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Phenotypic and genetic variability of elite spring bread wheat genotypes for hybrid wheat breeding and performance

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Preamble

The work presented in this manuscript was conducted at the international center of agricultural research in the dry areas (ICARDA) in collaboration with Bio-bio Center, Physiology and Plant Biotechnology Unit at Faculty of Sciences, Mohammed V University, Rabat, supervised by both Pr. Souad Cherkaoui and Pr. Najib Bendaou and co-supervised by doctor Wuletaw Tadesse Degu.

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Abstract

Hybrid breeding is one of the most promising technologies for further sustainable yield increases. However, for the autogamous wheat, the profitability of hybrid varieties strongly depends on an enhanced cross-pollination, improved male sterility system and adequate level of heterosis. Thus, we applied several phenotyping methods for floral and flowering traits, and we studied the phenotypic variability within 196 wheat genotypes. High heritability coupled with high genetic advance were observed for most of the floral traits such as anther extrusion (AE), pollen shedding (PSH), degree (OPF) and duration of floral opening (DFO). The methods based on visual selection have proven to be effective in applied plant breeding programs. However, phenotyping most of the floral traits is laborious and time consuming. Hence, we employed molecular tools such as genome-wide association study (GWAS) and genomic selection (GS), widely considered to hold promises for the dissection of genomic regions harboring genes for hybrid traits. GWAS revealed multi-marker-based associations among some of the floral traits, most likely linked markers, suggesting a potential genomic region controlling these complex traits. In addition, GS revealed medium to high genomic prediction accuracy indicating the feasibility to implement genomic selection to predict the performance of hybrid floral traits with high reliability.

To investigate the relationship between seed set and the floral traits, we assembled a panel of seven male and eleven female lines based on their performance for the important floral traits and we placed them in crossing block trials. Male sterility was induced on almost 95% of the female plants using a chemical hybridization agent (CHA, Croisor®100) at 13.5 l ha^{-1} rate and seed set showed large genetic variance and high heritability. Moreover, seed set showed tight correlation with most of the evaluated floral traits allowing direct selection of the associated traits.

The resulting 23 hybrids from the best parent combinations were then evaluated over their parental inbred lines through calculation of the heterosis and estimation of the combining ability. Maximum mid-parent heterosis (MPH) of 13.35%, 7.21%, and 12.5% was recorded for biomass (BM), thousand kernel weight (TKW) and grain yield (YLD), respectively. While maximum values of best-parent heterosis (BPH) were 7.20%, 11.26% and 24.04% for BM, TKW and YLD, respectively. The magnitude of general combining ability (GCA) variance was greater than the specific combining ability (SCA) variance suggesting the preponderance of additive gene action for BM, TKW and YLD. The favorable GCA estimates showed a simple attitude to predict additive effects contributing to high heterosis and thus could be an effective approach to be used for selection of excellent parents in early generations. The exploiting these results may help in implementing hybrid wheat breeding on a large scale through facilitated, sustainable and robust hybridization process.

Keywords CHA, floral traits, flowering traits, GCA, genomic selection, GWAS, hybrid, heterosis, marker, SCA, seed set, wheat

Résumé

La sélection hybride est une des technologies les plus prometteuses pour un rendement durable et accru. Cependant, pour le blé autogame, la productivité des variétés hybrides dépend fortement d'une meilleure pollinisation croisée entre des lignées endogames de blé soigneusement sélectionnées, d'un système de stérilité mâle amélioré pour la production de semences hybrides et d'un niveau adéquat d'hétérosis. Plusieurs méthodes de phénotypage pour les caractères floraux et de floraison ont été appliquées et ils ont montré une large variation phénotypique entre les 196 génotypes du blé. Des héritabilités élevées avec progrès génétique élevé ont été observées pour la plupart des traits floraux. Les méthodes conventionnelles basées sur la sélection visuelle se sont avérées efficaces dans les programmes d'amélioration des plantes. Cependant, le phénotypage de la plupart des traits floraux est laborieux et dévoreur de temps. Par conséquent, nous avons utilisé des outils moléculaires tels que l'étude d'association pangénomique (GWAS) et la sélection génomique (GS), largement considérées comme outils prometteurs pour la dissection des régions génomiques abritant des gènes/marqueurs pour les traits hybrides. GWAS a révélé des associations multiples des marqueurs parmi quelques traits floraux, très probablement des marqueurs liés, suggérant une région génomique potentielle contrôlant ces traits complexes. En outre, la sélection génomique a révélé une précision de prédiction génomique moyenne à élevée indiquant la faisabilité de la sélection génomique pour prévoir la performance des traits floraux hybrides avec une grande fiabilité.

Pour étudier la relation entre la production de graines et les traits floraux, nous avons rassemblé un panel de sept lignées mâles et onze femelles sélectionnées en fonction de leurs performances pour les traits floraux importants et nous les avons placées dans des essais de croisement en blocs. La stérilité mâle a été induite sur près de 95% des plantes femelles en utilisant un agent d'hybridation chimique (CHA, Croisor®100) à raison de 13,5 l ha^{-1} et la production de graines a montré une grande variance génétique et une héritabilité élevée. Par ailleurs, la grenaison a montré une corrélation étroite avec la plupart des traits mâles permettant une sélection directe des caractères associés.

Les 23 hybrides résultants des meilleures combinaisons parentales ont été évalués par rapport à leurs lignées parentales endogames par le calcul du niveau d'hétérosis et l'estimation de l'aptitude de la combinaison. Le maximum d'hétérosis par rapport à la moyenne des parents (MPH) pour la biomasse (BM), le poids de mille grains (TKW) et pour le rendement (YLD) a été de 13,35 %, 7,21 % et 12,5 %, respectivement. Les valeurs maximales de l'hétérosis par rapport au meilleur parent (HBP) ont été de 7,20 %, 11,26 % et 24,04 % pour BM, TKW et YLD, respectivement. La variance de l'aptitude générale à la combinaison (GCA) était supérieure à la variance de l'aptitude spécifique à la combinaison (SCA), suggérant la prépondérance de l'action génique additive pour BM, TKW et YLD. Les estimations GCA favorables ont montré une attitude simple pour prévoir les effets additifs contribuant à une forte hétérosis et pourraient donc être une approche efficace pour la sélection des meilleurs parents dans les premières générations.

Mots clés Blé, CHA, GCA, GWAS, hétérosis, hybride, marqueur, traits de floraison, traits floraux, SCA, sélection génomique, semence

Abbreviations

AE	Anther extrusion
AFLP	Amplified Fragment Length Polymorphism
BC	Bonferroni Correction
BLAST	Basic Local Alignment Search Tool
BLUEs	Best Linear Unbiased Estimates
BLUPs	Best Linear Unbiased Predictors
BM	Biomass
BPH	Best-Parent Heterosis
CHA	Chemical Hybridization Agent
CIMMYT	International Maize and Wheat Improvement Center
CMS	Cytoplasmic Male Sterility
CWANA	Central and West Asia and North Africa
DAPC	Discriminant Analysis of Principal Components
DArT	Diversity Arrays Technology
DFO	Duration of Floret Opening
DH	Double Haploid
DH	Days to Heading
DMA	Days to Maturity
DNA	Deoxyribonucleic acid
E	Environment
FDR	False Discovery Rate
FHB	Fusarium Head Blight
FLD	Flowering Duration
FLE	Flowering End
FLP	Flowering Peak
FLS	Flowering Start
GA	Genetic Advance
Gac	Gibberellin Acid
GAM	Genetic Advance as percentage of the Mean
GAPIT	Genome Association and Prediction Integrated Tool
G-BLUP	Genomic Best Linear Unbiased Predictor
GBS	Genotyping By Sequencing
GCA	General Combining Ability
GCV	Genotypic Coefficient of Variation

GEBVs	Genomic Estimated Breeding Values
GLM	General Linear Model
GP	Genome-wide Prediction
GS	Genomic Selection
GWAS	Genome Wide Association Mapping
GYS	Grain Yield per Spike
h^2 /H^2	Repeatability or narrow sense heritability/ broad sense heritability
ICARDA	International Center for Agricultural Research in Dry Areas
IKI	Iodine potassium iodide
IWGSC	International Wheat Genome Sequencing Consortium
K	Kinship matrix
KI	Potassium iodide
LD	Linkage Disequilibrium
LOD	Logarithm of the Odds
LSD	Least Significant Difference
MAF	Minor Allele Frequency
MAS	Marker-Assisted Selection
MCMC	Markov Chain Monte Carlo
MER	Merchouch
MLM	Mixed Linear Model
MPH	Mid-Parent Heterosis
MTA	Marker Trait Association
OPF	Openness of Floret
PA	Predicted Ability
Pacc	Prediction accuracy
PCA	Principal Component Analysis
PCMS	Photoperiod-Sensitive Cytoplasmic Male Sterility
PCV	Phenotypic Coefficient of Variation
PLH	Plant Height
PM	Pollen Mass
PMS	Photoperiod-sensitive Male Sterility
PSH	Pollen Shedding
PV	Pollen Viability
Q	Population structure
qq	quantile–quantile

