ILL 6363) were identified in cluster 2. This group had the highest GYP with a mean of

3.30 g and CP with an average of 24.7%, while Zn and Fe concentrations were 42.5 and 69.7 mg kg^{-1}, respectively. Thirteen genotypes including two heat-tolerant lines (ILL 8029 and ILL 7286), three combined temperature-drought tolerant genotypes (ILL 6362, ILL 7804 and ILL 6075), five moderately tolerant lines and three susceptible lines were found in cluster 3 (Figure S1B). This group had moderate GYP with an average of 1.97 g, HSW with 2.35 g and CP with a mean of 24.4%, whereas Zn and Fe concentrations were 39.3 and 64.8 mg kg^{-1}, respectively (Table 3). The biplot of PC1 and PC2 of the three clusters and traits under temperature stress conditions is shown in Figure 3.

Table 3. Grain yield, hundred-seed weight, crude protein, zinc content and iron content of the three clusters for normal, temperature stress and combined temperature-drought stress.

Cluster	Experiment	GYP	HSW	CP	Zn	Fe
Cluster I	Normal	5.55	3.27	29.78	44.12	69.50
	High-temperature	1.65	4.95	22.52	56.63	73.85
	Temperature-drought	1.84	2.00	12.92	41.26	65.02
Cluster II	Normal	4.06	2.44	27.94	54.13	85.15
	High-temperature	3.30	2.17	24.66	42.49	69.73
	Temperature-drought	1.28	3.56	14.25	33.85	58.65
Cluster III	Normal	3.30	2.54	29.94	39.94	72.39
	High-temperature	1.97	2.35	24.37	39.30	64.77
	Temperature-drought	2.97	1.96	13.87	37.12	59.99

GYP; grain yield, HSW; 100-seed weight, CP; crude protein, Zn; zinc content and Fe; iron content.

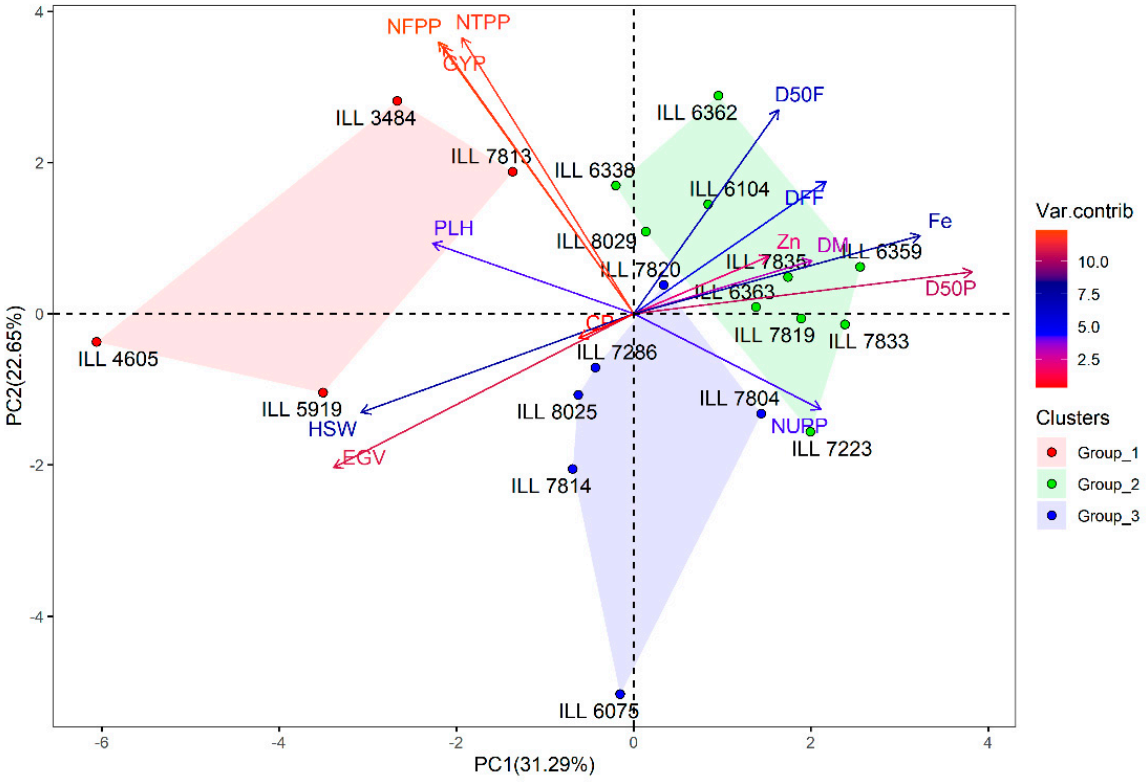

Figure 2. Biplot of the first two axes of the PCA analysis summarizing the relationship between the measured variables and the classification of lentil genotypes under normal conditions.

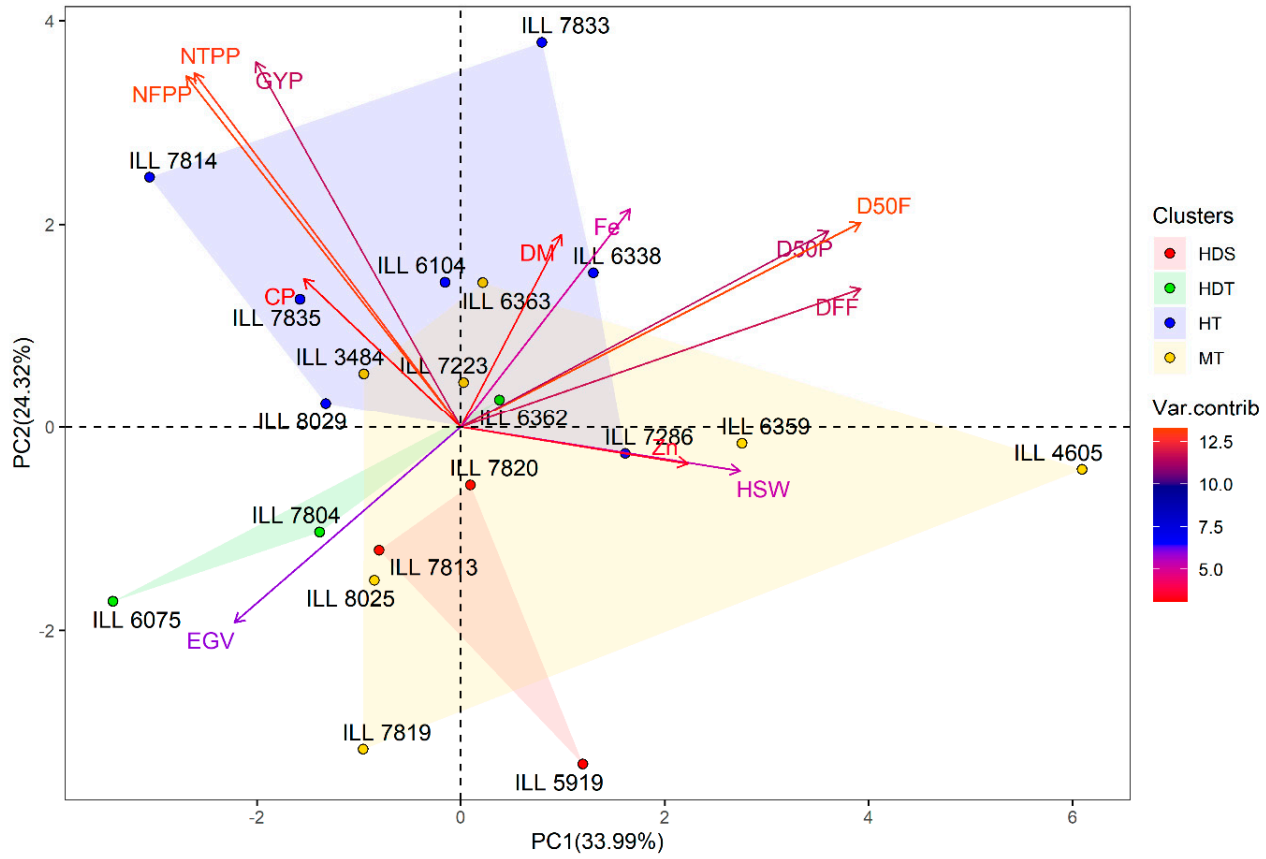

Figure 3. Biplot of the first two axes of the PCA analysis summarizing the relationship between the measured variables and the classification of lentil genotypes under temperature stress conditions. HDS, heat stress and combined heat-drought susceptible; HDT, heat-drought tolerant; HT; heat tolerant and MT, moderately tolerant to heat stress and combined heat-drought stress.

The first three PCAs in the combined temperature-drought stress explained 66.2% of the entire dissimilarity (PC1:34.5%, PC2:19.3% and PC3:12.4%). PC1 showed a significant positive correlation with D50F, D50P, DM, GYP, NTPP, NFPP and CP, and a negative correlation with Zn, Fe and EGV. PC2 was positively correlated with GYP, NTPP and NFPP, and negatively correlated with D50F and HSW (Table 2). Based on hierarchical cluster analysis (Figure S1C), 11 genotypes including two heat-tolerant lines (ILL 6104 and ILL 8029), four heat-drought tolerant lines (ILL 6075, ILL 7804, ILL 7835 and ILL 7814), three moderately tolerant lines (ILL 7223, ILL 7819 and ILL 8025) and three susceptible genotypes (ILL 5919, ILL 7813 and ILL 7820) were found in group 1. This group was characterized by the highest Zn and Fe concentrations with an average of 41.3 and 65.0 mg kg^{-1}, respectively, and medium GYP (1.84 g). Group 2 comprised two moderately tolerant genotypes (ILL 4605 and ILL 6359), and had the highest CP and HSW, with a mean of 14.2% and 3.56 g, respectively. Three heat-tolerant lines (ILL 7833, ILL 7286 and ILL 6338), two moderately tolerant (ILL 6363 and ILL 3484) and one heat-drought tolerant genotype (ILL 6362) were grouped in cluster 3. This group had the highest GYP with an average of 2.97 g, and moderate concentrations of Zn and Fe with a mean of 37.1 and 59.9 mg kg^{-1}, respectively (Table 3). The biplot of PC1 and PC2 of the three clusters and measured traits under the combined temperature-drought stress is illustrated in Figure 4.

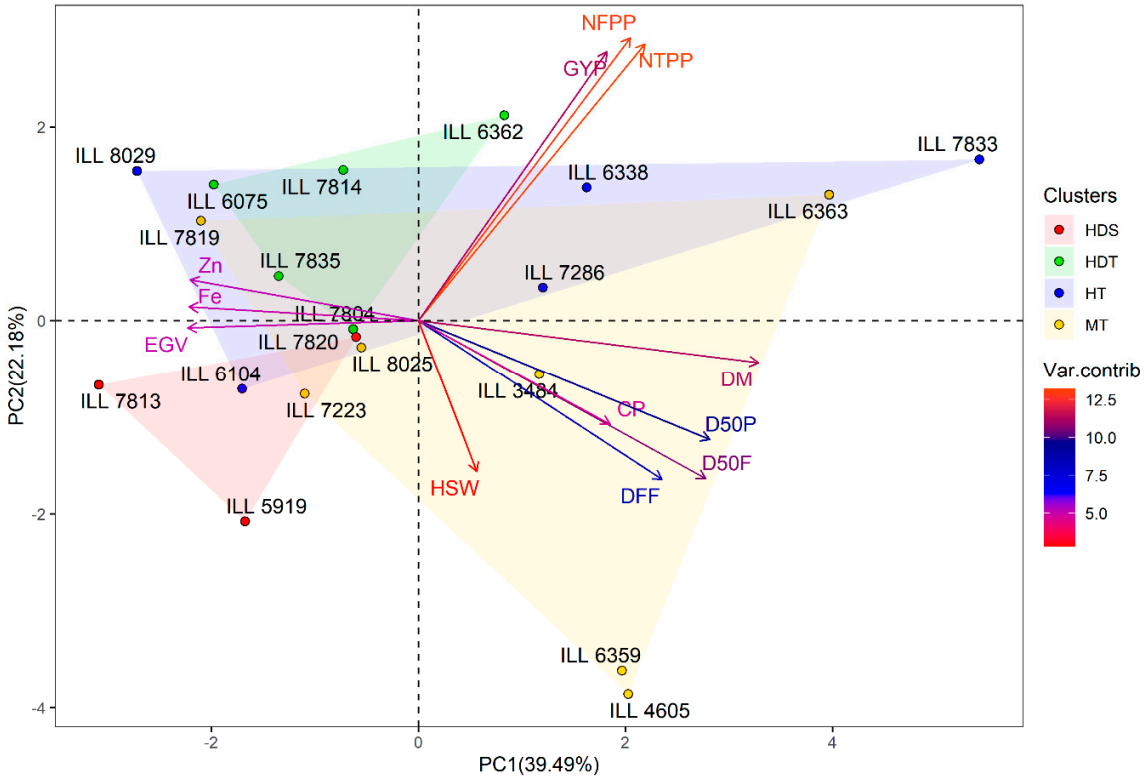

Figure 4. Biplot of the first two axes of the PCA analysis summarizing the relationship between the measured variables and the classification of lentil genotypes under the combined temperature-drought stress conditions. HDS, heat-drought susceptible; HDT, heat-drought tolerant; HT; heat tolerant and MT, moderately tolerant to heat-drought stress.

2.7. Canopy Temperature Variation under Normal and Stress Conditions

Analysis of variance for canopy temperature (CT) showed a significant difference between genotypes and treatments; however, there were no significant differences for genotype x treatment interaction (Table S5). Under normal conditions, the CT of plants increased significantly from an average of 29.1 °C at day 100 to 31.8 °C at day 115 after sowing. Under normal conditions, the minimum and maximum CT were 26.6–32.7 °C at day 100 and 29.7–33.4 °C at day 115 (Table S6). Under temperature stress, the plants recorded an average of 29.2 °C and 31.7 °C at day 65 and day 70, respectively. The average CT increased significantly from 31.5 °C at day 80 to 31.9 °C at day 90. The lowest maximum CT was recorded at day 65 with 31.3 °C, while the highest was achieved at day 90 with 33.8 °C. Under combined temperature-drought stress, the CT increased significantly from an average of 29.5 °C at day 65 to 31.9 at day 70. On day 80 and day 90, the CT was 31.6 and 31.7 °C, respectively. Plants at day 65 recorded the lowest maximum CT (31.3°C), while, the highest was recorded at day 90 with 34.0 °C.