QTL	Quantitative Trait Loci
R^2	Correlation estimates
RAPD	Random Amplification of Polymorphic DNA
REML	restricted maximum likelihood
Rf	Fertility-Restorer
RFLP	Restriction Fragment Length Polymorphism
RIL	Recombinant Inbred Lines
RP	Recurrent Parent
SCA	Specific Combining Ability
SE	Standard Deviation
SEA	Sidi El Aydi
SL	Stigma Length
SNP	Single Nucleotide Polymorphism
SPL	Spike Length
SPS	Spikelets per Spike
SSD	Single Seed Descent
SSR	Simple-Sequence Repeat
TGMS	Thermo-sensitive recessive male sterility
TKW	Thousand Kernel Weight
TLP	Tillers per Plant
TP	Training Population
UPGMA	Unweighted Pair Group Method with Arithmetic mean
VAE	Visual Anther Extrusion
Ve	Error Variance
Vg	Genotypic Variance
Vg/e	Genotype by Year Variance
Y	Year
YLD	Grain Yield

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General introduction

Climate variability and extreme weather events pose a considerable threat to the global food security due to its adverse impacts on crop productivity. It has a large effect in increasing the frequency and severity of abiotic (heat and drought) and biotic (pests and diseases) stresses Pandey et al. (2017). Crop improvement *via* conventional and modern breeding methods have contributed greatly to increase global crop production. Development and deployment of climate resilient crop varieties with stable yield and combining potential resistance to both biotic and biotic stresses is an excellent way forward to fulfill future food requirements (Dhankher and Foyer 2018).

In most countries, cereal crops such as maize, rice and wheat serve as principal component of human diet providing 55% to 70% of total calories for over 4.5 billion people (Palacios-Rojas et al. 2020). Wheat is the second most important cultivated crop at global level with annual production area of more than 220 million hectares cultivated on 766 million tons (FAOSTAT).

Bread wheat (*Triticum aestivum* L., spp. aestivum) can be grown around the globe under widely varying agro-ecological conditions, including different temperatures, altitudes and latitudes, land and soil types. Bread wheat is an autogamous hexaploid (AABBDD, 2n=6x=42) with three similar but not identical copies of most of its genes (Morris and Sears 1967). The flour of bread wheat is used to make French bread, Arabic bread, Chapati, biscuits, pastry products and for the production of commercial starch and gluten (Tadesse 2016).

Over the years, enhanced yield potential and stability have been observed. However, even with the remarkable progress achieved to date, wheat productivity needs to boost to meet 60% more food requirements for 9.6 billion-world population by 2050 (Kumar et al. 2020a). Therefore, new strategies and innovative breeding approaches to break yield barriers and substantially enhance the level of productivity might be applied (Jordaan et al. 2015). In this context, hybrid breeding technology has great potential to capture significant yield benefits through exploitation of heterosis (hybrid vigor) (Longin et al. 2014; Mette et al. 2015). Hybrid wheat has shown superior grain potential for enhanced yield performance and stability compared to pure-line varieties (Mühleisen et al. 2014). However, Edwards (2001) explored the factors limiting the growth of hybrid wheat production given that less than 1% of the global wheat area is planted with hybrids. The cleistogamous nature of wheat, limited genetic variability, inadequate levels of heterosis, lack of a cost-efficient and inefficient seed production systems,

and lack of defined heterotic groups are the major hindrance of successful hybrid seed production (Zhou et al. 2005). Thus, optimization of floral and flowering traits is the first prerequisite to ensure sufficient cross-pollination. Many traits such as flowering time, plant height, extrusion of anthers with viable pollen, shed pollen outside the florets, adequate pollen mass and wide and extended floret opening were seen as important traits for maximum pollination (de Vries 1971; Whitford et al. 2013). In addition, the respective male and female parental lines used for hybrid production need to possess specific trait combinations. The male should be taller than the female, with long extruded anthers producing large quantities of viable pollen over an extended time able to disperse meters away. In comparison, the female ideotype should be shorter with wide flower opening and long hairy stigmas that are receptive for extended periods (de Vries 1972; Longin et al. 2012; Whitford et al. 2013). Most importantly, the flowering time for both parents has to be synchronized. However, the identification of potential male and female lines with these trait combinations is very challenging due the intensive work and time requirements for phenotyping. Hence, suitable high-throughput phenotyping and application of genomic approaches can be used to simplify and expedite the selection.

Genome wide association mapping (GWAS) is a promising alternative to dissect the genetic architecture of complex traits (Yang et al. 2014). To date, little is known about the genetic mechanisms controlling male and female flower development (El Hanafi et al. 2021a). While the genetic architecture of male floral traits has been fairly studied, female traits are far less understood (El Hanafi et al. 2021a). For the few studies previously conducted in this regard, the genome-wide scan detected only few quantitative trait loci (QTL) with small or medium effects and no major QTL other than *Rht-D1* were associated with anther extrusion and pollen mass, indicating a complex genetic architecture of these two traits (Boeven et al. 2016). In this case, where the trait is complex and mainly governed by many loci with small effects, genomic selection (GS) can be a good approach to select superior genotypes with suitable trait combinations based on the genomic estimated breeding values (GEBVs) (Crossa et al. 2017). However, a successful genomic selection depends on degree of prediction accuracy generated by the best predictive model. As per the principle of GS, genome wide markers are used to capture all possible genetic variations in the population and each QTL governing a trait is in linkage disequilibrium (LD) with at least one marker. The accuracy of GS, in addition, relies on LD between specific alleles of markers and QTL (Krishnappa et al. 2021).

The genetic variability within a germplasm represents the initial steps towards implementing male and female pools for hybrids breeding program. Large genetic variation and high heritability of hybrid wheat-related traits allow adequate identification of best performing male and female genotypes for cross-pollination test. Improved floral architecture has been shown to be valuable to maximize seed set on female plants and thus tremendously improve the cost-efficiency of hybrid seed production. Higher seed set per head in female tester lines demonstrates the success of the hybridization system used (Whitford et al. 2013). Several fertility control systems have been explored to induce male sterility in wheat such as chemically induced male sterility mainly known as chemical hybridization agents (CHAs), cytoplasmic male sterility (CMS) system and thermo-photosensitive male sterility (Whitford et al. 2013). The ability to exploit adequate heterosis level is the important factor that drive hybrid seed production and make it economically feasible. Heterosis is a result of allelic and non-allelic gene interaction in either homozygotes or heterozygotes under a specific environmental influence. Although heterosis has been observed in wheat, its level is widely different among F1 crosses produced. Hence, advanced breeding technologies and more intense screening for lines are warranted to identify the cross combinations that express desirable heterosis compared with their parents (Koekemoer et al. 2011). The best performing parents and crosses provide information concerning the nature and the importance of gene effects influencing quantitative traits (Amiruzzaman et al. 2013; Fasahat et al. 2016).

For the above facts, the major objectives of this study were:

1- To evaluate genetic variability in bread wheat panel for male and female traits with potential relevance for hybrid wheat breeding programs, assess their correlations, heritability and genetic advance for hybrid related traits, and in order to identify time- and cost-effective proxies for good outcrossing ability. The phenotypic approaches and proxies applied in addition to the yellow rust disease score will help in assembling a panel for hybrid seed production.

2- To identify the genetic basis of various male and female hybrid potential traits by performing a genome-wide association study to identify loci associated with the traits of interest and investigating the potential of genome-wide prediction (GP).

3- To investigate the efficiency of the CHA Croisor®100 on the selected female in a crossing blocks, assess hybrid seed set of the successful hybrids produced in order to investigate its genetic variance and heritability and its correlation with male and female traits.

4- To evaluate hybrids performance by calculating the mid-parent (MPH) and best-parent heterosis (BPH) and determine patterns of general and specific combining ability.

Chapters description

Chapter I- Literature review: In this chapter, we gave description of wheat, its history and origin, its life cycle, the challenges that wheat production is facing, and we introduced how hybrid wheat breeding with genomic tools can help to overcome the production constraints.

Chapter II- Phenotypic evaluation of elite spring bread wheat genotypes for hybrid potential traits: This chapter has been already published in Euphytica journal in 2020. We have evaluated 196 genotypes for different floral and flowering traits. We have applied different phenotypic approaches in order to evaluate the genetic variability and assess the heritability, genetic advance and correlations among the traits. Selection criteria based on the performance *per se* were applied to select the best performing genotypes and identify time- and cost-effective proxies that enhance the outcrossing ability in wheat.

Chapter III- Genome-wide association and prediction of male and female floral hybrid potential traits in elite spring bread wheat genotypes: This chapter represents a published paper in Plants journal in 2021 in which we have investigated the genetic basis of 196 genotypes for hybrid potential traits using genome-wide association and prediction.

Chapter IV- Hybrid seed set in relation with floral traits and estimation of heterosis and combining abilities for yield and its components in wheat: This chapter is under review in Crop Science journal in which we have evaluated seed set in relationship with the floral traits in a panel composed of parents selected based on their performance as described in chapters II, III, and also based on the response of the genotypes to yellow rust infection (article published in Euphytica and referenced in the annex 2). We investigated the genetic variance and heritability of hybrid seed set and its correlation with male traits. A chemical hybridization agent (CHA, Croisor®) was applied as a male sterility system and allowed a production of 23 hybrids. These hybrids were evaluated for their heterosis level and combining ability.