2.8. Transpiration Response to VPD under Controlled Environments

The results revealed that nine lentil genotypes were well represented by the two-segment regression with a breakpoint (BP) (Table 4). However, 11 genotypes exhibited a linear response and failed to express the limited transpiration (TR_{lim}) trait during the increase in VPD (Table 5). The R^2 of the genotypes with BP ranged from 0.60 to 0.87 and varied from 0.66 to 0.95 for the genotypes without TR_{lim}. The results displayed high variation between the assessed genotypes in TR to increasing VPD. The value of BP ± SE of the genotypes expressing TR_{lim} varied from 2.76 ± 0.43 to 3.51 ± 0.54 kPa with an average of 3.14 ± 0.66 (Table 4).

Table 4. Outputs from the analysis of the two-segment linear regression used for fitting the TR to the VPD variations.

Genotype	Breakpoint		Left Slope		Right Slope		R^2
	BP ± SE	CI (95%)	S1	CI (95%)	S2	CI (95%)	
ILL 3484	3.27 ± 0.95	3.01 to 4.50	44.48	32.68 to 59.19	24.95	3.45 to 39.94	0.87
ILL 6075	3.15 ± 1.14	2.73 to 4.51	28.91	21.74 to 45.84	17.37	0.25 to 28.46	0.82
ILL 6104	3.00 ± 0.25	3.00 to 3.45	36.40	25.72 to 47.06	12.26	−2.57 to 26.43	0.83
ILL 6338	3.37 ± 0.61	3.00 to 3.45	43.73	31.41 to 60.63	15.42	−9.64 to 38.61	0.81
ILL 6362	3.01 ± 0.99	2.76 to 3.46	44.71	34.40 to 71.39	28.14	2.88 to 41.59	0.87
ILL 7814	3.36 ± 0.56	2.79 to 4.17	34.42	25.90 to 77.81	5.59	−26.00 to 28.45	0.85
ILL 7833	2.81 ± 0.47	1.67 to 3.57	18.14	9.59 to 38.87	−1.63	−13.49 to 8.32	0.60
ILL 7835	2.76 ± 0.43	2.23 to 3.30	37.32	23.76 to 50.88	10.79	−3.32 to 24.91	0.79
ILL 8029	3.51 ± 0.54	3.07 to 4.01	22.69	14.10 to 31.29	13.10	−10.92 to 37.13	0.70

The BP is the breakpoint (kPa), S1 and S2 are the left slope and right slope, respectively (mg H_2O m^{-2} s^{-1} kPa^{-1}), CI is the confidence interval.

Table 5. Results of the 11 genotypes exhibiting single linear regression fits of TR response to vapor pressure deficit. The table includes slope with standard error (SE), X-intercept and R^2 values.

Genotype	Slope ± SE	Slope CI (95%)	X-Intercept	X-Intercept CI (95%)	R^2
ILL 5919	27.23 ± 2.59	21.96 to 32.50	−0.58	−1.34 to −0.06	0.77
ILL 4605	16.53 ± 1.51	13.46 to 19.61	−0.39	−1.13 to 0.13	0.79
ILL 6359	31.18 ± 2.82	25.44 to 36.92	−0.13	−0.82 to 0.36	0.79
ILL 6363	27.07 ± 2.72	21.55 to 32.58	−0.75	−1.65 to −0.15	0.74
ILL 7223	25.95 ± 1.01	23.88 to 28.02	0.06	−0.20 to 0.28	0.95
ILL 7286	22.04 ± 2.98	15.94 to 28.13	−1.32	−2.84 to −0.44	0.66
ILL 7804	30.32 ± 2.17	25.93 to 34.71	0.12	−0.35 to 0.49	0.82
ILL 7813	29.08 ± 3.67	21.54 to 36.62	−0.15	−1.19 to 0.48	0.70
ILL 7819	24.22 ± 2.27	19.62 to 28.83	−0.20	−0.92 to 0.30	0.76
ILL 7820	23.01 ± 2.61	17.60 to 28.43	−0.61	−1.62 to 0.03	0.78
ILL 8025	36.48 ± 2.78	30.81 to 42.16	0.22	−0.27 to 0.59	0.85

High dissimilarity was distinguished for the slopes below and above BP increasing TR with further increases in VPD. The slope at VPD above BP ranged from −1.63 ± 5.34 to 28.1 ± 11.3 mg with an average of 14 mg H_2O m^{-2} s^{-1} kPa^{-1}. In contrast, the slope below BP varied from 18.1 ± 5.49 to 44.7 ± 7.37 mg with an average of 34.5 mg H_2O m^{-2} s^{-1} kPa^{-1}. Genotype ILL 8029 showed the highest BP at about 3.51 kPa, while the two genotypes ILL 7835 and ILL 7833 recorded the lowest BP at around 2.76 and 2.81 kPa, respectively (Figure 5). The genotype ILL 7833 expressed the lowest TR during the increase in VPD, with 18.1 and −1.63 mg H_2O m^{-2} s^{-1} kPa^{-1} for left and right slopes, respectively. Similarly, ILL 7814 and ILL 7835 were characterized by a very low TR after expressing the BP, with slopes greater than the BP ranging from 5.59 to 10.8 mg H_2O m^{-2} s^{-1} kPa^{-1}. Among genotypes that did not express a BP, the check Bakria (ILL 4605) had the lowest slope with a TR of 16.5 ± 1.51 mg H_2O m^{-2} s^{-1} kPa^{-1}. Oppositely, ILL 8025 exhibited the highest slope with 36.5 ± 2.78 mg H_2O m^{-2} s^{-1} kPa^{-1}. The results also revealed that ILL 5919, ILL 7813 and ILL 8025 had the maximum TR at around 175 mg H_2O m^{-2} s^{-1} kPa^{-1}, which lost TR_{lim} trait expression during the increase in VPD. ILL 6262 recorded the highest TR with 210 mg H_2O m^{-2} s^{-1} kPa^{-1}, followed by the genotypes ILL 6338 and ILL 3484, which had a TR of 190 mg H_2O m^{-2} s^{-1} kPa^{-1} (Table 4).

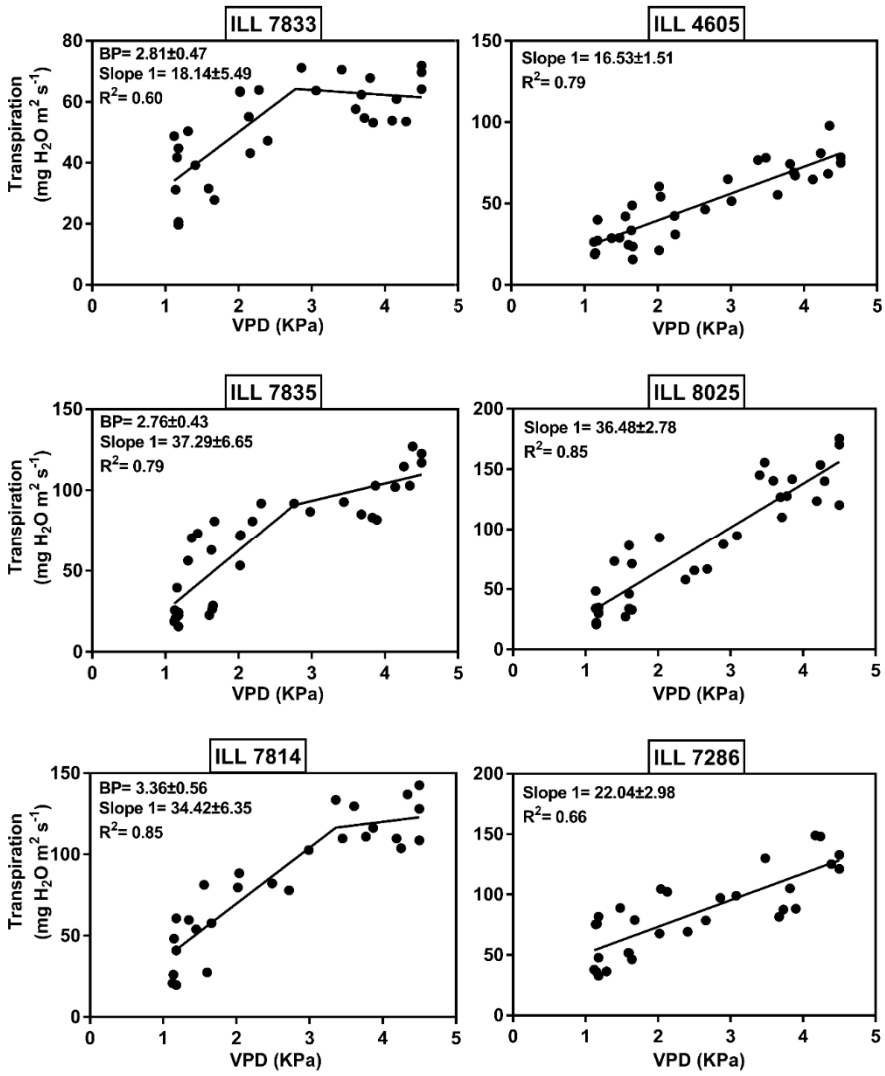

Figure 5. Transpiration rate (mg H_2O m^{-2} s^{-1}) response to increasing vapor pressure deficit (VPD in kPa). Genotypes ILL 7833, ILL 7835 and ILL 7814 represent tolerant accessions with two segmental regression, while ILL 4605, ILL 8025 and ILL 7286 represent susceptible genotypes with linear transpiration response to increasing VPD. The breakpoint (BP), slopes and R^2 are indicated.

3. Discussion

3.1. Phenology, Yield and Nutritional Quality under High-Temperature and Drought Stress

In this study, delayed sowing to expose the reproductive stage to heat stress significantly affected phenological traits, grain yield components, nutritional quality parameters and canopy temperature in lentil genotypes. However, combining drought with heat stress through limited irrigation had a more severe impact on plant growth, resulting in significantly lower grain yield and nutritional quality. These results are consistent with our previous studies that confirmed the influence of water limitation and high temperature on lentil [14,35]. Similar findings were also observed in chickpea [61], lentil [31,36], soybean [62] and groundnut [63] where combined temperature-drought stress was more detrimental than temperature stress alone.

Our findings revealed that the number of filled pods decreased because of high-temperature and combined temperature-drought stress. This is in agreement with earlier studies that showed temperature and/or drought stress negatively influenced the reproductive processes, mainly pollen and ovule fertility [64,65], resulting in the reduction of filled pods. Recently, Jiang et al. [66] described that temperature stress reduced the number of pollen grains per anther, induced smaller pollen grains and increased reactive oxygen

species (ROS) production in pollen grains in pea. Still, it did not affect ROS accumulation in ovules and ovule number per ovary. Furthermore, high-temperature exposure when young floral buds were visible at the first formed reproductive node was more destructive to flower retention, seed set, pod development and seed yield compared to heat exposure started later, when flowers at the second reproductive node were fully open. Temperature and drought stress also impact pollen vacuolization, which plays a vital role in increasing the volume of pollen grains with the accumulation of cytoplasmic components [67]. A recent study by Fábián et al. [29] showed that the combination of high temperature and water stress altered the phenology of the plants, reduced pollen viability, shortened the duration of gametogenesis and grain filling, and modified the morphology and anatomy of the pistils. Functionality of female and male reproductive parts was reduced by 34 and 66%, respectively.

Our study showed a decrease in seed weight due to temperature stress, while combined temperature-drought stress magnified the impact. It is a fact that during the seed-filling stage, the decline in photosynthesis and leaf sucrose metabolism in response to temperature stress and drought stress led to lower carbohydrate availability for import into developing seeds [68]. Previous investigations suggested seed weight is reduced in response to high-temperature and/or drought stress during grain filling by altering endosperm cells [69] and reducing starch accumulation [70]. Starch is the central component in seeds for major global staple crops. A decrease in source strength and carbon availability for starch biosynthesis through the grain filling period will definitely reduce seed size and weight [71,72]. Recent studies revealed a decline in the activity of enzymes involved in starch biosynthesis under high-temperature or water stress. Eventually, their combination contributed to a decrease in the starch accumulation rate and duration and starch content [73,74]. Sehgal et al. [36] indicated a significant decrease in starch content under temperature stress and water stress in lentil seeds. However, the combined temperature-drought stress caused the most severe reduction due to the inhibition of starch synthesizing enzyme (starch synthase) and sucrose synthesizing activity (sucrose synthase), which were previously identified to have a correlated effect on seed size in chickpea [75].

A significant reduction in protein content was reported under temperature stress; however, the combined temperature-drought stress caused more decrease, which is in agreement with previous observations in lentil [14,36], and in chickpea [76] through reduced function of PSII, weakened nitrogen anabolism and strengthened protein catabolism. Previous findings by Zahedi et al. [77] and Triboï et al. [78] revealed a stable relation between protein content and the total quantity of nitrogen in wheat grain. They suggested that the synthesis of storage proteins is limited mainly by the availability of nitrogen. Similarly, Liu et al. [79] also reported a decrease in crude protein fractions in drought-stressed alfalfa (*Medicago sativa* L.) due to water limitation in addition to a reduction in N fixation. For that, improvement of N fixation could lead to higher biomass production and water-use efficiency and may increase pod yield under drought stress conditions.

High temperature induced a significant reduction in grain micronutrient concentrations. The combined temperature-drought stress further aggravated the decline, by 18% for Zn and 20% for Fe, compared to that under normal conditions. Similar findings were observed in other legume crops such as lentil, where iron and zinc contents were dramatically reduced in response to temperature and drought stress due to decreased root nutrient uptake, with reduced root biomass and metabolic rate [80] or by direct damage to roots [81]. In addition, the reduction in transpiration rate because of water deficit may also decrease nutrient absorption and the effectiveness of their use by the plants [36,82]. Choukri et al. [14] suggested that the decrease in iron and zinc was attributed to decreasing water availability under heat and drought stress conditions. Furthermore, Hummel et al. [83] reported that the variations in zinc, iron and protein under drought stress conditions are more affected by weather conditions than genotype. The present study also revealed a negative correlation between Zn and protein content in high-temperature stress and heat-drought stress conditions, and Fe and protein content in the combined temperature-drought stress,

which are in disagreement with previous studies conducted by Ghanbari et al. [84] and Impa et al. [85]. They reported a positive correlation between protein content and micronutrients under temperature and drought stress conditions. However, Fe and Zn were positively correlated under the three treatments, which was also reported in earlier findings in lentil [86], chickpea [87,88] and pea [89]. Furthermore, our results showed a significant increase in canopy temperature of plants under high-temperature stress and combined temperature-drought stress. This effect might be explained by inhibition of stomatal conductance and transpiration reducing leaf water content, which resulted from an increase in leaf temperatures [90,91].