Chapter I: Literature review

I-1. History and origin of bread wheat

Since the Agricultural Revolution for about 12,000 years ago in the Fertile Crescent of the Middle East, route of developing domesticated wheat from wild emmer (reported variously as *T. araraticum*, *T. turgidum* ssp. dicoccoides, or *T. dicocoides*) is no doubt a landmark in the history of wheat evolution (Bell 1987; Shewry 2009). The transition from wild form with natural seed dispersal to non-brittle rachis type represented a key adaptative change in early stages of wheat domestication. Followed by complex evolutionary changes such as grain retention and threshability, changes in plant architecture, dwarf and semi-dwarf plants, yield improvement, changes to photoperiod sensitivity and nutritional value (lower grain protein and mineral concentrations), modern wheat has become a fodder and a widely grown cereal in the world with more than 220 million ha planted annually. Cultivated wheat is classified into two major categories. The tetraploid durum wheat (2n = 4x = 28, AABB) and the hexaploid bread wheat (2n = 6x = 42, AABBDD) (Figure I-1). The donor of the A genome is the wild wheat *Triticum urartu* (2n = 2x = 14, AuAu) known as einkorn (*Triticum monococcum* L.) and the B genome comes from the wild diploid *Aegilops speltoides Tausch*. *Aegilops tauschii*, also known as Tausch's goatgrass is the diploid progenitor of the D genome. Finally, spontaneous hybridization between *Triticum turgidum* and *Aegilops tauschii* gave rise to the hexaploid *Triticum aestivum* which represents the cultivated bread wheat form after domestication and centuries of cultivation and selection.

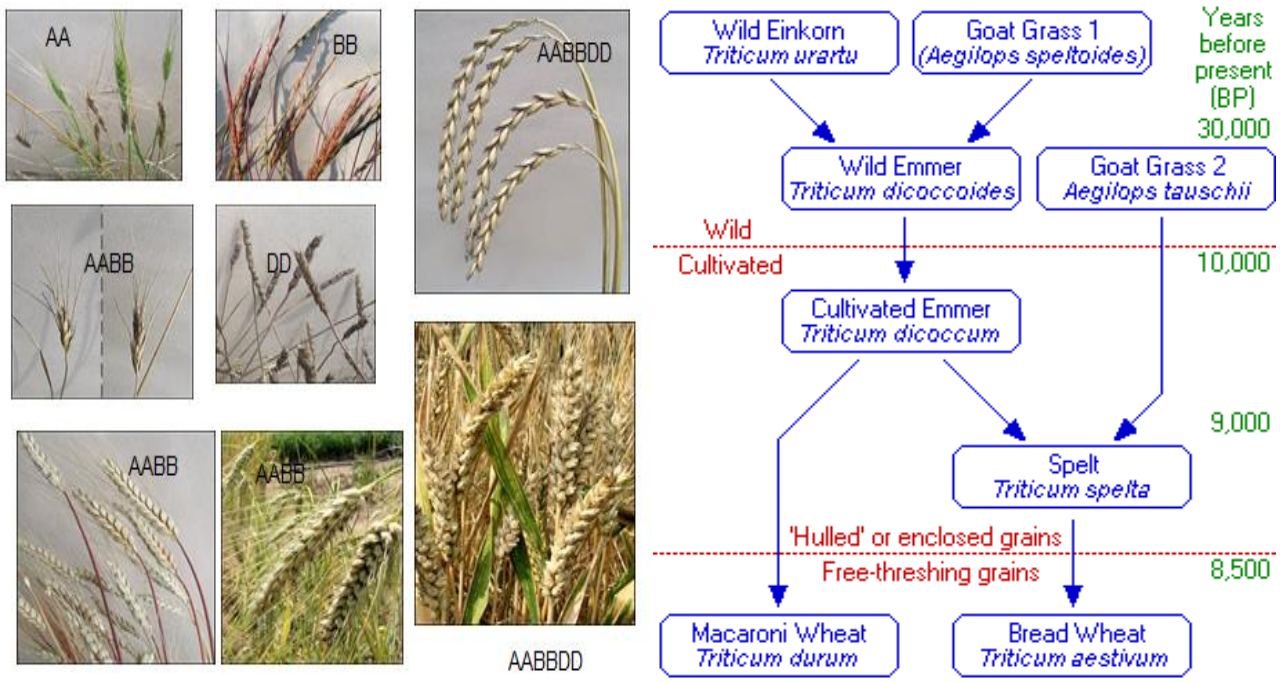


Figure I- 1: The evolutionary and genome relationships between cultivated bread and durum wheats and related wild diploid grasses.

I-2. Bread wheat life cycle

The performance of a wheat genotype, which is normally assessed by its yield potential and adaptability, is dependent on various environmental factors that adversely affect the growth. Biotic and abiotic stresses affect wheat differently during each development stage. Therefore, understanding the different growth stages is crucial to help farmers in optimizing the yield. There are six basic stages (Figure I-2):

- Germination and emergence: when seed sown, the embryo pushes out the first seminal root into the soil and increase the surface area for maximum water absorption. The emergence requires the seed coats to split by the expanding coleorhiza and form the primary roots (Symons et al. 1984). At the same time, the coleoptile which is a sheath that facilitates the first leaf emergence elongates towards the surface.
- Tillering: after the first three leaves emergence from the soil, the crown of the plant usually becomes noticeably distinct, and the development of new tillers and secondary root crown system starts. The first tiller is formed at the base of the first leaf and later tillers are usually synchronized with the emergence of each subsequent new leaf that develops on the main shoot. The axillary buds in the axils of each leaf of a tiller may produce its own subtillers once the three leaves are fully developed. Therefore, each additional tiller produced may

grow its own stalk and head then the numbers of tillers will determine the potential yield of the plant.

- Jointing and booting: jointing starts when the stalk form its second node. At this point, the internode region elongates and pushes the four nodes that are stacked in the crown upward producing a long stiff stem that carry the head. The developing head within the flag leaf sheath is pushed as the peduncle and sheath elongate. The booting stage ends when the awns start appearing from the flag sheath and the floral organs are well formed at this level.
- Heading/flowering: the heading stage begins when the awns emerge from the sheath of the flag leaf. To determine the heading stage of a field or a plot, at least 50% of the plants must have 50% of the emerged spikes. The flowering starts 4-5 days later after a complete heading and lasts for 5-7 days depending on the environment and on the weather conditions. Wheat flower usually remain closed due its cleistogamous nature and the flowering begins slightly above the middle portion of the spike and continues towards the top and the base. The flowering is marked by the extrusion of the three anthers of each floret that burst and release the pollen, commonly known as anthesis, and the anthers fade to white as flowering completes. The duration of the stigma receptivity is variable depending on the cultivar/variety and the weather conditions, but it's reported to be 3-13 days only (de Vries 1971). Once the fertilization occurs, the embryo and endosperm begin to form.
- Grain filling: the developing endosperm begins as a milky fluid marking the beginning of the grain filling stage. The kernel accumulates most of its dry weight during the dough development and takes all the nutrients from the stalk and leaves. Kernels reach their maximum dry weight (kernel physiologically mature) at the hard dough stage even though it still contains approximately 30% of moisture content.
- Maturity: the kernel continues to lose the rest of its moisture and the grain is ripe and ready for harvest and the green plant tissue fades to straw.

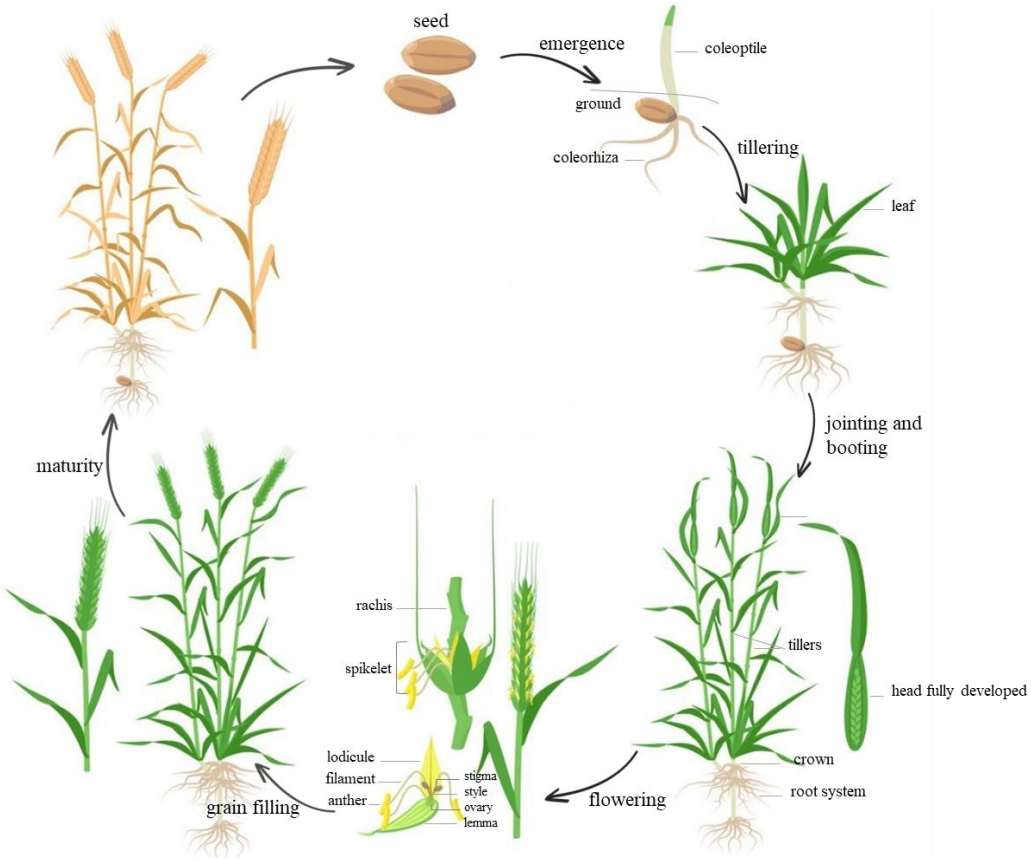


Figure I- 2: Life cycle of wheat.