3.2. Transpiration Response to High VPD under Controlled Conditions

Our findings showed significant genetic variation among lentil genotypes in transpiration response to increasing VPD under controlled environments. Eleven genotypes consistently increased TR with increasing VPD, while nine exhibited a distinct response by limiting their TR when VPD reached about 3.1 kPa. Our outcomes indicated that most of the genotypes displayed lower BP compared to what was reported by Guiguitant et al. [57], who showed a BP at approximately 3.4 kPa in the majority of lentil genotypes, with the lowest BP at 3.31 kPa. In our study, two tolerant genotypes (ILL 7833 and ILL 7835) limited their TR at early VPD (2.8 kPa), representing the lowest BP over all the already published VPD experiments in lentil. Restriction of TR under high VPD was also reported in many other species such as chickpea [56], peanut [51], soybean [49], sorghum [92] and maize [93], in which the BP values mostly fluctuated from 1.1 to 2.7 kPa. However, Schoppach and Sadok [94] showed high BP ranging from 2.4 to 3.9 kPa in wheat, while Sinclair et al. [95] described a VPD threshold ranging from 2.6 to 3.38 kPa in peanut.

Over the whole range of tested VPD, almost all of the tolerant genotypes showed lower TR_{lim} compared to the sensitive lentil lines, which exhibited a continuously increasing TR with increasing VPD. These results support our field findings [35], and they are in good agreement with the conclusion of Belko et al. [54] in cowpea. Genotypes with TR_{lim} may have considerable potential for increased soil water conservation, indicating somehow more effective control of TR under limited-water conditions [43]. Several findings reported that genotypes with a conservative water behavior have the opportunity of using the conserved soil water to preserve physiological activity during the grain filling period and produce higher grain yield than genotypes without the TR_{lim} trait in late-season water deficit conditions [37,42,43,57,60,96].

Interestingly, the check variety Bakria, a moderately tolerant line to drought and heat stress, exhibited the lowest TR among all tested lines with 16 mg H_2O m^{-2} s^{-1} kPa^{-1} under increasing VPD, and probably had the lowest conductance among the studied genotypes. Among the tolerant lines, ILL 7833 and ILL 8029 had the lower TR presented in slope 1 with 18.1 and 22.7 H_2O m^{-2} s^{-1} kPa^{-1}, respectively. However, genotype ILL 8029 registered the maximum BP at 3.51 kPa, which was likely to be disadvantageous in expressing the TR_{lim} trait under high levels of VPD [94,97]. Variation in TR shown in the two slopes, which normalized water use efficiency, can be explained by the difference in the hydraulic conductance to water flux in the plants. Several studies have indicated that the TR_{lim} trait under high evaporative conditions of VPD is likely due to low hydraulic conductivity in leaves between the xylem and into the guard cells, which increased water use efficiency [93,98,99]. The data described here do not allow support for the claim of a hydraulic conductance limitation in leaves; however, the difference in VPD breakpoint values among lentil genotypes may indicate variation in stomatal conductance between studied genotypes.

Since the experiment was conducted under controlled conditions, the variations in temperatures and relative humidity were the main key factors to reach higher VPD levels; however, the high temperatures (>35 °C) may influence TR response of the plants. A recent study by Kar et al. [100] revealed that an increase in VPD had a powerful positive impact on plant water loss irrespective of the effect of temperature, light and relative

humidity. Although previous studies have reported similar observations, the effects of temperature, relative humidity, radiation and water on TR had minor effects compared to VPD [94,101,102].

Genotypes with TR_{lim} under high VPD offer an essential candidate for breeding programs to improve lentil yields in water deficit areas [57]. A low BP represents an imperative approach by the plants to maintain the greatest water conservation during critical drought periods when high VPD promotes TR. Our outcomes indicate that studied lentil genotypes could be used to improve yield potential over a range of drought-stressed environments. Hence, tolerant genotypes such as ILL 7833 and ILL 7835, which had the lowest BP, may be desirable for drought-prone conditions and improve lentil productivity in regions affected by drought stress. The TR profiles generated from the current investigation present an opportunity to understand the physiological behaviors of tolerant and sensitive lentil genotypes under controlled conditions. However, additional studies are needed to examine more physiological and genetic responses of lentil material under field and controlled conditions.

4. Materials and Methods

4.1. Plant Material

The material consisted of 20 lentil genotypes selected from our previous field screening study [35], where we evaluated a focused identification of germplasm strategy (FIGS) set against drought and temperature stresses. In the present study, we included moderately heat tolerant, moderately drought tolerant, heat tolerant, heat sensitive, drought tolerant and drought sensitive lines (Table S7).

4.2. Field Experiment

The germplasm was assessed under three independent experiments at the ICARDA experimental station Marchouch (33.56° N, 6.63° W, 392 m altitude) during the 2016–17 cropping season. These three experiments were deemed to represent three treatments, namely (i) normal date of planting (Treatment A), (ii) late planting with irrigation (Treatment B) and (iii) late planting without irrigation (Treatment C). All of the treatments were planted in an alpha lattice design with two replications. In the three treatments, each genotype was planted in a three-row plot of 1 m length, with a spacing of 0.30 m between rows. In each row, seeds were sown at 2 cm depth, maintaining 10 cm space between plants. Treatment A resulted in optimal growing conditions (150 mm well-distributed rainfall and temperature below 27 °C, without any heat or water stress to the plants). Treatment B (planted 65 days after normal planting date with irrigation at field capacity throughout crop duration) imposed high-temperature stress. In both late planting treatments, the plants were synchronized with temperatures above 32 °C during the reproductive stage. Regular irrigation at field capacity avoided any water stress to the plants during their growth and development. Treatment C (planted 65 days after normal planting date without irrigation during the reproductive stage) imposed combined high-temperature and drought stress. Treatment A was planted on 27 December 2016, without any supplementary irrigation during the cropping period, as the crop received enough well-distributed rainfall during the cropping season. Treatments B and C were planted on 1 March 2017. Irrigation was performed regularly to sustain water supply at field capacity using a sprinkler system throughout the crop duration in treatment B. In contrast, irrigation was stopped at the flower initiation stage onward in treatment C to impose water stress (<5 mm rainfall during the reproductive stage) combined with the temperature stress. All field management followed standard agricultural practices in lentil production [103].

4.3. Data Collection

Data were collected on early growth vigor (EGV) after 1 month of sowing, plant height (PLH), days to first flowering (DFF), days to 50% of flowering (D50F), days to 50% of podding (D50P) and days to physiological maturity (DM) on a plot basis. Five plants were

randomly selected from each plot to measure number of total pods plant^{-1} (NTPP), number of filled pods plant^{-1} (NFPP) and number of unfilled pods plant^{-1} (NUPP), grain yield plant^{-1} (GYP) and hundred-seed weight (HSW). Canopy temperature (CT) was determined using a thermal infrared camera FLIR T4xx-series (Model T420-KIT-15). Thermal images were captured between 11:00 and 13:00 GMT time on a sunny day and analyzed using FLIR tools+ software (FLIR systems, Teledyne FLIR, Wilsonville, OR, USA). The seeds harvested from these experiments were analyzed for protein content and micronutrient contents (zinc and iron).

4.4. Crude Protein Content

A 0.3 g amount of ground lentil seeds from each treatment was digested with sulfuric acid and selenium mixture following the modified Kjeldahl procedure [104]. Based on the color reaction between ammonium and a weakly alkaline mixture of sodium salicylate and sodium hypochlorite, a UV visible spectrophotometer was used to determine the color development in the samples at 560 nm. Next, crude protein content (CP) was measured using nitrogen values multiplied by 6.25, and triplicate analyses were performed for each sample.

4.5. Iron and Zinc Determination

Seeds were ground by a Cyclone mill (Twister, 10 mm–250 um, Retsch). Iron (Fe) and zinc (Zn) concentrations were measured using a modified HNO_3 and H_2O_2 method [105]. In the digestion block (QBlock series, Horiba), 0.5 g of each ground sample was placed in individual tubes and digested with 6 mL nitric acid (HNO_3), followed by heat treatments at 90 °C for 1 h. To each tube, 3 mL of 30% hydrogen peroxide (H_2O_2) was added, and sample digestion was continued by heating for 15 min at 90 °C, and then 3 mL of 6 M hydrochloric acid (HCL) was added. Once samples were cooled, the solutions were filtered and diluted to 10 mL with distilled water. The mineral content analysis was carried out by inductivity coupled plasma-optical emission spectrometry (ICP-OES); (iCAP-7000 Duo, Thermo Fisher Scientific, Waltham, MA, USA) at the Cereals and Legumes Quality Laboratory, ICARDA, Rabat, Morocco.

4.6. Plant Growth Conditions in the Greenhouse

An independent experiment was conducted during summer 2018 in the greenhouse at ICARDA, Rabat, Morocco. Three seeds were sown at a depth of 2.5 cm in plastic pots (15 cm in diameter and 20 cm in height) filled with 1.5 kg of 50% sandy loam soil and 50% of compost garden soil (Floragard Vertriebs-GmbH product, Oldenburg) that included 18-10-20 N-P-K fertilizer. Here, the same set of 20 genotypes assessed under field conditions were studied under controlled conditions. Five replications were used for each genotype. The pots were positioned in a randomized design on steel grid platform installed at mid-height of 1.3 m. In each pot, a small hole was opened at the bottom of the end cap to facilitate drainage. Plants were grown under well-watered conditions, and the temperature in the greenhouse was maintained at 25 °C/18 °C (day/night) for 1 month. The photosynthetic photon flux density in the greenhouse was approximately 600 μmol m^{-2} s^{-1}.

The day before the measurement, pots were watered to full capacity until their water flowed from the bottom of the pots, then the pots were allowed to drain overnight. After drainage, the pots were bagged with plastic bags and covered with polyethylene balls to keep soil evaporation to a minimum. A thermo-hygrograph sensor (Tinytag Ultra 2 TGU-4500 Gemini Datalogger Ltd., Chichester, UK) was positioned over the plant to measure the temperature and RH at 5 min intervals. Hourly atmospheric VPD was calculated on the basis of average temperature and RH. Plants were exposed to each humidity and temperature treatment for 1 h and then reweighed to measure the final weight. On an hourly basis, the transpiration rate (TR) was calculated as the weight difference between successive measurements using a balance with a resolution of 0.1 g. The measurement was

conducted in the morning from 7:00 am (Morocco standard time) at low VPD until 7:00 pm, when the VPD decreased following the midday maximum. Measurements were first started with the low VPD (0–1.5 kPa) treatment, the medium VPD treatment (1.5–2.5 kPa), and finally, the high VPD (2.5–4.5 kPa) treatment. The experiment in the greenhouse allowed us to control the temperature and RH, exposing the plants to the expected ranges of VPD (1.18 to 4.5 kPa). The average temperature and RH during the experiment fluctuated from 19 to 40 °C and from 30 to 80%, respectively. A high VPD level was achieved by increasing temperature and decreasing RH inside the greenhouse. At the end of the experiment, the leaf area was measured using a leaf area meter, and the TR was expressed as water loss per unit of leaf area and time (g H_2O_2 cm^{-2} h^{-1}).

4.7. Statistical Analysis

4.7.1. Field Data Analysis

Analysis of variance was carried out using the general linear model (GLM) in IBM SPSS statistics 23. Duncan's post-hoc was applied to compare differences between the mean values at $p < 0.05$. Pearson's correlation coefficient was determined for the three treatments. Principal component analysis (PCA) was performed using the *Factoextra* and *FactoMineR* packages in R version 4.1.0 and RStudio version 1. 3.1093 [106,107]. In addition, hierarchical cluster analysis was performed using Ward's squared Euclidean distance method with the *dendextend* R package [108].

4.7.2. Vapor Pressure Deficit Analysis

The individual data for each VPD treatment of each genotype were used in the regression analysis of transpiration response. The data were first tested to fit the data to a two-segment linear regression using GraphPad Prism 7 (GraphPad Software Inc.). The results of a successful regression fit to the two-segment model were the coefficients defining two intersecting linear regressions:

$$\text{If VPD} < \text{BP, TR} = \text{intercept 1} + \text{slope 1 (VPD)} \quad (1)$$

$$\text{If VPD} \geq \text{BP, TR} = \text{intercept 2} + \text{slope 2 (VPD)} \quad (2)$$

where BP is the breakpoint between the two linear segments; it is also an output of the GraphPad analysis and an estimate of the standard error for BP. The intercept represents the constant of the first and second line segments; the two slopes were statistically compared within GraphPad for a significant difference at $p \leq 0.05$ level. In case there was a significant difference, the two-segment model characterized the outcomes for that genotype. All data for a genotype were assumed to fit a single linear regression model if two slopes were not identified to be significantly different.