I-3. Wheat production trends

Wheat is one of the principal cereal grains produced and consumed globally and its demand is almost synonymous with demand for food. World wheat production is ranked second after maize accounting for 29.8% on total cereal production (FAOSTAT). It is grown on 220 million hectares with an annual production 766 million tons produced in 2019 (Figure I-3). In general, the production and the harvested area showed a relatively flat trend pattern. From the 1960s through to 2019, global production reached the peak in 2017 with 772.3 Mt grown somewhat in the same total harvested area dedicated to wheat. China (134 Mt), India (102 Mt), and Russia (75 Mt) are the highest producer countries in 2019, together accounting for 41% of global production (Figure I-4) (FAOSTAT). Followed by U.S.A, France, Canada, Ukraine, Pakistan, Australia, Turkey, Germany, and Argentina, which together accounted for a further 34%. However, despite the significant increase observed in wheat production, many of the developing countries that depend on wheat as staple crop are not yet self-sufficient in production. Egypt, among the northern African countries, is the larger importer with an average of 10.5 Mt during the period 2010-2019 of a total value of 2.8 billion $US (Figure I-5). Morocco, as fourth large importer, relies heavily on wheat import to cover its consumption due

to the large gap between production and consumption with an average of 4 Mt during for the same period (Figure I-5).

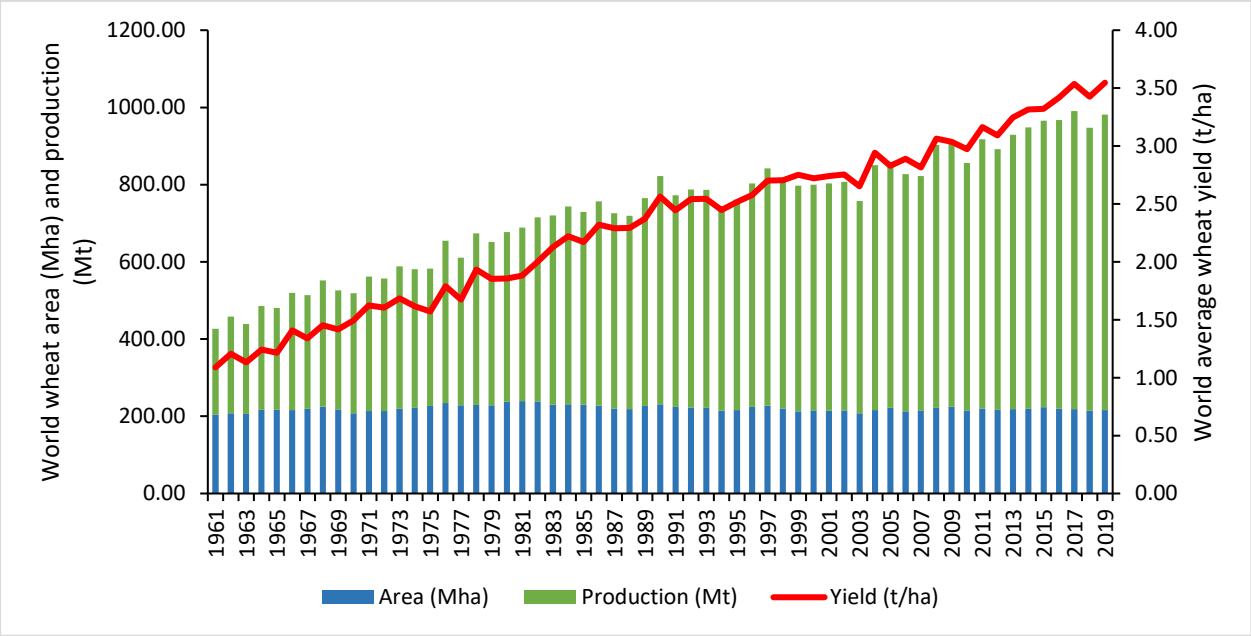


Figure I- 3: World wheat yield, production, and area from 1961 to 2019 (FAOSTAT).

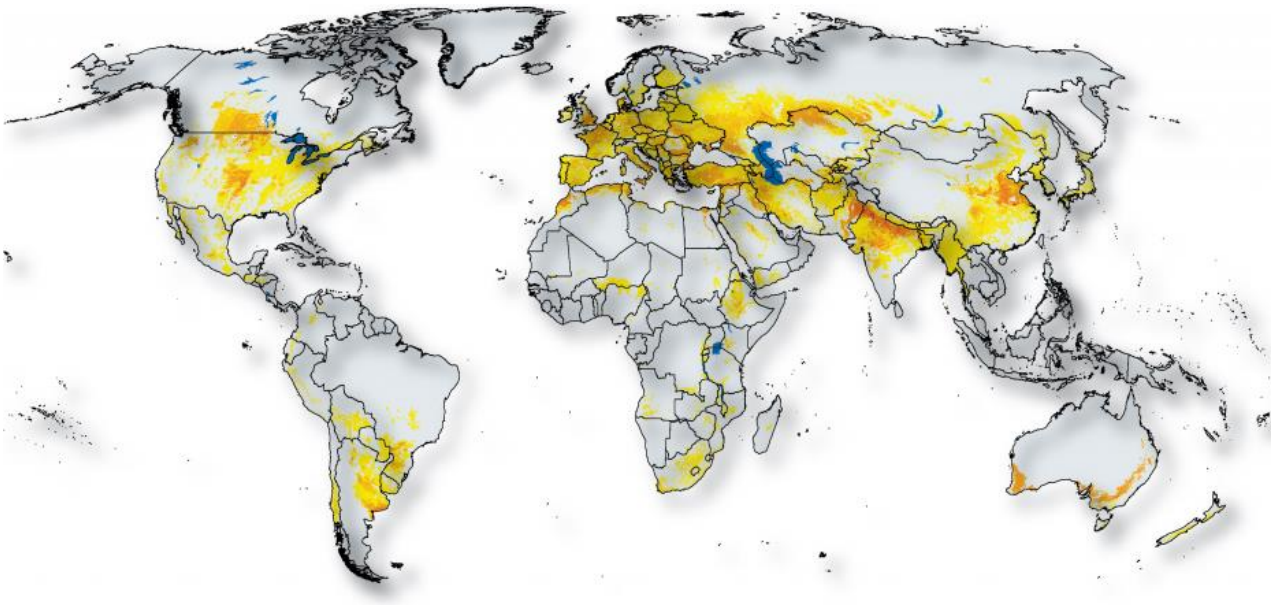


Figure I- 4: Global Wheat Cultivation. Dark yellow and brown colors indicate areas where more wheat is grown. Map based on You et al. (2014). (Source: wheat.org).

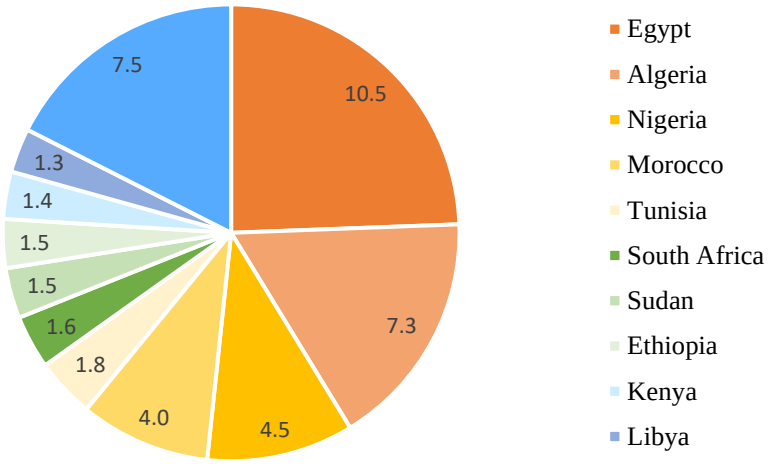


Figure I- 5: Wheat import in the developing countries (Mt) (FAOSTAT)

I-4. Challenges to wheat production

The demand for wheat is increasing with the increasing population. Fulfilling this demand, requires increased level of wheat production through increasing genetic gains and controlling stresses. Basically, wheat production is constrained by two types of environmental stresses (biotic and abiotic stresses) at different levels of intensity.

Climate change has a negative impact overall wheat grain and yield and on protein concentration due to rising temperature that shorten the growing cycle and thus less time to intercept light for photosynthesis to be supplied. Asseng et al. (2019) have shown that grain and protein yield are expected to be lower and more variable in most of the low-rainfall regions, with nitrogen availability limiting growth stimulus from elevated CO_2 concentration. It is therefore important to introduce genotypes with delayed anthesis time and increased grain filling rate to overcome the negative effects of increasing temperature on yield.

In addition, a changing climate is impacting the planet in different ways resulting in increased temperatures, infrequent and erratic precipitations, and affected land areas under flood or water deficit which consequently hampers wheat productivity. Heat and drought are the most multidimensional and unpredictable abiotic stresses that can affect wheat plant at any growth stage. Both are more likely to occur simultaneously in semi-arid and hot growing regions in North Africa, Argentina, Mexico, Australia, South Africa, and the Mediterranean countries, and in high latitude, semi-arid growing regions of central and eastern Asia, Canada, the USA, and Kazakhstan (Johnson 1973; Araus et al. 2002; Pradhan et al. 2012)

High temperature and drought together limit the development of the root system which result in decreased leaf area, leaf number per plant, leaf size, and leaf longevity (Boonjung and Fukai 1996; Huang et al. 2012). It shortens the tillering phase and affects survivability of the productive tillers resulting poor number and unfertile tillers (Barnabás et al. 2008; Riaz et al. 2018). During the flowering period, heat and drought stresses provoke an early stage of gametogenesis and negatively affects the microspore and pollen cell development (Saini et al. 1984). Moreover, if the temperature is above the range from 12 to 22°C in droughty areas, reduced grain filling rate and duration occur leading to lower seed weight and thus grain yield loss up to 69% (Ji et al. 2010; Pradhan et al. 2012). In developing countries, the impact of the combined heat and drought stresses is huge. Morocco as an example, harvested in 2015-2016 season 2.7 Mt (1.9 Mt bread wheat and 0.9 Mt durum wheat) in a very droughty season which represents approximately 38% of the total production (7.1 Mt) in 2016-2017 (FAOSTAT). Besides the above constraints, cold/frost, salinity, water logging and preharvest sprouting stand also as important abiotic stresses that limit wheat productivity. Therefore, it is essential to improve the adaption to abiotic stresses through trait-based selection and promotion of tolerant varieties.

Moreover, biotic stresses pose a great threat on wheat production worldwide. Fungal diseases such as rusts (Puccinia spp.), Helminthosporium, septoria (*Septoria tritici*), tan spot (*Pyrenophora tritici repentis*), fusarium (Fusarium spp.), the bunts and smuts, root rot, along with pests like wheat stem sawfly, russian wheat aphids, sunn pest and hessian fly, limit the potential yield performance of wheat. Stripe (yellow) rust caused by *Puccinia graminis f.* sp. tritici is the most prevalent and devastating disease globally, causing yield losses of about 5.5 million tones, or 979 million dollars, per year (Beddow et al. 2015). In 2016, 88% of the global wheat production is assumed to be covered by susceptible cultivars (Beddow et al. 2015). Consequentially, future yellow rust epidemic outbreaks remain a constant threat to yield stability. A prominent example is the breakdown in resistance conferred by *Yr27* that forced farmers in Ethiopia to spend over 3.2 million dollars in fungicides in 2010 to save their crop (Ali et al. 2017). Later on, in 2017, severe epidemics in major wheat growing area in the Central Asia Central and West Asia and North Africa (CWANA) region like Algeria, Morocco, Syria and Turkey were reported (Morgounov et al. 2012). Similar cases were reported in Ethiopia and Kenya for wheat stem rust which mutates frequently in the regions (Pretorius et al. 2000; Pretorius at al. 2000; Prank et al. 2019).

I-5. Wheat breeding objectives

The main objectives of any wheat breeding program have been similar over many decades i.e development of high yielding, widely adapted varieties combining resistance /tolerance to the major biotic and abiotic stresses with acceptable end-use quality. Over the years, wheat breeding has significantly contributed to enhancing yield potential and stability. Comparing the 1961–1965 and 2013–2017 periods, average yield rose from 1.18 to 3.15 t/ha, and wheat production increased from 247 to 741 million tons (FAOSTAT). National programs and international research centers such as International Center for Agricultural Research in Dry Areas (ICARDA) and International Maize and Wheat Improvement Center (CIMMYT) played important roles in developing high yielding and widely adapted germplasm with resistance and/or tolerance to the major biotic and abiotic challenges prevailing in the developing world.

I-5-1. Breeding strategies

Conventional breeding is referred to traditional or classic breeding. Two genetically different plants are crossed to create an array of variation in segregating generations. The recombinants generated in the crosses either recombine the good traits of two parents or superior to either of the parental lines. In the hybridization program, breeder can choose more than two different parents and the number of the parent involved in crosses will determine the type of the hybrids produced (simple, double, and three-way crosses) (Oliveira 2007). Various breeding methods have been used to select the best recombinant. The breeding method chosen depends on the nature of the trait(s) of interest, its inheritance and the amount of the variability present (Acquaah 2015). The available financial resources will also be important in influencing the approach taken.

I-5.1.1. Pedigree selection:

The pedigree method has by far been the most common and established breeding method. Individual plants are selected from the F2 and the selfed seed from that individuals are tested for their performance as single plant progenies. Over successive generations, efficient selections are made and the desired plants carrying the trait of interest are advanced to the next generation by selfing and harvesting separately. The maximum genetic variation for the traits is observed in the F2 generation, and thus the population size should be maximum at that stage. These is no standard number, but a reasonable 2000 plants should be present (Singh 2004). In early generations (F3 and F4), selection is practiced both within and between families and only

the best appearing and uniform lines are usually advanced, but in the later generations (F6 and F7) selection is mainly between families and not within families. Many modifications exist based on the pedigree method. Yield testing may be done as early as the F3 or F4 generation. Often the residual seed for a progeny row is used for yield test after plant selections have been made. Individual plant selection may be terminated earlier if lines appear uniform.

I-5.1.2. Bulk selection:

Bulk method is simple, convenient and inexpensive that allows breeders to handle more crosses than they could with the pedigree selection method (Nilsson-Ehle 1908). Natural selection can be enhanced by growing each selected progeny and harvested in bulk over the generations. At some generation (mainly at F4 generation), single plants are harvested separately in the next generation in rows basis to select for families. The selected head-rows family will be promoted to a preliminary yield trial and to an advanced yield trial for multi-environment testing.

I-5.1.3. Single seed descent method (SSD)

SSD is the modification of bulk method which consists of generation advancement of the crosses without any selection applied. To maintain the existing variation within a cross, only a single seed from each generation of selfing is promoted to the next generation (Acquaah 2015). Basically at least 2-3 seeds from each plant are sown and then thinned randomly to one. So, the objective of this method is to maintain descendants from a maximum number of F2 plants in the population during the segregating generations (catch maximum of variation generated in the cross) in order to reduce loss of superior genotypes for characters, which have low heritability such as yield (i.e., maintain genetic variation). SSD method can use of off-season nurseries in the greenhouse to advance the generations and thus, increase the number of generations per year. It can be also modified to fit a breeding scheme, such as doing selection in the early generations which would be more advantageous to select better ones or discard obvious off-types. This method is useful in development of Recombinant Inbred Lines (RILs) mapping populations for several economically important traits such as pest and disease resistance.

I-5.1.4. Backcross method

The backcross is a form of recurrent hybridization by which a superior characteristic may be added to an otherwise desirable variety, that could be one or few traits (Harlan and Pope 1922). The F1 hybrid and the progenies in subsequent generations are repeatedly backcrossed to one

of the parents. The expected recurrent parent (RP) genome recovery would be 99.2% by six backcrosses, which is most similar to improved variety (Hasan et al. 2015). The Final backcross generation is followed by a selfing after which the selected lines will be homozygous for the desired allele from the donor line. If the trait of interest is produced by a dominant gene, this process involves four rounds of backcrossing within seven seasons. If the gene is recessive, this process requires more generations of selfing and thus nine or more seasons are needed (Vogel 2009). However, in cases where the recognition of the recessive allele is phenotypically difficult, markers linked to the gene of interest might be used.

I-5.1.5. Double haploid production

Double haploid (DH) technology ensures the production of complete homozygous plant in a single year which may significantly reduce cultivar development time. The development of wheat haploids includes two major steps: haploid induction and chromosome doubling. Chromosome doubling of haploid plants has been routinely and successfully performed using colchicine (Kisana et al. 1993). Anther culture and wheat × maize cross are the most systems commonly used double haploid production in wheat. In general, the anther culture system exploits the fact that certain proportion of pollen grains in situ are embryogenic which they develop into embryos once placed in artificial medium. While for wheat × maize cross method, embryo rescue is very essential to recover haploid plantlets (Santra et al. 2017). However, this method is laborious and maintaining maize pollen can be challenging over the course of wheat flowering period.

DH has proven to be a very successful for rapidly obtaining homozygous lines. The time gain is especially important for winter wheat which is limited on a single generation advancement per year contrary to spring wheat where at least two generations per year might be possible through SSD method or shuttle breeding approach. However, it can be expensive, labor intense, and it has a variable success rate (Dwivedi et al. 2015).