5. Conclusions

The outcomes of the present study validate our previous field screening results. Almost all tolerant genotypes showed a high adaptation to high temperatures and combined temperature-drought stress under field conditions and exhibited a fairly clear TR_{lim} at high VPD under controlled conditions. These genotypes would contribute to water saving in soil and improve the productivity under terminal heat or water-limited environments. Interestingly, two tolerant genotypes (ILL 7833 and ILL 7835) exhibited the lowest breakpoint found in lentil, which may be exclusively desirable for harsh regions affected by water deficit. Genotypes such as ILL 7835, ILL 7814 and Bakria (ILL 4605) combine good nutritional quality with moderate to high yield under both heat and combined heat-drought stress, which is of great interest for breeding programs. The parameters of the tested lentil genotypes in response to high temperatures, water deficit and higher VPD would facilitate formulating new experimental strategies to shed light on the molecular and genetic basis of heat and drought tolerance mechanisms in the future. Hence, combining crop physiology

with molecular techniques could be a promising approach to improve lentils under high temperatures, water deficit and higher VPD.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/plants11010095/s1, Table S1: ANOVA analysis for 20 lines under normal and late planting trials at Marchouch, Table S2: Correlation between tested traits under normal conditions at Marchouch, Table S3: Correlation for late planting under combined temperature-drought stress (above) and temperature stress (below), Table S4: Contribution of traits to the first three PCA dimensions under normal conditions, temperature stress, and combined temperature-drought stress, Table S5: Analysis of variance for canopy temperature between normal conditions, temperature stress and combined temperature-drought stress at Marchouch, Table S6: Minimum, maximum, mean of canopy temperature under normal conditions, temperature stress and combined temperature-drought stress at Marchouch, Table S7: Description of origin, IG number and classification of lentil genotypes used in the current study. MHT: Moderately heat tolerant; MDT: Moderately drought tolerant, HT: heat tolerant, HS: heat sensitive, DT: drought tolerant, DS: drought sensitive, Figure S1. Dendrogram of normal (A), temperature stress (B) and combined temperature-drought stress (C) conditions.

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Metabolic Mechanisms Underlying Heat and Drought Tolerance in Lentil Accessions: Implications for Stress Tolerance Breeding

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Abstract: Climate change has significantly exacerbated the effects of abiotic stresses, particularly high temperatures and drought stresses. This study aims to uncover the mechanisms underlying heat and drought tolerance in lentil accessions. To achieve this objective, twelve accessions were subjected to high-temperature stress (32/20 °C), while seven accessions underwent assessment under drought stress conditions (50% of field capacity) during the reproductive stage. Our findings revealed a significant increase in catalase activity across all accessions under both stress conditions, with ILL7814 and ILL7835 recording the highest accumulations of 10.18 and 9.33 under drought stress, respectively, and 14 µmol H_2O_2 mg protein^{-1} min^{-1} under high temperature. Similarly, ascorbate peroxidase significantly increased in all tolerant accessions due to high temperatures, with ILL6359, ILL7835, and ILL8029 accumulating the highest values with up 50 µmol ascorbate mg protein^{-1} min^{-1}. In contrast, no significant increase was obtained for all accessions subjected to water stress, although the drought-tolerant accessions accumulated more APX activity (16.59 t to 25.08 µmol ascorbate mg protein^{-1} min^{-1}) than the sensitive accessions. The accessions ILL6075, ILL7814, and ILL8029 significantly had the highest superoxide dismutase activity under high temperature, while ILL6363, ILL7814, and ILL7835 accumulated the highest values under drought stress, each with 22 to 25 units mg protein^{-1}. Under both stress conditions, ILL7814 and ILL7835 recorded the highest contents in proline (38 to 45 µmol proline/g FW), total flavonoids (0.22 to 0.77 mg QE g^{-1} FW), total phenolics (7.50 to 8.79 mg GAE g^{-1} FW), total tannins (5.07 to 20 µg CE g^{-1} FW), and total antioxidant activity (60 to 70%). Further, ILL7814 and ILL6338 significantly recorded the highest total soluble sugar content under high temperature (71.57 and 74.24 mg g^{-1}, respectively), while ILL7835 achieved the maximum concentration (125 mg g^{-1}) under drought stress. The accessions ILL8029, ILL6104, and ILL7814 had the highest values of reducing sugar under high temperature with 0.62 to 0.79 mg g^{-1}, whereas ILL6075, ILL6363, and ILL6362 accumulated the highest levels of this component under

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drought stress with 0.54 to 0.66 mg g^{-1}. Overall, our findings contribute to a deeper understanding of the metabolomic responses of lentil to drought and heat stresses, serving as a valuable reference for lentil stress tolerance breeding.

Keywords: antioxidant activities; catalase; ascorbate peroxidase; superoxide dismutase; proline; flavonoids; sugars; drought stress; heat stress

1. Introduction

Crops are subjected to a wide range of abiotic stresses such as drought, heavy metals, UV rays, heat, and salinity, concurrently throughout their life cycles due to constantly changing environmental conditions, which reduce and limit their growth and productivity [1,2]. Extreme temperatures and drought stress have been identified as two of the most severe environmental risks restricting crop development, productivity, and, consequently, food security in a changing climate [3]. These two stresses contribute significantly to reducing the production of several important staple food crops, which provide 60% of the world's food energy [4–7]. Drought stress and high temperatures have been on the rise in recent decades, and this trend is expected to continue and worsen in the future [8]. Consequently, the resulting conditions are likely to have a significant impact, leading to increased malnutrition and micronutrient deficiencies, particularly in regions such as sub-Saharan Africa, South Asia, Central and South America, and small islands.

Lentil (*Lens culinaris* Medik.) holds a significant position as the third most vital cool-season food legume globally, after chickpea and pea [9], with an area of 5.5 million ha, a production of 5.6 million tons, and a productivity of 1004 Kg ha^{-1} in 2021 [10]. The cultivation of lentil predominantly takes place in rainfed environments, where drought and extreme temperatures are the two most challenging abiotic stresses that reduce its production in arid and semi-arid areas [11,12]. Compared to other leguminous crops, lentil has been found to exhibit greater sensitivity to high temperatures and drought stress, particularly during the reproductive stage, significantly diminishing its yield and productivity [13].

The impacts of extreme temperatures and water stress are receiving increased attention due to the substantial threat to the productivity of leguminous crops. These stresses adversely affect crucial factors such as pollen viability, fertilization, and ultimately, the formation of pods [14]. Elevated temperatures exceeding 32/20 °C (max/min) during the flowering and pod-filling stages have a harmful effect on the growth and grain filling of lentil, and by consequence, negatively impact both yield and nutritional quality [15,16]. Extensive research has been conducted on the effects of high temperatures and drought stress across various plant species, including wheat [17], barley [18], maize [19], chickpea [20], lentil [21], and soybean [22].

Both high temperatures and drought stress induce an imbalance between reactive oxygen species (ROS), such as superoxide radical ($O^{\bullet -}{}_2$), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH•), singlet oxygen ($^1O_2$), and the scavenging activities of antioxidant system components in plants [23]. Abiotic stress causes excessive ROS production, which interferes with photosynthesis, increases lipid peroxidation and electrolyte leakage, damages the structures of cell membranes, and disrupts nucleic acids and proteins. As a result, cells are unable to perform their normal functions [24]. The accumulation of free amino acids, proteins, solutes proline, soluble sugars, and protective heat shock proteins and activation of enzymatic (ascorbate peroxidase, catalase, superoxide dismutase, and peroxidase) and non-enzymatic (glutathione, tocopherols, carotenoids, and ascorbate) antioxidant systems are some of the mechanisms used by plants [25,26]. In addition, secondary metabolites such as phenols, flavonoids, and tannins play a pivotal role in the adaptation and defense of plants against deleterious environmental factors like heat and drought stresses [27]. Accumulation of secondary metabolites in plants during stress conditions contributed

to enhance physiological processes and stress tolerance via detoxifying ROS, ultimately promoting plant growth and development [28,29]. Hence, it is important to investigate and understand the enzymatic and non-enzymatic responses to high temperatures and drought stress to improve plants' stress tolerance.

In order to increase lentil production in areas that are severely impacted by climate change, it is essential to investigate the mechanisms linked to tolerance of high temperatures (>32/20 °C) and drought stress. Despite the importance of enzymatic and non-enzymatic responses in the lentil defense system against the increasing frequency of high temperatures and drought stress, there have been limited studies conducted on this aspect [30–32]. In this context, this investigation is the continuation of our previous efforts, and it provides additional insight into the responses of different metabolic traits to drought and heat stresses in lentil accessions. The objectives of the current study were (i) to uncover the metabolic mechanisms underlying heat and drought tolerance in lentil accessions selected from our previous research findings [33,34] and to (ii) identify superior lentil accessions best suited for heat and drought tolerance improvement in lentil germplasm for future programs.

2. Results

2.1. Antioxidant Enzyme Activity (CAT, APX, and SOD)

Seven accessions were subjected to the drought stress (50% field capacity), while twelve accessions were exposed to high-temperature stress (32/20 °C) during the reproductive phase. Our findings showed that stress significantly increased catalase (CAT) activity to higher than in the control plants, especially in the heat-tolerant accessions (6.91 to 16.32 µmol H_2O_2 mg protein^{-1} min^{-1}) and drought-tolerant accessions (4.12 to 10.18 µmol H_2O_2 mg protein^{-1} min^{-1}) compared to the sensitive accessions (Figure 1a,b). The accessions ILL6359, ILL7814, and ILL7835 maintained the highest CAT accumulation with an increase of 56 to 65%; however, ILL5919 and ILL6075 recorded the lowest values under high-temperature conditions with 6.24 and 6.91 µmol H_2O_2 mg protein^{-1} min^{-1}, respectively. In response to drought stress, the accessions ILL6363, ILL7835, and ILL7814 retained the highest CAT activity (9.32 to 10.18 µmol H_2O_2 mg protein^{-1} min^{-1}) with an increase of 27 to 47% compared to the control plants. In contrast, our findings showed that drought stress decreased CAT activity in the sensitive accessions ILL5919 and ILL7820 by 21 and 36%.

Under high temperature, ascorbate peroxidase (APX) significantly increased ($p < 0.05$) in all accessions except ILL5919, ILL6075, ILL6104, and ILL6338, which had values statistically similar to the control plants (Figure 1c). The tolerant accessions ILL6359, ILL8029, and ILL7835 were able to retain the highest APX activity (52.55 to 55.21 µmol ascorbate mg protein^{-1} min^{-1}) in response to high-temperature stress with a 39 to 69% increase compared to the control. In contrast, no significant increase was obtained for all accessions subjected to water stress, although the drought-tolerant accessions accumulated more APX activity (16.59 t to 25.08 µmol ascorbate mg protein^{-1} min^{-1}) than the sensitive accessions. The highest APX activity was recorded for the accessions ILL7835 and ILL7814 with 21.31 and 25.08 µmol ascorbate mg protein^{-1} min^{-1}, respectively (Figure 1d). In addition, our findings indicated that APX activity in the accessions ILL5919, ILL7820, ILL6075, and ILL6363 decreased by 46 to 51% as a result of drought stress.

Superoxide dismutase (SOD) activity significantly increased in all accessions except ILL5919 under heat stress and ILL7820 under drought stress when compared to the control (Figure 1e,f). Under high-temperature stress, the accessions ILL6075, ILL6104, ILL7223, ILL7814, and ILL8029 accumulated the highest SOD activity (20 to 25 units mg protein^{-1}) with a 39 to 65% increase compared to control plants. However, the drought-tolerant accessions ILL6362, ILL6363, ILL7814, and ILL7835 retained higher SOD activity 21.82 to 27.43 units mg protein^{-1} than sensitive accessions (9.79 to 16.02 units mg protein^{-1}) in response to drought stress conditions.

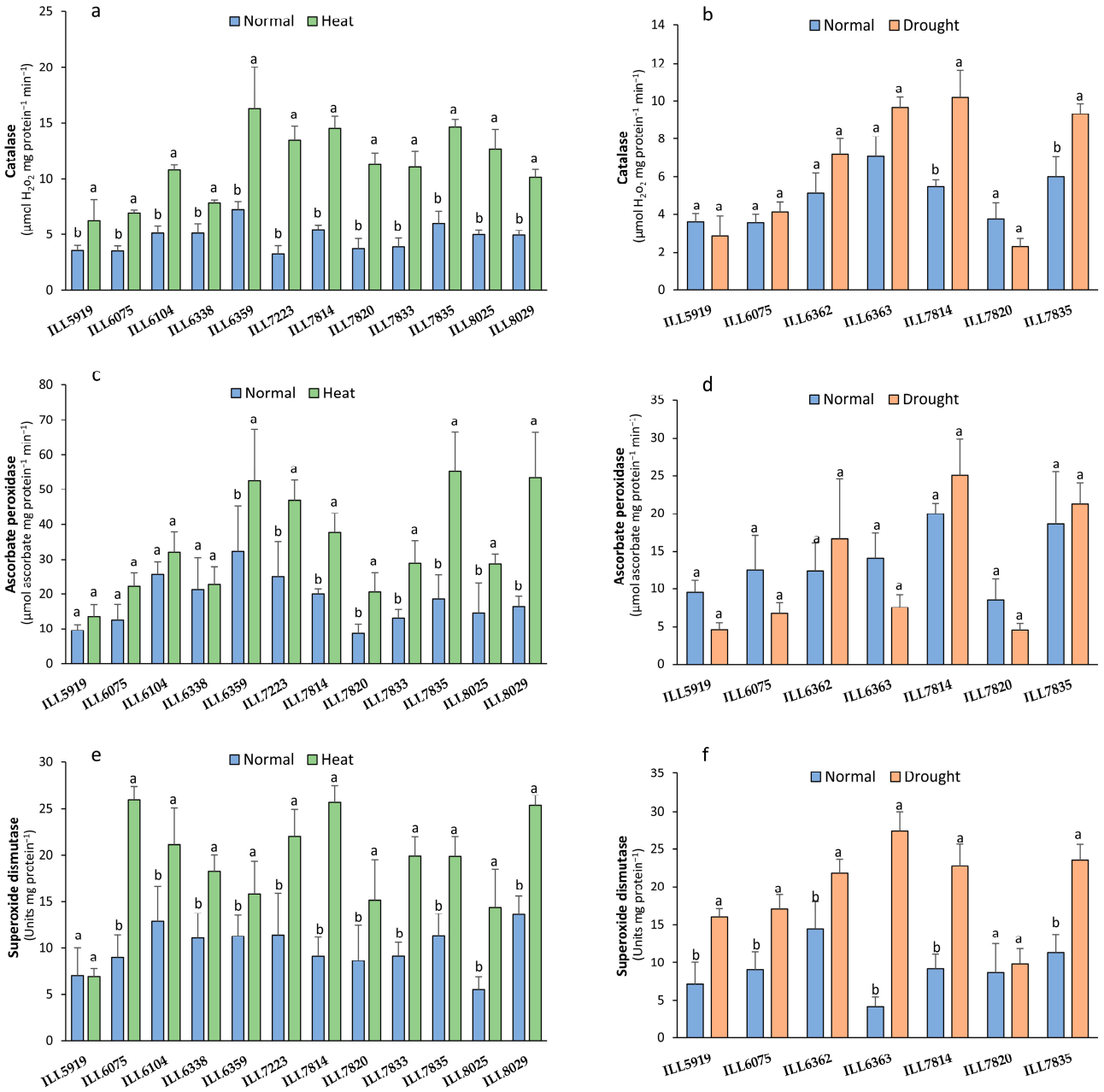

Figure 1. Catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD) activities of lentil accessions in response to high-temperature (**a**,**c**,**e**) and drought stress (**b**,**d**,**f**). Significant differences between means of tested accessions under not-stressed and stressed conditions using the LSD test at $p < 0.05$ are indicated by different letters on the bars.