I-5-2. Application of molecular tools

In spite of the significant progress of wheat breeding, to date, investment for sustaining agricultural growth and food security continue to be critical and necessitates to accelerate the breeding process and increase the genetic gains through the application of molecular markers, genomics, proteomics, and other laboratory-based assays for marker assisted selection, and gene pyramiding purposes.

Efficient and effective breeding strategy is key for timely delivery of any product profile. The application of molecular markers for QTL mapping and marker-assisted selection (MAS) is being widely used. A molecular marker is a specific fragment of deoxyribonucleic acid (DNA) that can be identified within the whole genome through the polymorphism present between the nucleotide sequences of different individuals (Nadeem et al. 2017). From the 1990s to 2000s, Random Amplification of Polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), and simple-sequence repeats (SSR) were the most used markers (Yasui 2020). However, with the rapid evolution of next-generation genotyping and the concurrent development of informatics and data management tools, single nucleotide polymorphism (SNP) and diversity arrays technology (DArT) markers provide powerful resources for plant breeding (Appleby et al. 2009; Mammadov et al. 2012). SNP markers are DNA sequence variations that occur in single bases of nucleotide (adenine, thymine, cytosine or guanine) (Koopaee and Koshkoiyeh 2014). They are the most abundant molecular markers in crop genomes and more amenable to high-throughput genotyping. Wide range of genotyping platforms are available to address different purposes in different crops. Illumina® 9 K iSelect Beadchip Assay, Illumina® iSelect 90 K SNP Assay (Wang et al. 2014) and Axiom® 820 K SNP array (Winfield et al. 2016), in which respectively 9000 to nearly 820,000 SNPs can be evaluated in a single analysis. Also, using genotyping by sequencing (GBS), thanks to the arrival of next generation sequencing technologies, maps containing 20 to 450 K loci have already been generated for wheat (Poland and Rife 2012; Saintenac et al. 2013). The deployment of high-density genotyping platforms served for creating a resource for diversity studies, high resolution dissection of complex traits in wheat, identification of plant varieties and cultivars, germplasm enhancement, developing resistance to biotic and abiotic stresses, QTL analysis, construction of high-density genetic map, and genome-wide association analysis (Wang et al. 2014; Singh et al. 2019, 2020).

I-5-2-1. Genome-wide association study (GWAS)

The development of bioinformatics and data management tools have offered new possibilities and set cornerstones for modern plant breeding. Genome wide association mapping is a study design used to detect associations between specific genetic variants (i.e., loci, alleles) and common diseases or traits in a population (Tam et al. 2019). The genetic variants can be genotyped using any of the different high-density genotyping platforms (e.g SNP) enabling genetic dissection for further GWAS analyses. GWAS and bioparental quantitative trait locus

mapping have been widely used for identifying gene function (Mackay et al. 2009). However, GWAS distinct from biparental QTL mapping in two ways. First, because traditional QTL mapping relies on mapping populations derived from only two parents and consequently limited amount of variation can be assessed. Second, only limited recombination events take place during the F1 generation and thus it will be hard to locate the with high resolution (Rakshit et al. 2012). In contrast, larger genetic variation might be assessed using association mapping panel, and thus various allelic and cytoplasmic interactions might be assessed too allowing finer resolution of QTL location (Rakshit et al. 2012).

There are many considerations that should be taken into account when performing a GWAS (Figure I- 6):

- The phenotypic data with considerable population size, 100–500 individuals, should be well organized and filtered from the outliers (Alqudah et al. 2020). For simple check, data visualization using a boxplot may show the extreme outliers. Meanwhile, the phenotypic variation is an important part of the association analysis and removing outliers should not affect it. Moreover, only traits with moderate to high heritability estimates should be considered in GWAS because heritability is a good indicator of how much the genetic variance contributed to the phenotype and how much the phenotype is linked to the genotype (Alqudah et al. 2020).

- The population structure called also as population stratification is an important covariate to run GWAS. It is a statistical approach that aim to calculate relatedness correlation among the individuals within the population used due to the admixture (Alqudah et al. 2020). The selection of the population size and which material to be included define its structure based on geographic or growth habit, etc. The STRUCTURE software is a free available program that can be used to define the population structure and then estimate the proportion of clusters within the population so-called Q-matrix and then estimates which individual belongs to which subpopulation (Pritchard et al. 2000). STRUCTURE uses a systematic Bayesian clustering approach applying Markov Chain Monte Carlo (MCMC) estimation. The MCMC process begins by randomly assigning individuals to a pre-determined number of genetic groups, then variant frequencies are estimated in each group and individuals re-assigned based on those frequency estimates. The process is repeated many times, typically comprising 100,000 iterations, in the burnin process that results in a progressive convergence toward reliable allele frequency estimates in each population and membership probabilities of individuals to a population (Porras-Hurtado et al. 2013). Alternatively, principal component analysis (PCA),

which is a standard method for estimating population structure, can be easily applied for GWAS (Price et al. 2006). The PCA is presented in a scatter plot of PCA1 and PCA2 which presents most of the total variation between the individuals based on their genotypic data. If the genotypes are randomly distributed in the plot and form no clear groups, then there is no population structure, and vice versa. Most of the association mapping studies use both methods (STRUCTURE and PCA) to confirm their results (Wang et al. 2017; Ledesma-Ramírez et al. 2019).

- Kinship matrix (K) or genetic relationship matrix represents a widespread confounding factor in GWAS. The kinship matrix can calculate the relatedness between pairs of individuals using genotypic information (Yu et al. 2005). The high value of relationships among the individuals indicates high genetic similarity; the individuals clustered in the same group might be from the same geographical region or sharing the same ancestors. TASSEL 5 software provides a function to estimate the kinship matrix (Bradbury et al. 2007) or can be estimated using SPAGeDi software (Hardy and Vekemans 2002).

- Linkage disequilibrium (LD) is the nonrandom association of alleles of different loci in a given population. LD throughout the genome reflects the population history, the breeding system and the pattern of geographic subdivision, whereas LD in each genomic region reflects the history of natural selection, gene conversion, mutation and other forces that cause gene-frequency evolution (Slatkin 2008). LD can be measured by calculating the squared correlation coefficient (r^2) between each pair of SNPs using TASSEL 5 (Bradbury et al. 2007) and the LD decay plot can be plotted using R software and GGT 2.0 (van Berloo 2008), using data from SNPs anchored in the genetic map. The basic of any GWAS is to detect markers that are either associated with the trait of interest directly or are in linkage disequilibrium. The markers which are in LD or in the same haplotype block are usually dependent, whereas those in different blocks are mostly in weak LD or in linkage equilibrium (Li et al. 2021)

The success of GWAS can be attributed to the above requirements depending on the model used. The general linear model (GLM) or called naïve model and mixed linear model (MLM) are statistical models often proposed for performing GWAS. The GLM does not take the population structure into account. The MLM, on the other hand, considers the population structure in its model (Kinship or kinship + Q matrix + PCAs). It can be performed using TASSEL, GenStat, PLINK, or the genome association and prediction integrated tool package (GAPIT) in R. Each software program gives slightly different parameters as output results. The

main output can be presented in the Manhattan plot that illustrates, on a genomic scale, the p-values of all markers used in GWAS. The p-values should be ordered by chromosome and position and plotted in the x-axis, while the values in the y-axis represent the (–log10) of the p-value. The associated significant SNPs (lowest significant p-values) tend to show up as a signal on the Manhattan plot based on the chosen threshold. The most common and reliable threshold value is the $-\log10 \geq 3$ or it can be recalculated using different statistical procedures including the Bonferroni correction, Sidak correction, False Discovery Rate (FDR), permutation test, and Bayesian approaches (Kaler and Purcell 2019).

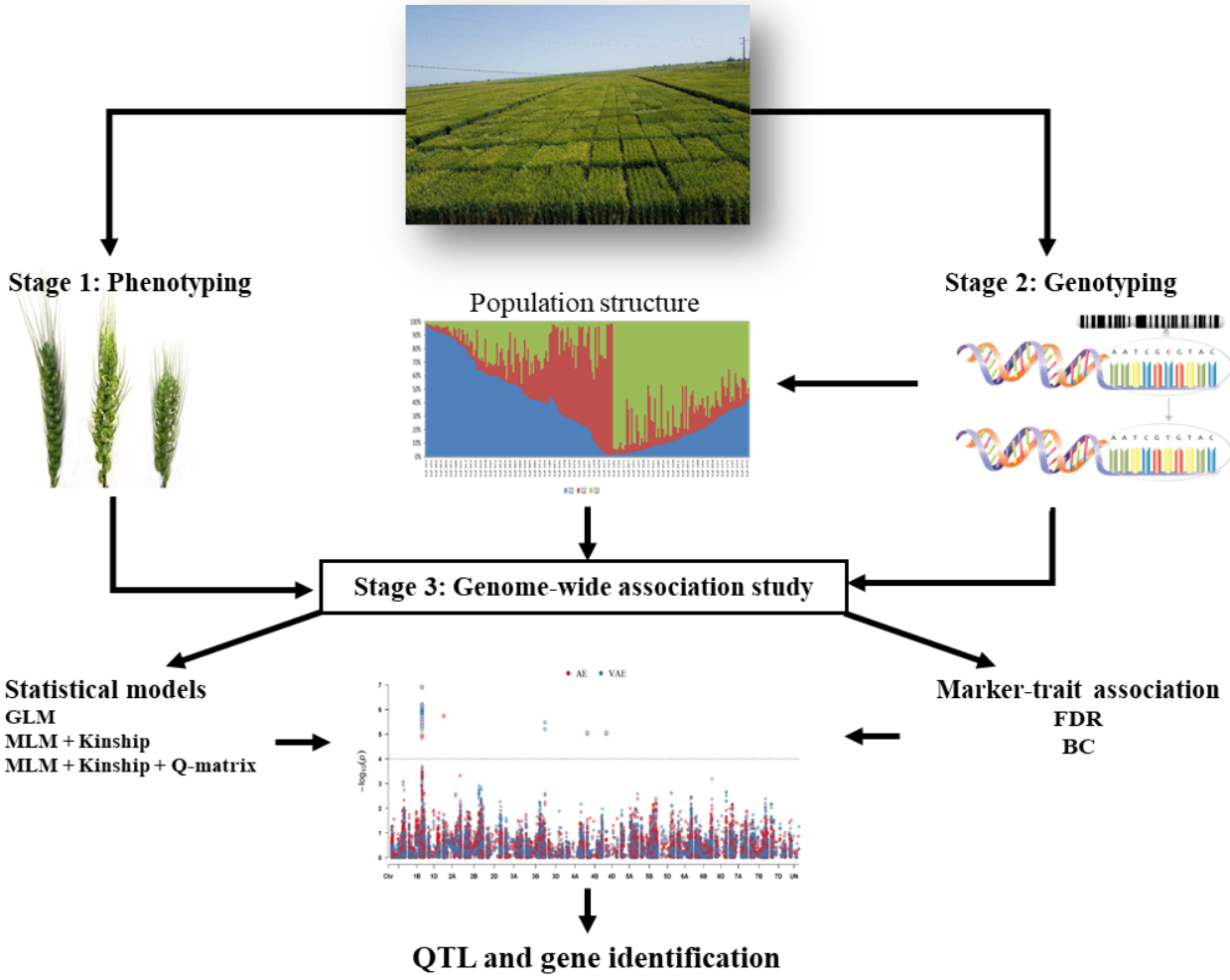


Figure I- 6: Genome wide association mapping principle.

Stage 1: Phenotyping, stage 2: Genotyping and stage 3: Genome-wide association study including statistical models, multiple-testing analyses, and software/packages for QTL and gene identification.

I-4-2-2. Genomic selection

Genomic selection (GS) or genome-wide prediction (GP) is an advanced form of marker-assisted selection that was first developed by (Meuwissen et al. 2001). It simultaneously

predicts the genetic values of selected candidates depending on genome-estimated breeding values (GEBVs) predicted from high density of markers that are distributed throughout the genome. These GEBVs allow breeders to select superior genotypes that would be suitable either as parent or for next generation advancement of the breeding program. GS contrasts with traditional MAS given that there is no defined subset of significant markers used for selection and MAS comprises selecting individuals with QTL-associated markers that have major effects. Instead, GS requires dense marker coverage across the genome to explain the total genetic variance and no gene or QTL identification is needed prior to selection (Crossa et al. 2017). Moreover, GWAS is about identification of marker trait associations (MTAs), while GS considers all possible phenotypic traits at the same time and associates them with genome wide markers and determines a breeding value.

The application of GS requires phenotypic data and marker information of a training population (TP) of individuals that is used to train a model that takes genotypic information from a candidate population of untested individuals. This second set of individuals is considered as a validation set to estimate the prediction accuracy of the model used. GS has been extensively applied in animal and plant breeding to predict traits using the molecular markers and pedigree information to facilitates the rapid selection of superior genotypes and accelerates the breeding cycle (Crossa et al. 2017).

I-4-2-3. Gene annotation

The rapid evolution of next-generation sequencing opened up unprecedented opportunities to sequence and analyze a wide range of crops. Efforts to generate a reference genome of wheat for the scientific community began in 2005 with the establishment of the International Wheat Genome Sequencing Consortium (IWGSC). The genome assembly of *Triticum aestivum*. Chinese Spring estimated in 14.5 Gb chromosome scale in total has been assembled, improved, and released by IWGSC through its platform for public access. Recently, the 21 wheat chromosomes were re-constructed for the new reference sequence, IWGSC RefSeq v2.1 with approximately 10% sequence length of the previous version IWGSC RefSeq v1.0 (Zhu et al. 2021). Wheat genome annotation aims to identify and assign positions in Chinese spring reference bread wheat sequence to the linkage regions of the significant marker-trait association based on basic local alignment search tool (BLAST) in the server. Therefore, gene annotation is a powerful tool for plant breeding being used to understand wheat evolution

(Marcussen et al. 2014; El Baidouri et al. 2017) and many genome wide association studies (Arruda et al. 2016; El Hanafi et al. 2021a).

I-6. Hybrid breeding

Global wheat production needs to increase by at least 50% before 2050 to meet the demand of a growing population (https://www.openaccessgovernment.org/demand-for-wheat/83189/). However, with the numerous biotic and abiotic stresses that limits wheat productivity and the limited genetic variability in wheat germplasm, there is a need to explore new innovative approaches to break yield barriers and enhance wheat sustainability (Venske et al. 2019). Hybrid wheat has shown to be one of the most promising options to capture significant yield benefits through exploitation of heterosis. Heterosis or hybrid vigor is the superiority of a hybrid performance over its corresponding parental inbred lines (mid- or best parent heterosis), and it is often expressed in the form of higher yield and other desirable attributes (Koemel et al. 2004). Hybrid wheat occupies less than 1% of the total world wheat area and produced mainly in Europe, China and India (Kempe et al. 2014; Gupta et al. 2019). In Europe, the area under hybrid wheat is increased from about 100,000 ha in 2002 to 560,000 ha in the year 2017–2018 (http://www.bcwagric.co.uk/hybrid_wheat) The main hybrid wheat-growing European countries are France and Germany with the main players in breeding companies being ASUR Plant Breeding SAS and Nordsaat Saatzuchtgesellschaft mbH, respectively (Longin et al. 2012). In India and China, hybrid wheat is cultivated in about 35,000 ha (Longin et al. 2012) and 66,700 ha area (Gupta et al. 2019), respectively. For long term success of hybrid breeding programs, the cleistogamous nature of wheat has to be addressed through cost-effective hybridization system, adequate exploitation of heterosis, high seed density, and development of high-yielding heterotic groups and patterns (Dreisigacker et al. 2005; Longin et al. 2012, 2013; Selva et al. 2020). There are several systems that induce male sterility; the European Hybrids are currently produced using chemical hybridization agents (CHAs), most commonly Croisor® 100 (Sintofen; former Dupont-Hybrinova, ASUR Plant Breeding SAS, France). In China, photoperiodic sensitivity and cytoplasmic male sterility (CMS) systems are being successfully used (Whitford et al. 2013). Wheat hybrids in India are developed based on cytoplasmic male sterility systems derived from *Triticum timopheevii* and CHA approach (Longin et al. 2012; Gupta et al. 2019).

I-6-1. Fertility control systems

I-6-1-1. Chemical hybridizing agents (CHAs)

Chemical hybridization agents, also known as gametocides, induce physiological abnormalities, which in turn prevents pollen development, pollen shedding, and/or pollen viability without affecting female fertility (McRae 2011). An advantage inherent to CHA use is that any inbred line may be used as female parent by simply spraying the chemical and therefore significantly reduce the production costs (Kofoid 1991).

The first report of CHA published in spring bread wheat was the effect of maleic hydrazide on suppressing pollen development (Hoagland et al. 1953). The first chemical compounds tested as CHA included anti-lodging and height-reducing agents like ethephon (Ethrel) (Wilson and Driscoll 1983), gibberellins (Porter and Wiese 1961), and RH531 and RH532 (Jan et al. 1976a, b). However, all these chemicals showed high level of phytotoxicity, inadequate level of male sterility across the environments and high risk of their commercial use. Therefore, research to cope with these negative attributes led to the development of the next generation CHAs such as fenridazon-potassium (RH-0007, HYBREX®) which is shown to be the most important chemical inducing 95-100% male sterility in diverse winter and spring wheats (Mizelle et al. 1989). This CHA became, at that time, the widely used in commercial hybrid wheat production both in Europe and USA. However, its association with reduction in hybrid seed quality and its narrow application window discouraged its use for long-term basis (Cisar and Cooper, 2002). Then after, several different pyridine mono-carboxylates were tested for pollen sterility in wheat. Among these, 4-hydroxy, 2, 6-bis (trifluoromethyl) methyl ester and 2, 4-bis (trifluoromethyl) benzoic acid induced complete pollen sterility in wheat (Ciha and Ruminski 2002). During the 1990s, two novel CHAs, namely "Genesis™" (marketed by HybriTech-Monsanto) registered as Clofencet by the US Environmental Protection Agency (Parodi and Gaju 2009) and "Croisor® 100" (sintofen, marketed by DuPont-Hybrinova, Saaten Union, now Asur Plant Breeding), were authorized and are currently used in Europe. These two modern CHAs, Genesis™ and Croisor® 100, are effective across a wide range of genotypes with less phytotoxicity effects but their narrow window application is their major limitation. Genesis® was used in wheat for commercial hybrid seed production in the USA and Europe until 2007 and Croisor® 100 is currently the most widely used CHA (Parodi and Gaju 2009). Many commercially viable hybrid wheat cultivars using CHAs were developed, and hybrid seed sales

was launched in several European countries including France, Germany, UK, Poland, Czech Republic, Hungary, Portugal, etc (Gupta et al. 2019).