2.2. Effect of High-Temperature and Drought Stress on Proline Content

Proline content (PC) significantly increased ($p < 0.05$) due to high-temperature and drought stress in all accessions except the susceptible accessions (ILL5919 and ILL7820), which had statistically similar PC under both stresses in comparison with the control (Figure 2). Under high-temperature stress, the maximum value of PC was found in ILL7814, ILL7833, and ILL7835 (up to 45 µmol proline/g FW with a 55 to 71% increase compared to control plants). However, ILL6362, ILL7814, and ILL7835 had the highest PC with 34.63, 38.09, and 35.78 µmol proline/g FW, respectively, under drought stress conditions.

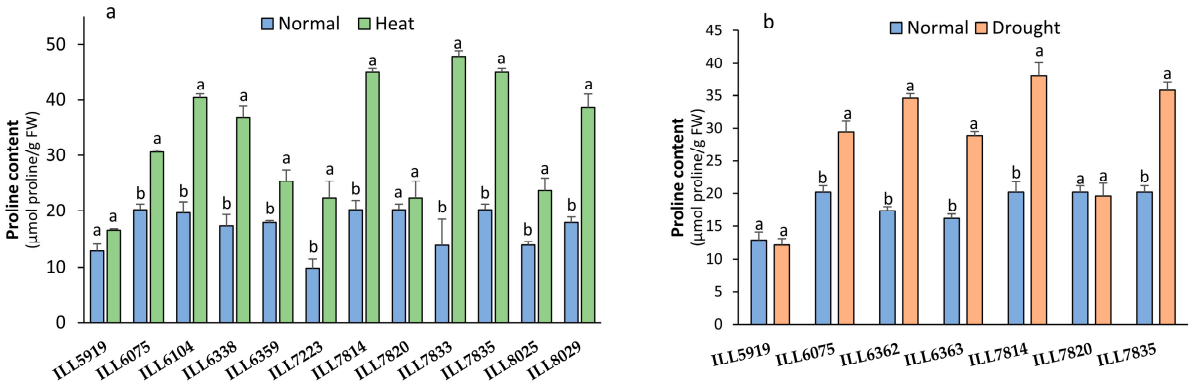

Figure 2. Proline content (PC) response to heat stress (**a**) and drought stress (**b**) in lentil accessions. Significant differences between means of tested accessions under not-stressed and stressed conditions using the LSD test at $p < 0.05$ are indicated by different letters on the bars.

2.3. Total Flavonoid Content Response to High-Temperature and Drought Stress

Total flavonoid content (TFC) significantly increased ($p < 0.05$) in five accessions (ILL6075, ILL6104, ILL6338, ILL7833, and ILL7835) in response to high temperature, whereas all accessions except ILL5919 exposed to drought stress increased TFC compared to normal conditions (Figure 3). Under high-temperature stress conditions, ILL6104, ILL6338, and ILL7835 increased TFC by up 70% and accumulated 0.46, 0.64, and 0.77 mg QE g^{-1} FW, respectively. Under drought stress, accessions ILL6362, ILL6363, and ILL7814 had the highest TFC (up to 0.22 mg QE g^{-1} FW) with an increase of 8 to 60% compared to control plants.

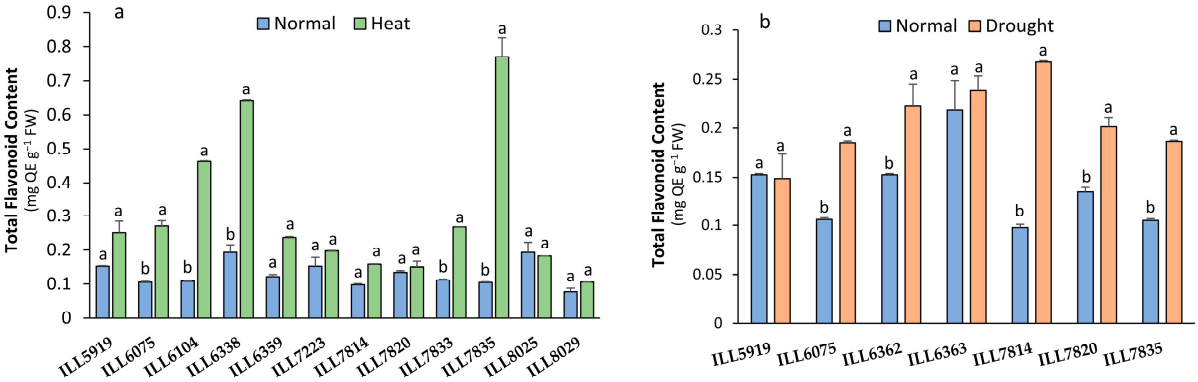

Figure 3. Total flavonoid content (TFC) of lentil accessions in response to heat stress (**a**) and drought stress (**b**). Significant differences between means of tested accessions under not-stressed and stressed conditions using the LSD test at $p < 0.05$ are indicated by different letters on the bars.

2.4. Total Phenolics Content Response to High-Temperature and Drought Stress

When subjected to high-temperature stress, all accessions displayed a significant increase in their total phenolics content (TPC), with the exception of ILL5919, which did not exhibit a significant increase compared to the control (Figure 4a). The TPC accumulation was greatest in ILL6104, ILL6338, ILL7814, ILL7835, and ILL8029 (7.50 to 8.79 mg GAE g^{-1} FW) with a 77 to 90% increase compared to control plants. However, only the two tolerant accessions ILL7814 and ILL7835 significantly increased TPC by up to 85% in response to drought stress (Figure 4b). In drought conditions, the average TPCs of ILL7814 and ILL7835 were 7.67 and 8.79 mg GAE g^{-1} FW, respectively.

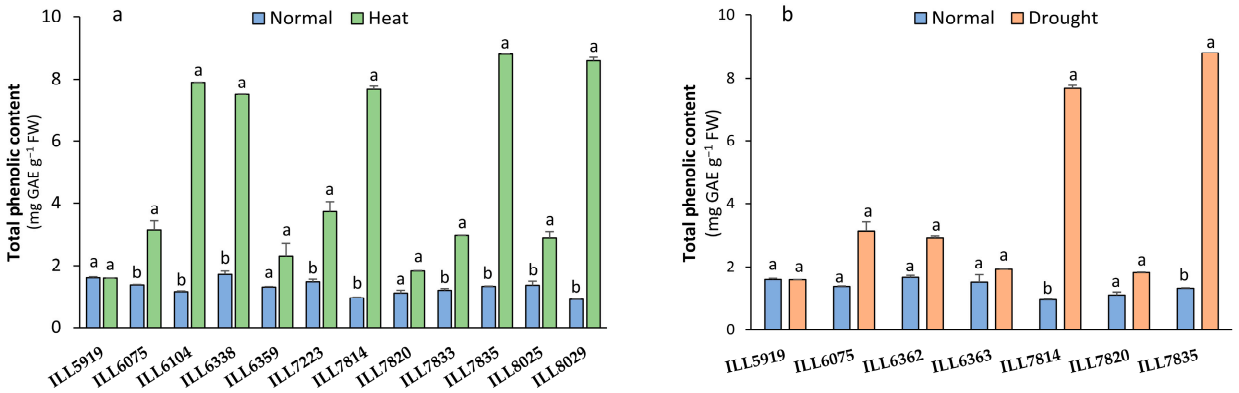

Figure 4. Total phenolic content (TPC) of lentil accessions in response to heat stress (**a**) and drought stress (**b**). Significant differences between means of tested accessions under not-stressed and stressed conditions using the LSD test at $p < 0.05$ are indicated by different letters on the bars.

2.5. Tannin Content Response to High-Temperature and Drought Stress

High-temperature stress caused tannin content (TC) to increase significantly ($p < 0.05$) in comparison to the control in all accessions, while ILL5919 showed no significant difference (Figure 5a). The highest TC accumulation under high-temperature stress was found in accessions ILL6104, ILL7814, ILL7835, and ILL8029 (20.31 to 23.92 µg CE g^{-1} FW), which increased their TC by 80 to 90% compared to control. With drought stress, on the other hand, TC significantly decreased in accessions ILL5919, ILL6075, and ILL6362 by 65 to 78%, while it increased TC in ILL7814 and ILL7835 by up 50% with an average of 5.07 and 8.37 µg CE g^{-1} FW, respectively (Figure 5b).

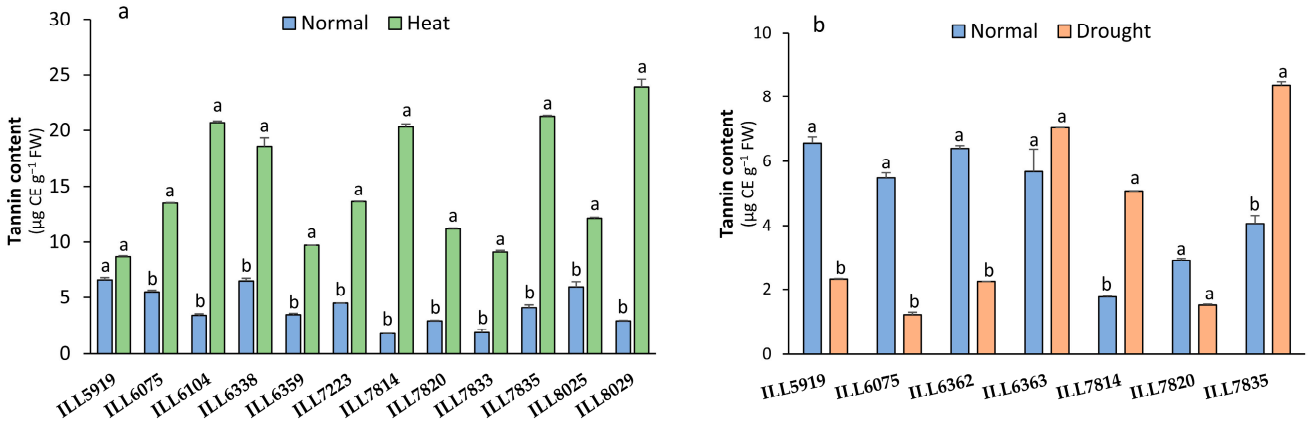

Figure 5. Tannin content (TC) in response to heat stress (**a**) and drought stress (**b**) in lentil accessions. Significant differences between means of tested accessions under not-stressed and stressed conditions using the LSD test at $p < 0.05$ are indicated by different letters on the bars.

2.6. Total Soluble Sugar and Reducing Sugar under Stressed Conditions

Total soluble sugar (TSS) were significantly augmented by 30 to 66% in eight accessions in response to high-temperature conditions, with the highest accumulation recorded in ILL7814 and ILL6338 with 71.57 and 74.24 mg g^{-1}, respectively (Figure 6a). Furthermore, TSS accumulation significantly increased by up 60% in three accessions (ILL5919, ILL6075, and 7835) in response to drought stress (Figure 6b). Among all the accessions, ILL7835 demonstrated the highest TSS content, with an average of 125 mg g^{-1} in response to drought stress conditions.

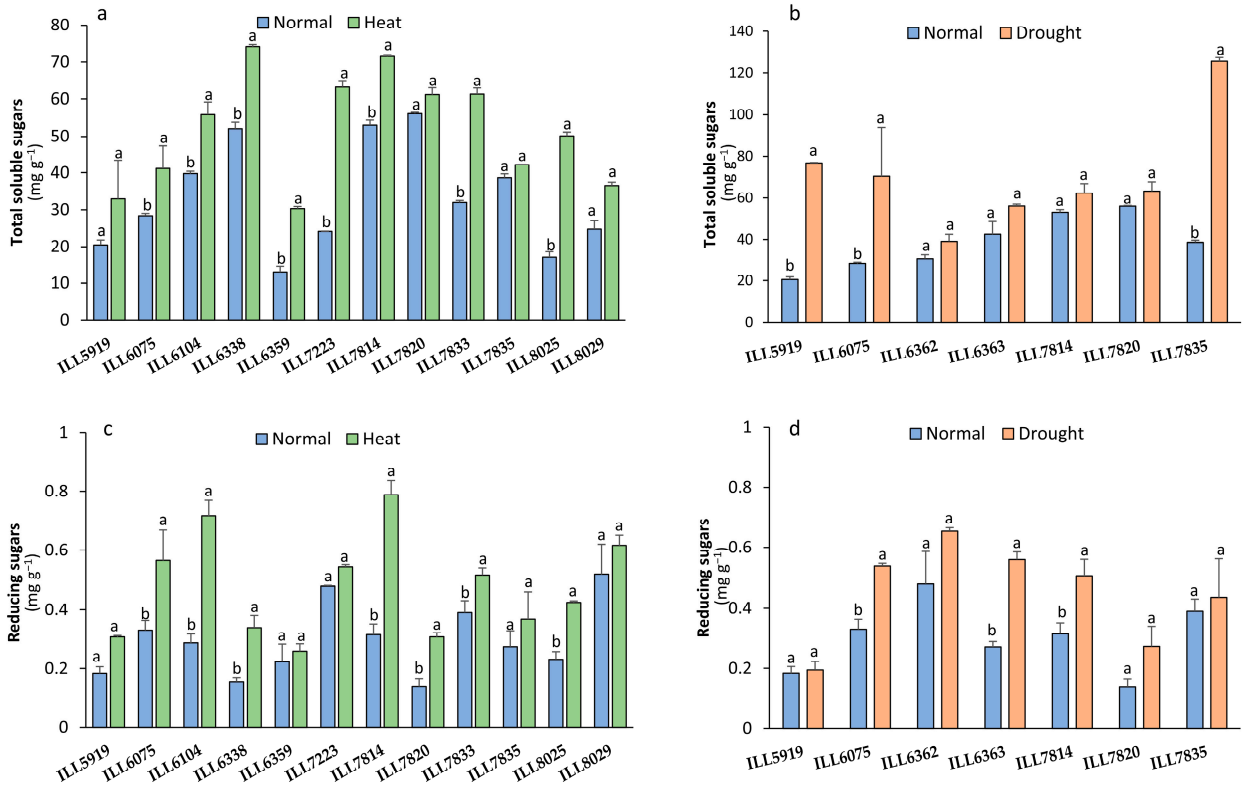

Figure 6. Total soluble sugar (TSS) and reducing sugar (RS) in response to heat stress (**a**,**c**) and drought stress (**b**,**d**) in lentil accessions. Significant differences between means of tested accessions under not-stressed and stressed conditions using the LSD test at $p < 0.05$ are indicated by different letters on the bars. The colored bars represent the treatments as blue for non-stress conditions, green for high temperatures, and orange for drought stress.

Compared to normal conditions, reducing sugar (RS) significantly increased ($p < 0.05$) by 40 to 60% in response to high-temperature stress in seven accessions (Figure 6c). The accessions ILL8029, ILL6104, and ILL7814 accumulated the highest RS under high temperature, with 0.62, 0.72, and 0.79 mg g^{-1}, respectively. On the other hand, the accessions ILL6075, ILL6363, and ILL7814 significantly increased RS by 30 to 50% under drought stress, whereas ILL5919, ILL6362, ILL7820, and ILL7835 had statistically similar RS compared to normal conditions (Figure 6d). Under drought stress, ILL6075, ILL6363, and ILL6362 had the highest RS with 0.54, 0.56, and 0.66 mg g^{-1}, respectively, whereas ILL5919 and ILL7820 had the lowest RS with 0.20 and 0.27 mg g^{-1}, respectively.

2.7. Total Antioxidant Activity Response to High-Temperature and Drought Stress

Total antioxidant activity (TAA) measurements in all 12 accessions under high-temperature stress showed that seven accessions had significantly ($p < 0.05$) higher TAA than the control. The heat-tolerant accessions ILL6104, ILL6338, ILL7814, ILL7835, and ILL8029 had the top TAA with 60 to 74% of DPPH inhibition in high-temperature conditions (Figure 7). However, TAA of accessions ILL7814, ILL6363, and ILL7835 significantly increased by 50 to 67% in response to drought stress, with 60.05, 62.79, and 65.82% of DPPH inhibition, respectively. Under the influence of both high-temperature and drought stress, the susceptible accessions ILL5919 and ILL7820 displayed the lowest accumulation of TAA.

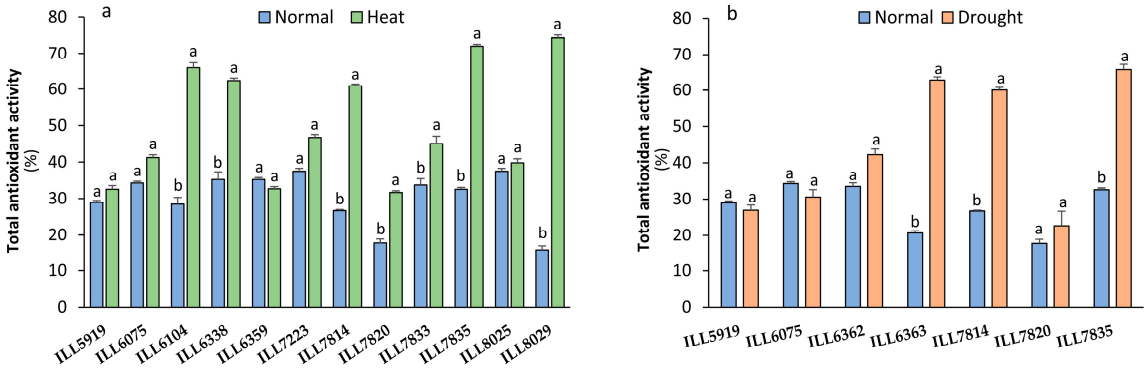

Figure 7. Total antioxidant activity (TAA) in lentil accessions expressed as a percentage of DPPH inhibition in response to heat stress (**a**) and drought stress (**b**). Significant differences between means of tested accessions under not-stressed and stressed conditions using the LSD test at $p < 0.05$ are indicated by different letters on the bars.

2.8. Genotypic Correlation under Normal, High-Temperature, and Drought-Stress Conditions

In normal conditions, TFC exhibited a highly significant positive correlation ($p < 0.01$) with TC and TPC, while it was negatively correlated at $p < 0.05$ with SOD (r = −0.57) (Figure 8a). Furthermore, a remarkably significant positive correlation ($p < 0.001$) between TPC and TC was found (r = 0.88). The heatmap analysis showed that CAT, APX, SOD, and RS were grouped together in the same cluster, while TSS and PC were found in the second cluster (Figure 8b). Under normal conditions, TC, TPC, TFC, and TAA are grouped together. Additionally, genotypic correlation identified three groups. The first group included two heat-tolerant accessions (ILL8029 and ILL6104), two heat- and drought-tolerant accessions (ILL7814 and ILL7835), and one susceptible accession (ILL7820). The second group consisted of two moderately heat- and drought-tolerant accessions (ILL7223 and ILL8025), one drought-tolerant accession (ILL6075), one heat-tolerant accession (ILL7833), and one susceptible accession ILL5919. Three accessions were grouped in one group: two moderately tolerant (ILL6363 and ILL6359), one drought-tolerant (ILL6362), and one heat-tolerant (ILL6338).

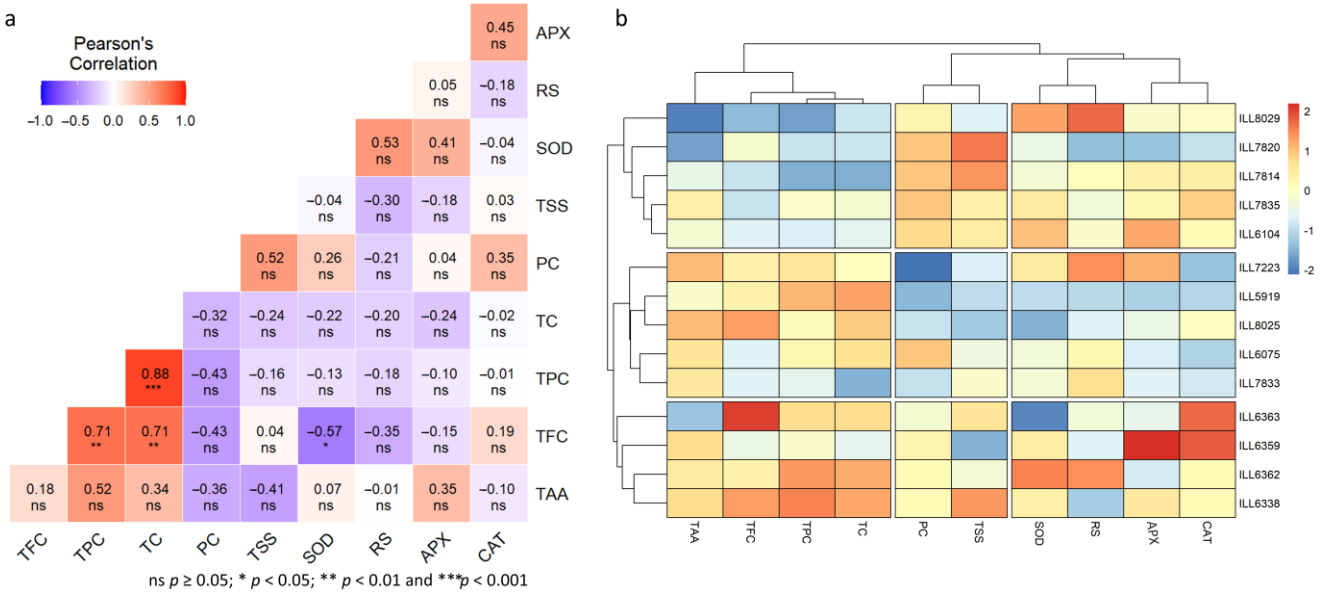

Figure 8. Pearson's correlation coefficients (**a**) and dendrogram along with heatmap (**b**) between various traits of lentil accessions under normal conditions.

In high-temperature conditions, SOD displayed a notable positive correlation with TAA, TC, PC, and RS, whereas CAT had positive correlation ($p < 0.01$) with APX (r = 0.72) (Figure 9a). Moreover, TPC, TAA, and TC were found to have a highly positive correlation ($p < 0.001$). The heatmap analysis identified that CAT and APX were clustered together, while TFC was clustered independently (Figure 9b). The remaining components formed the third group. Three heat-tolerant accessions (ILL8029, ILL6104, and ILL6338) and two accessions tolerant to high-temperature and drought stress (ILL7814 and ILL7835) were clustered together in the same group based on genotypic correlation. For almost all the tested components, these accessions had the highest accumulations. The second group contained two moderately tolerant (ILL7223 and ILL8025), one heat-tolerant (ILL7833), one drought-tolerant (ILL6075), and one sensitive (ILL7820) accession. The third group included one susceptible accession (ILL5919) and one moderately tolerant accession (ILL6359).

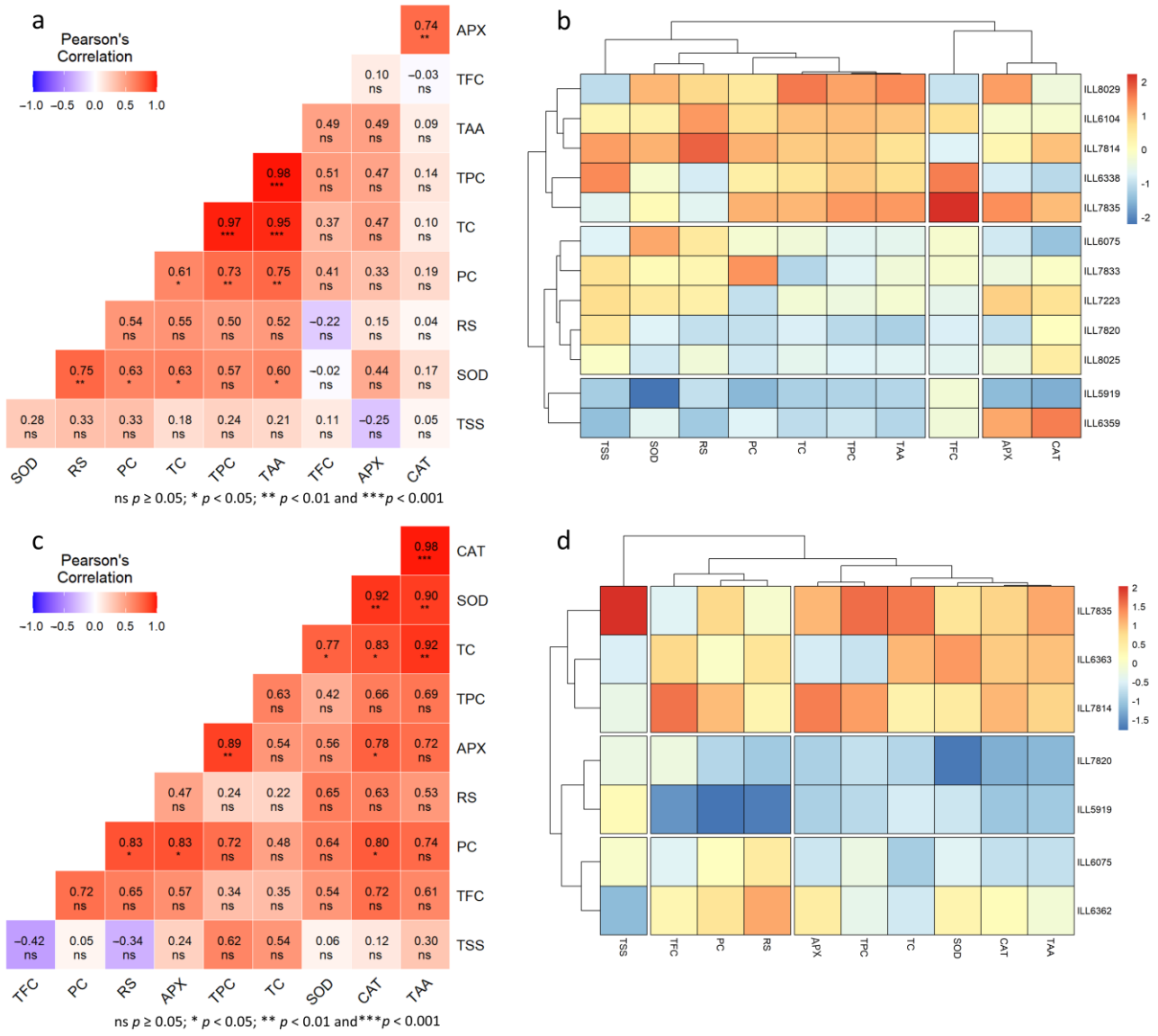

Figure 9. Pearson's correlation coefficients (**left**) and dendrogram along with heatmap (**right**) between various traits for high-temperature (**a**,**b**) and drought-stress (**c**,**d**) conditions.

Under drought stress conditions, SOD was positively correlated with CAT, TAA, and TC (Figure 9c). In addition, APX was found to be positively related to CAT, PC ($p < 0.05$), and TPC ($p < 0.01$). Furthermore, TAA exhibited a positive correlation with TC at $p < 0.001$,

whereas PC had a positive correlation with RS (r = 0.83) under drought stress. The heatmap analysis revealed that CAT, SOD, APX, TPC, TAA, and TC were clustered in the same group, whereas RS, PC, and TFC formed the second group (Figure 9d). In the third group, TSS was clustered individually. Furthermore, accessions ILL7835, ILL6363, and ILL7814 were found in the same cluster, while susceptible accessions ILL7820 and ILL5919 were found in a separate group. The accessions ILL6075 and ILL6362, which are drought-tolerant, were found in the third group.

3. Discussion

In the near future, alterations in climatic conditions are anticipated to lead to a frequent occurrences of droughts and extreme temperatures, thereby posing a substantial threat to food security [35]. Both stress factors impose a detrimental effect on metabolic processes, affecting vital aspects like photosynthesis, water relations, nutrient absorption, and consequently, grain yield [36]. Hence, the selection of tolerant genotypes to heat and drought stresses and the comprehension of the underlying mechanisms by plant breeding scientists are extremely important for increasing agricultural productivity in arid and semi-arid environments. In the current study, fourteen lentil accessions were subjected to high-temperature stress (32/20 °C) throughout the critical reproductive stage, while seven accessions were assessed under drought stress by reducing field capacity to 50%. The two sets of accessions were chosen based on their tolerance to high temperatures and drought stress discovered from our previous investigations [33,34]. Under both stressed conditions, the accessions ILL5919 and ILL7820 were used as susceptible controls. The outcomes of our study revealed that the application of high-temperature and drought-stress conditions induced substantial modifications in both enzymatic and biochemical processes in the tested lentil accessions. Remarkably, the concentrations of ascorbate peroxidase, catalase, and superoxide dismutase exhibited a significant increase in the heat-tolerant accessions as compared to the heat-sensitive ones. This suggests a stronger enzymatic response to high-temperature stress in the heat-tolerant accessions. Tolerant accessions ILL7814 and ILL7835 significantly increased CAT and SOD activity in response to heat and drought stresses; however, there was no significant difference in APX across all accessions exposed to drought stress. The moderately tolerant accession ILL6363 accumulated the highest SOD under drought stress, whereas the tolerant accessions ILL6075 and ILL6362 had significant SOD activity. Additionally, it was observed that under both high-temperature and drought-stress conditions, the susceptible accessions ILL5919 and ILL7820 exhibited the lowest antioxidant activity, which suggests their reduced capacity to combat oxidative stress under these challenging conditions. When subjected to high-temperature stress, the moderately heat-tolerant accessions (ILL6075, ILL6359, ILL7223, and ILL8025) displayed a significant increase in the levels of CAT, APX, and SOD enzymes compared to normal conditions. SOD activity was also significantly increased in the ILL6363 accession, which is moderately drought-tolerant.

Both drought and high-temperature stresses can result in elevated production of reactive oxygen species, which hinder various metabolic processes in plants, including those related to photosynthetic components. As a protective mechanism, plants deploy antioxidant enzymes such as CAT, APX, and SOD as their primary defense to scavenge H_2O_2 with different mechanisms and suppress its toxic impacts [37]. Our study observed heightened activities of antioxidant enzymes in nearly all tolerant and moderately tolerant lentil accessions, as a response to the stresses of high temperature and drought, which was in agreement with the outcomes of other investigations [38–42]. Under adverse environmental conditions, complex antioxidant enzyme activities play a critical role in overcoming uncontrolled reactive oxygen species generation and protecting plants from oxidative damage [23]. Through our research, we discovered significant variations in CAT, APX, and SOD activities between tolerant and sensitive accessions. In fact, the tolerant accessions exhibited higher levels of antioxidant enzyme activities compared to the susceptible ones. In lentil, Chakraborty and Pradhan [43] also observed an elevation in APX,

SOD, and CAT levels in response to high-temperature stress, with the tolerant accessions demonstrating a more pronounced increase. However, our findings indicate that APX activity did not exhibit a significant increase in lentil accessions under drought stress. Furthermore, the APX activity for four specific accessions (ILL5919, ILL7820, ILL6363, and ILL6075) displayed a non-significant decrease, suggesting a lower level of oxidative stress due to induced antioxidant enzyme activities and detoxified reactive oxygen species, as elucidated by Singh et al. [41]. Reduction in CAT and APX activities was also reported in two grasses and two legume species that were subjected to high temperatures and drought stress [44]. Nonetheless, in comparison to the sensitive controls, the majority of drought-tolerant accessions exhibited notably higher levels of APX and CAT. Similar results regarding antioxidant enzyme activities were reported in other studies in response to heat and drought stresses [45,46]. In addition, our findings confirmed the results of Sarker and Oba [42] in demonstrating that accessions with higher tolerance to drought stress had higher SOD activity contribution compared to accessions with lower tolerance to water stress. Therefore, these enzymatic antioxidants (CAT, APX, and SOD) can be used as economical and effective metabolic indicators for either screening or enhancing lentil germplasm for heat and drought tolerance in the early growth stages.

In lentil, Hosseini et al. [47] identified two groups of SOD genes (Cu/Zn-SOD and Mn-SOD) up-regulated in mitochondria, chloroplast, and cytosol under drought- and heat-stress conditions. Furthermore, previous investigations also showed that SOD activity increased in drought-tolerant genotypes of lentil under drought stress [48], and chloroplastic and cytosolic Cu/Zn-SOD and Mn-SOD were up-regulated under drought stress in soybean [49], wheat [50], and rice [51]. In addition, several cytosolic and chloroplastic APX genes such as lentil homolog of Atperoxidase 1, 12, 52, 67, and 17 were commonly up-regulated under drought and heat stress, and they were found to be important genes used as indicators of tolerance to heat and drought-stress conditions [47]. Rahman et al. [52] also reported that the expressions of APX, Cu/Zn-SOD, and CAT genes are the key candidates in antioxidant defense, which might be useful in molecular breeding strategies for heat and drought tolerance.

Proline stands as one of the most crucial osmoprotectants in plants, serving as a solute that adapts to alterations in the cell's water environment and mitigating the adverse impacts of high temperatures and drought stress on plants [53]. After the removal of stress relief, proline generated during stressful conditions can additionally function as a reservoir for energy and ammonia sources directly influencing plant metabolism [4]. Therefore, higher accumulations of proline are associated with increased stress tolerance. Proline also acts as an antioxidative defense molecule, and it scavenges ROS to activate specific gene functions that are essential for the plant's recovery from stresses [54]. In the current investigation, we observed a significant increase in proline content in all tested accessions subjected to high-temperature and drought stresses, except for the susceptible accessions ILL5919 and ILL7820, which displayed non-significant increases under these stress conditions. These findings align with the observations of Singh et al. [41] who reported higher proline levels in heat-tolerant lentil accessions when compared to sensitive ones. Increasing proline content was also reported by Hosseini et al. [47] in response to heat and drought stresses, and it was identified the hub gene P5CS2 (delta 1-pyrroline-5-carboxylate synthase 2) as the major transcription factor involved in lentil tolerance to these stresses. Furthermore, Hayat et al. [55] highlighted the role of proline in preventing photodamage to chloroplast thylakoid membranes by effectively scavenging and/or reducing the production of reactive oxygen species. Moreover, the utilization of exogenous proline has been shown to regulate drought stress by stimulating plant growth, which is achieved by triggering the antioxidant mechanism, reducing oxidative damage, enhancing the synthesis of compatible solutes, and accelerating proline accumulation, resulting in enhanced photosynthesis and yield attributes [56]. El-Beltagi et al. [57] found that the sequence combination of antioxidant and proline increased the antioxidant defense system and osmolyte synthesis, resulting in accelerated plant growth in chickpea under drought stress. Exogenous proline has the

remarkable capacity to interact with enzymes, effectively preserving protein aggregation and preventing thermal denaturation [58].

The soluble sugar and reducing sugar are also among the most important osmotic regulators in plants, serving diverse cellular functions including energy storage, osmoprotectants, and signals for adapting to abiotic stresses [59]. Under the influence of high-temperature and drought stress, the concentrations of total soluble sugar increased in almost all examined accessions within our study. The same trend was also reported by Shah et al. [31] who observed a significant rise in soluble sugar content and proline content in lentil cultivars exposed to water-deficit stress conditions. Furthermore, Sehgal et al. [60] reported similar outcomes, demonstrating a significant increase in soluble sugar in response to heat and drought stresses in lentil. These results confirmed that heat and drought stresses increased the soluble sugar content in plants and thus reduced the osmotic potential and maintained the normal water demand, as described by Li et al. [61]. On the other hand, reducing sugar content was reported to be correlated with grain yield under heat and drought stresses [32]. Sita et al. [62] found that heat-tolerant lentil accessions accumulated more reducing sugar than sensitive ones, which they attributed to an increase in acid invertase activity (hydrolysis of sucrose into fructose and glucose) in the tolerant accessions, which sustained their redox ionic homeostasis.

In addition, our results revealed that tolerant accessions exposed higher concentrations of total flavonoids, total phenolics, and total tannins than sensitive accessions under both high-temperature and drought-stress conditions. A drastic difference has been also observed in the concentrations of phenols, flavonoids, and tannins of control and drought-stress soybean cultivars [49]. These secondary metabolites function as osmoprotectants and play a crucial role in scavenging free radicals against oxidative damage caused by abiotic stress [63]. According to Hassan et al. [64], total phenolic and flavonoid contents are strong antioxidants, and their accumulation in plants has a strong association with drought-stress tolerance. Ghazghazi et al. [54] concluded that polyphenolic compounds serve as subtracts for the synthesis of enzymes in the antioxidant defense network. Further, the second hypothesis suggests that plants use antioxidant enzymes as their primary method of reducing ROS levels rather than phenolic compounds [65]. Total antioxidant activity significantly increased due to both high-temperature and drought stress. Under stressful conditions, tolerant accessions accumulated a greater total antioxidant concentration than susceptible ones. The heat tolerant accessions ILL6104, ILL6338, ILL7814, and ILL8029 had the highest accumulations of total antioxidant activity in high-temperature conditions, while ILL6363, ILL7814, and ILL7835 were the top accumulators under drought stress, inhibiting DPPH by up to 60%. In the presence of high-temperature and drought stress, the increased antioxidant capacity in tolerant accessions can help mitigate oxidative damage to membranes and proteins, thereby preserving the functionality of organelles and promoting overall plant performance [66].

Based on our findings, proline had a significant positive correlation with CAT and APX under drought stress, and with SOD and TAA under high temperatures. These results may be related to the adequate actions of these antioxidants, which can remove ROS and reduce the harmful effects of stress conditions. These outcomes are consistent with the findings of previous reports in lentil [41,67] and other species [38,68,69]. In addition, we observed a significant correlation between TPC and TAA under high-temperature stress conditions. Similar findings were found in several studies [70,71], suggesting that the total antioxidant activity largely depends on the presence of large amounts of phenolic compounds [72]. Our results also revealed a positive correlation between APX and both CAT and TPC under drought stress. Furthermore, TPC accumulation in plants has been shown to have a strong correlation with their resistance to stress [4]. Similarly, TC was positively correlated with TAA, CAT, and SOD under drought stress and with TAA, SOD, PCA, and PC under conditions of heat stress. Total tannin content in plants has been shown to have significant antioxidant activity for removing and preventing ROS formation [73]. Reducing sugar also had a positive correlation with SOD at high-temperature stress and

proline content during drought stress. Hussain et al. [4] found that reducing sugar played a significant role in osmoregulation, which protects plants from environmental conditions.

4. Materials and Methods

4.1. Plant Material

A set of 14 diverse lentil accessions were selected based on findings from our previous studies [33,34]. The material included four heat-tolerant accessions, two drought-tolerant accessions, two heat- and drought-tolerant accessions, four moderately heat- and drought-tolerant accessions, and two accessions sensitive to both stresses. The material was obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA). The information regarding these accessions can be found in Table 1.

Table 1. Description of origin, IG number, and classification of lentil accessions used in the current study.

Accession	IG Number	Origin	Classification
ILL5919	69528	Ethiopia	HDS
ILL6075	70130	Pakistan	DT
ILL6104	70159	Pakistan	HT
ILL6338	71270	Pakistan	HT
ILL6359	71291	Pakistan	MT
ILL6362	71294	Pakistan	DT
ILL6363	71295	Pakistan	MT
ILL7223	75942	Nepal	MT
ILL7814	114931	Nepal	HDT
ILL7820	114951	Nepal	HDS
ILL7833	115006	Nepal	HT
ILL7835	115010	Nepal	HDT
ILL8025	117726	Pakistan	MT
ILL8029	117734	Pakistan	HT

HT, heat-tolerant; DT, drought-tolerant; MT, moderately tolerant to heat and drought; HDT, tolerant to heat and drought; HDS, susceptible to heat and drought; IG, ICARDA germplasm.

4.2. Experiments Details

The experiment was conducted in a controlled glasshouse at ICARDA, Rabat, Morocco (latitude 33°58′45.7″ N longitude 6°51′44.9″ W, and altitude 52 m). In plastic pots (20 cm in diameter and 18 cm in height) filled with compost garden soil and sandy-loam soil in a ratio of 2:2 w/w, seeds were planted, and each filled pot weighed a total of 3 Kg. In each pot, five seeds were initially sown at a depth of 2 cm and were thinned to three per pot after emergence. Three treatments were used: control (C), drought stress (D), and heat stress (H). Randomized complete block design with four replications was used for each treatment. Plants were kept in normal conditions, which included 25/18 °C (day/night) temperature, 90/60% relative humidity (RH), and 450 µmol m^{-2} s^{-1} light intensity. During development, plants were fully irrigated daily between 09:00 and 10:00 h; however, irrigation was restricted during the flower initiation stage for drought treatment to impose water stress. At the first day of anthesis phase (i.e., first flower appearance), plants in heat treatment were exposed to temperatures above 32 °C for four hours in a growth chamber at 65–70% RH for a period of seven days. By maintaining 50% of the relative water content of the control conditions for nine days, plants under drought stress were subjected to severe water stress. Whole plants were collected in liquid nitrogen and kept at −80 °C for various analyses after being subjected to high temperatures and drought treatments during the reproductive period. Concurrently, plants grown under controlled conditions were also collected.

4.3. Total Phenolic Content

Total phenolic content was measured using the technique outlined by Waterhouse [74]. In a 2 mL Eppendorf tube, 200 µL of extract from whole plant and 1 mL of Folin–Ciocalteu reagent were added. After five minutes of incubation, 800 µL of aqueous sodium carbonate 20% was added to the mixture. The mixture was incubated in the dark at room temperature for 60 min. At 765 nm, the absorbance was measured with a Thermo scientific spectrophotometer. The total phenolic concentration was calculated using a gallic acid standard curve. The results were expressed as mg gallic acid equivalents (GAE) per gram of fresh weight. Total phenolic content (TPC) was calculated as follows [33]:

$$(\mathrm{TPC})_{(\mathrm{mg}\,\frac{\mathrm{GAE}}{\mathrm{g}}\mathrm{dw})} = (\mathrm{C} \times \mathrm{V})/\mathrm{m} \quad (1)$$

where C is the total phenolic concentration (mg L^{-1}), V is the volume of the extraction, and m is the fresh weight of the plant material (g).

4.4. Estimation of DPPH-Scavenging Activity

The antioxidant potential of the extracts from whole plants was determined by their ability to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, according to Williams et al. [75]. Briefly, 200 µL of each extract preparation was added to 1.8 mL of 0.1 mM DPPH dissolved in methanol. The absorbance of the resulting solution was measured at 517 nm after the agitation of the mixture for 30 min at room temperature. A decrease in absorbance indicates an increase in DPPH-scavenging activity. The scavenging effect was characterized using the following equation:

$$\text{scavenging activity} = \left(\frac{\text{Blank absorbance} - \text{Sample absorbance}}{\text{Blank absorbance}}\right) \times 100 \quad (2)$$

4.5. Total Soluble Sugar Content

Total soluble sugar concentration from whole plants of each accession was measured using the colorimetric method developed by Dubois et al. [76]. An amount of 2 mL aliquot of a carbohydrate solution was mixed with 1 mL of 5% aqueous solution of phenol in test tubes. Furthermore, a total of 5 mL of concentrated sulfuric acid was rapidly added to the mixture. After allowing the test tubes to stand for 10 min, they were vortexed for 30 s and placed in a water bath at room temperature for 20 min to develop the color. Then, a spectrophotometer was used to record the light absorption at 490 nm. Reference solutions were prepared using the same procedure as described above, except that the 2 mL aliquot of carbohydrate was replaced with bi-distilled water. The phenol used in this procedure was redistilled, and a solution of 5% phenol in water (*w/w*) was prepared immediately before the measurements.

4.6. Reducing Sugar Content

The analysis of residual reducing sugar from whole plants of each accession were analyzed using the 3,5-dinitro-salicylic acid (DNS) method described by Miller [77]. In a 25 mL test tube, 1 mL of standard or test sample was added, followed by 3 mL of DNS reagent. The reaction mixture was heated in boiling water for 5 min. The absorbance spectra of the test samples were measured at 540 nm using 2 mm path length quartz cuvettes. The absorbance measurements were compared to a standard mannose curve (180.2 mw) ranging from 0 to 20 mM to identify the presence of reducing sugar released in each extract.

4.7. Total Flavonoid Content

Total flavonoid constituents of whole plant extracts were determined using an aluminum chloride colorimetric assay and Quercetine as the standard [78]. An amount of 1 mL of extract was added to a 10 mL flask containing 4 mL of distilled deionized water. A reagent blank using double-distilled H_2O (ddH_2O) instead of the extract was prepared

at this stage. After adding 0.3 mL of 5% $NaNO_2$ to the flask, it was vortexed and kept for five minutes. Then, the mixture was treated with 0.3 mL of 10% AlCl3 solution and left for five minutes. At the sixth minute, 2 mL of 1 M NaOH was added, and the total volume was diluted to 10 mL with ddH_2O. The solution was vigorously vortexed, and absorbance was measured at 510 nm against the blank using a spectrophotometer. The procedure was repeated with all standard solutions of Quercetine (20–100 mg/L, Sigma-Aldrich, St. Louis, MO, USA) to obtain a standard curve. The total flavonoid content of the extracts was determined and expressed as milligrams of Quercetine equivalents (QE) per gram of extract. All samples were examined in triplicate.

4.8. Total Tannin Content

Total tannin content was calculated using the vanillin/HCl method developed by Price et al. [79]. The reagent was prepared by mixing equal parts of methanol solutions of 8% HCl and 1% vanillin, just before the reaction. Aliquots of 0.1 mL of lentil leaf extracts and 0.2 mL of vanillin/HCl reagent were mixed and incubated at 30 °C for 20 min. The absorbances were measured at 500 nm. A standard curve was created by using Catechin methanol solutions ranging from 0.1–2.5 mg mL^{-1}. The total tannin content of the extracts was determined and expressed as micrograms of Catechin equivalent (CE) per gram of extracts.

4.9. Total Soluble Protein

The method developed by Lowry [80] was utilized to predict the concentration of soluble proteins. An amount of 100 mg from the whole plant was macerated in 0.1 M phosphate buffer (pH = 7.0) and centrifuged at 513.16× *g* for 15 min to obtain a supernatant. The supernatant was treated with 5 mL of TCA (trichloroacetic acid; 15%) and kept at 4 °C for 24 h. The mixture was then centrifuged at 513.16× *g* for 15 min to separate the precipitates. The supernatant was discarded, and the precipitate was dissolved in 0.1 N NaOH (1 mL), allowed to dissolve completely for 18 h, and then treated as an extract.

4.10. Proline Content

The proline content was extracted and quantified using the methodology described by Bates et al. [81]. A total of 100 mg of leaf was homogenized in 10 mL of 3% sulphosalicyclic acid and centrifuged at 3000 rpm for 10 min. Filtrate was used for estimation by adding 2 mL of acid ninhydrin and 2 mL of glacial acetic acid. After one hour in a 100 °C water bath, the mixture was transferred to a separating funnel. Then, 4 mL of toluene was added. Two distinct layers were formed, and the absorbance of the upper layer was measured at 520 nm using the toluene as the blank reference.

4.11. Antioxidant Enzyme Activity Assays

The activity of catalase (CAT) was determined following the method of Chance and Maehly [82]. Whole plant was ground in mortars using liquid nitrogen. The enzyme was extracted from 0.5 g of tissue using chilled 50 mM sodium phosphate buffer (pH 7.0), containing 1% (*w*/*v*) polyvinylpyrrolidone (PVP), and was centrifuged at 12,000 rpm for 20 min at 4 °C. Enzyme estimation was performed using supernatant. The reaction mixture contained 1.9 mL of 50 mM sodium phosphate buffer (pH 7.5), 0.1 mL enzyme extract, and 1 mL of hydrogen peroxide. The decrease in absorbance after every 30 s interval for 2 min was recorded at 240 nm against a blank. Enzyme activity was calculated using the extinction coefficient of H_2O_2 (0.036 mM^{-1} cm^{-1}) and was expressed as µmol mg $protein^{-1}$ min^{-1}.

Ascorbate peroxidase (APX) activity was measured using the method of Nakano and Asada [83]. A total of 0.5 g of powdered sample was extracted with 2 mL of 50 mM sodium phosphate buffer (pH 7.5) containing 1% PVP and 2 mM ascorbate. The homogenized material was centrifuged at 12,000× *g* for 20 min at 4 °C. Supernatants were used as the crude enzyme source for the enzymatic assays. The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 2% H_2O_2, and 0.1 mL

enzyme extract in a final volume of 3 mL. The decrease in absorbance at 290 nm for 1 min was recorded, and the amount of ascorbate oxidized was calculated using the extinction coefficient (e = 2.8 mM^{-1} APX was defined as 1 mmol mL^{-1} per min at 25 °C, cm^{-1}). Enzyme activity was expressed as µmol mg $protein^{-1}$ min^{-1}.

Superoxide dismutase (SOD) activity was assayed by monitoring its ability to inhibit the photochemical reduction of nitro-blue tetrazolium chloride (NBT). One unit of SOD is defined as the amount of enzyme required to inhibit the rate of NBT reduction by 50 percent at 560 nm. In 3 mL of reaction mixture, 75 µM NBT, 1.5 mM riboflavin, 50 mM sodium bicarbonate, 13 mM methionine, 0.1 mM EDTA, 50 mM potassium phosphate buffer (pH 7.5), and 100 µL of enzyme extraction were present. The samples containing the mixture were shaken for 15 min under light at 78 µmol photons s^{-1} m^{-1}, and the absorbance at 560 nm was measured. A control reaction mixture that did not exhibit any color development was used as a reference, and its absorbance at 560 nm was subtracted from the absorbance of the reaction solution [84].

4.12. Statistical Analysis

A least significant difference (LSD) test was applied at a significance level of $p < 0.05$ to compare the mean values and determine significant differences. Pearson's correlation coefficient was calculated for the three treatments using metan package in R version 4.1.3 and RStudio version 1.3.31093 [85]. A dendrogram along with a heatmap was performed with the pheatmap R package (Version 1) [86].

5. Conclusions

The current study concluded that tolerant lentil accessions exhibited superior responses to high-temperature and drought-stress conditions than sensitive accessions and produced high enzymatic activities for almost all components. These results align with the classification of these accessions as either tolerant or sensitive to high-temperature or drought stress. In addition, we also observed a moderate response of moderately tolerant accessions under both stresses. The inclusion of differentially sensitive accessions allowed us to delve into the mechanisms associated with tolerance to high-temperature and drought stress, which was one of our primary objectives. Under stress, tolerant accessions maintained higher antioxidant activity, osmolytes, and phenolics metabolism. Consequently, these accessions hold promising potential for maintaining essential physiological processes and achieving high yield levels in regions that are significantly affected by increasing temperatures and drought events. However, more research is needed to investigate the underlying mechanism and identify genes associated with heat or drought tolerance in lentil.

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Abstract

This thesis explores a critical challenge facing lentil production globally: climate change, focusing particularly on the impact of heat and drought stresses. The aim was to identify new sources of tolerance to these stresses in lentil using the Focused Germplasm Identification Strategy. To achieve this, a set of 162 accessions was screened for heat and combined heat-drought stress tolerance under field conditions at two diverse locations (Marchouch and Tessaout) in Morocco. Novel potential sources of heat tolerance, such as ILL 7833, ILL 6338, and ILL 6104 at Marchouch, and ILL 7814 and ILL 8029 at Tessaout, were identified based on the heat tolerance index. Similarly, using the stress tolerance index, ILL 7835, ILL 6075, and ILL 6362 were found as the most tolerant accessions at Marchouch, and ILL 7814, ILL 7835, and ILL 7804 at Tessaout, under combined heat-drought stress conditions. Subsequently, 20 lentil accessions were selected for further evaluation under field conditions to explore the impact of both stresses on various traits related to phenology, grain yield, nutritional quality, and canopy temperature. These accessions were also assessed for genotypic variability in the limited transpiration (TRlim) trait in response to increased vapor pressure deficit (VPD) under controlled conditions. The study validated previous field screening results, showing that all tolerant genotypes exhibited high adaptation to high temperatures and combined temperature-drought stress under field conditions, along with clear TRlim at high VPD under controlled conditions. Two tolerant accessions (ILL 7833 and ILL 7835) demonstrated the lowest breakpoint observed in cultivated lentil. Additionally, accessions like ILL 7835, ILL 7814, and Bakria (ILL 4605) demonstrated a good nutritional quality alongside moderate to high yields under both heat and combined heat-drought stress. Afterward, twelve accessions were selected to elucidate the mechanisms underlying heat and drought tolerance in lentil accessions. Results revealed that tolerant lentil accessions exhibited higher antioxidant activity, osmolytes, and secondary metabolites under high-temperature and drought-stress conditions compared to sensitive accessions. This work contributes to a deeper understanding of lentil mechanism responses to drought and heat stresses, serving as a valuable resource for lentil stress tolerance breeding efforts.

Keywords: lentil, heat tolerance, drought tolerance, vapor pressure deficit, metabolite mechanisms, physiological responses, grain yield, nutritional quality

Résumé

Cette thèse explore un défi critique auquel est confrontée la production mondiale de lentille : le changement climatique, en mettant particulièrement l'accent sur l'impact des contraintes de chaleur et de sécheresse. L'objectif était d'identifier de nouvelles sources de tolérance à ces contraintes chez la lentille en utilisant la Stratégie d'Identification de Germplasm Ciblé. Pour ce faire, un ensemble de 162 accessions a été examiné pour la tolérance à la chaleur et à la combinaison chaleur-sécheresse dans des conditions de terrain à deux sites (Marchouch et Tessaout) au Maroc. De nouvelles sources potentielles de tolérance à la chaleur, telles que ILL 7833, ILL 6338 et ILL 6104 à Marchouch, et ILL 7814 et ILL 8029 à Tessaout, ont été identifiées sur la base de l'indice de tolérance à la chaleur. De même, en utilisant l'indice de tolérance au stress, ILL 7835, ILL 6075 et ILL 6362 ont été identifiés comme les accessions les plus tolérantes à Marchouch, et ILL 7814, ILL 7835 et ILL 7804 à Tessaout, sous des conditions de stress combiné chaleur-sécheresse. Par la suite, 20 accessions ont été sélectionnées pour une évaluation plus approfondie en conditions de terrain afin d'explorer l'impact des deux contraintes sur divers traits liés à la phénologie, au rendement en grain, à la qualité nutritionnelle et à la température du couvert végétal. Ces accessions ont également été évaluées pour la variabilité génotypique dans le trait de transpiration limitée (TRlim) en réponse à une augmentation du déficit de pression de vapeur (DPV) dans des conditions contrôlées. L'étude a validé les résultats précédents de criblage sur le terrain, montrant que tous les accessions tolérants ont présenté une forte adaptation aux températures élevées et au stress combiné chaleur-sécheresse sur le terrain, ainsi qu'une TRlim claire à des DPV élevés dans des conditions contrôlées. Deux accessions tolérantes (ILL 7833 et ILL 7835) ont démontré le plus bas point de rupture observé chez les lentilles cultivées. De plus, des accessions comme ILL 7835, ILL 7814 et Bakria (ILL 4605) ont présenté une bonne qualité nutritionnelle ainsi que des rendements modérés à élevés sous des contraintes de chaleur et de sécheresse combinées. Ensuite, douze accessions ont été sélectionnées pour élucider les mécanismes sous-jacents de tolérance à la chaleur et à la sécheresse. Les résultats ont révélé que les accessions tolérantes de lentilles ont présenté des réponses plus élevée pour l'activité antioxydante, les osmolytes et les métabolite secondaires sous des conditions de température et de sécheresse par rapport aux accessions sensibles. Cette étude contribue à une compréhension plus approfondie des réponses métaboliques des lentilles au stress hydrique et thermique, servant de ressource précieuse pour les efforts de sélection de la tolérance au stress des lentilles.

Mots-clés: lentille, tolérance à la chaleur, tolérance à la sécheresse, déficit de pression de vapeur, mécanismes métaboliques, réponses physiologiques, rendement en grains, qualité nutritionnelle

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