I-6-1-2. Cytoplasmic male sterility (CMS)

Cytoplasmic male sterility in plants is based on rearrangements of mitochondrial DNA, which lead to chimeric genes and can result in the inability to produce fertile pollen (Hanson and Bentolila, 2004; Horn 2006). CMS can arise both spontaneously and following mutagenesis, or be the result of interspecific, intraspecific, and intergeneric crosses (Kaul 1988). In cultivated wheats, CMS lines can be produced by crossing initially a common wheat as pollen donor with a wild wheat (e.g. *Triticum timopheevii* Zhuk.) or related species such as Aegilops, Hordeum, and Secale, and then backcrossed to common wheat (Mukai and Tsunewaki, 1979; Wilson and Ross, 1962; Martín et al. 2008). The first effective cytoplasmic male sterility induced into wheat is *Aegilops caudata* cytoplasm reported in 1951 (Kihara 1951), opened up new avenues for commercially feasible hybrid seed production. Since then, more than 70 male-sterile cytoplasms have been studied in detail for incompatibility and cytoplasmic male sterility in wheat (Singh et al. 2010). Based on the type of the cytoplasm, CMS has been classified into several groups like T-CMS, K-CMS, and V-CMS (Table I- 1). The T-type is the male-sterile line with the cytoplasm of *T. timopheevi* (Wilson and Ross, 1962). K-CMS is *A. kotschyi Bioss* cytoplasm, and V-CMS is having cytoplasm of A. variabilis (Lucken 1987). *T. timopheevii* CMS system for hybrid seed production in wheat is the best among the available CMS and thus it has been widely used for hybrid wheat and triticale breeding (Adugna et al. 2004; Singh et al. 2010; Würschum et al. 2017). However, fertility restoration in lines carrying *timopheevii* cytoplasms shown to be partial and it was necessary to have at least two or more fertility-restorer (*Rf*) genes in the same male parent. This can be verified using marker-assisted selection.

Table I- 1: Types of CMS based on source of cytoplasm contributing to male sterility

Type of CMS	Species contributing male-sterile cytoplasm	Reference
T-type	*T. timopheevi*	Wilson and Ross (1962)
K-type	*Ae. kotschyi Boiss*	Mukai and Tsunewaki (1979)
V-type	*Ae. variabilis*	Mukai and Tsunewaki (1979) and Lucken (1987)
D^2-type	*Ae. crassa*	Murai and Tsunewaki (1993)

Nian type	*A. kotschyi*, *A. variabilis*, *A. ventricoca*, and *A. bicornis*	Niu et al (2003)
AL-type	*Triticum aestivum* L. alborubrum Körn	Xiaoke Zhang, Northwest, A and F University, Yangling, Shaanxi, China, personal communication
YA-type	*Triticum aestivum* var. CA805	Liu et al. (2006)
msH1	*Hordeum chilense*	Martin et al. (2008, 2010)

CMS involves three types of parental lines, the male sterile line (A-line) which is used as female line; the maintainer line (B-line) which is isogenic line of A-line without which the A-line can never be maintained; and the fertility restorer line (R-line) which represents the male fertile line used as pollen source in commercial seed production plot. Hybrids are the resultant of A x R crosses. Wheat male fertility can be resorted by nuclear-encoded fertility-restorer (*Rf*) genes. *Rf* genes are classified as either sporophytic or gametophytic in action depending on the affected tissues. Sporophytic *Rfs* are more practical for hybrid breeding because heterozygotes (*Rfrf*) produce 100% viable pollen grains whereas only 50% of pollen grains are viable in gametophytic heterozygotes. This may have undesired yield penalties in the hybrids produced. To date, perhaps no cytoplasmic male sterility system is able to provide stable sterility without any deleterious side effects from the cytoplasm, and with a successful fertility restoration of F1 hybrid.

For *timopheevii*-based CMS systems, eight *Rf* genes (*Rf1*-*Rf8*) located in 1A, 7D, 1B, 6B, 6D, 5D, 7B and 2D were reported (Schmidt et al. 1962; Robertson and Curtis 1967; Yen et al. 1969; Bahl and Maan 1973; Maan et al. 1984; Tahir and Tsunewaki 1969; Mukai and Tsunewaki 1979; Du et al. 1991; Zhou et al. 2005; Sinha et al. 2013). *Rf1*, *Rf2*, *Rf4*, *Rf5* and *Rf7* had their origin on *T. timopheevii* (Livers 1964; Bahl and Maan 1973; Ma and Sorrells 1995; Yen et al. 1969). While *Rf3* was discovered in *T. spelta* var. *duhamelianum* (Kihara and Tsunewaki 1966) and Rf6 and Rf8 were discovered in wheat (Bahl and Maan 1973; Sinha et al. 2013). Rf^{multi} is another gene located on chromosome 1B identified when the male sterility due to cytoplasms from *Ae. kotschyi*, *Ae. mutica* and *Ae. uniaristata* lacked in the arm 1BS instead of 1BL.1RS translocation in common wheat. This gene was assigned to a 2.9 cM segment on 1BS through the use of a number of 1BS/1RS recombinant lines (Tsunewaki 2015).

I-6-1-3. Genic male sterility (GMS)

Genic male sterility is controlled by nuclear genes that can broaden the choice of parental lines when compared with the CMS. Mutations in nuclear-encoded genes, that cause male sterility, can be spontaneous or induced. There were five nuclear genes from which three are dominant *Ms2* on 4DS, *Ms3* on 5AS and *Ms4* on 4DS (McIntosh et al. 2013; http://www.shigen.nig.ac.jp/wheat/komug i/genes/symbol Class List.jsp). The two genes *ms1* on 4BS and *ms5* on 3AL are recessive (Singh et al. 2015). In addition, six allelic mutants Pugsley's (*ms1a*), Probus (*ms1b*), Cornerst one (*ms1c*), FS2 (*ms1d*), FS3 (*ms1e*), FS24 (*ms1f*) (Singh et al. 2021), and *ms1g* (LZ mutant) (Zhou et al. 2008) were identified at ms1 locus. The first monogenic recessive mutation, which led to partial male sterility, has been identified in wheat cultivar Moulin (Trottet et al. 2010). Wheat lines carrying *Ms2* gene, which is a spontaneous mutation in common wheat "233" shown in Taigu in China, are 100% male sterile and are called as Taigu genic male-sterile wheat (Ni et al. 2017). Probus (*ms1b*) and Cornerstone (*ms1c*) are a result of ionizing radiation, whereas ethylmethane sulfonate-induced male-sterile wheats include FS2 (*ms1d*), FS20 (*ms5*, 3AL), and KS87UP9 (*ms3*, 5AS) (Maan et al. 1987). The GMS systems are useful in wheat breeding programs such as recurrent selection, which increases the frequency of favorable alleles while maintaining genetic variation in the breeding population (Yang et al. 2009).

I-6-1-4. Chromosomal male sterility (ChMS): XYZ-4E-ms system

Chromosomal male sterility is a hybridization system that involves the use of three lines (X, Y, and Z). The XYZ system utilizes the fertility restoring chromosome-5R from Secale cereale (Driscoll 1977). All the three lines are homozygous (ms1/ms1), located on 4BS, for the recessive allele. In addition, an alien chromosome 4E from *Agropyron elongatum*, carrying dominant allele *Ms1* (suppressing the activity of ms1), is also involved in the system XYZ-4E. X line has two doses (*Ms1/Ms1*), Y has one dose (*Ms1*/–) and Z having 0 dose (–/–) of the alien chromosome (4E) as an addition to the normal complement (Zhou et al. 2006). X and Y lines are fertile due to the presence of the *Ms1* on chromosome 4E, while line Z is sterile due to the absence of the chromosome 4E (Whitford et al. 2013). X line will be a true breeding line, while Y line on selfing will segregate into three types: 3% fertile disomic 4E addition X line, 33% monosomic 4E addition line (line Y) and 64% male sterile (line Z carrying no 4E). Seed of the Y line that is monosomic for 4E (*Ms1*) is identifiable by light blue aleurone (Ba), which is used (selfed) again for the production of male-sterile Z line, whereas disomic seed (X line) is

identifiable by a dark blue colour and discarded. Alien chromosome 4E is poorly transmitted through the male germ line, when plants monosomic for 4E are selfed, resulting in 64% white-seeded male-sterile progeny (Z line), which is used as male-sterile female line in the hybrid seed production field. Although GMS system looks attractive for commercial deployment, some limitations may discourage the use of this system; females that need to be ms1/ms1 in order to transfer the ms1 allele to the desired female parent and the desired males will need to be converted into monosomic alien addition lines carrying alien chromosome 4E with dominant allele Ms1.

I-6-1-5. Photoperiod-sensitive cytoplasmic male sterility (PCMS)

Photoperiod-sensitive cytoplasmic male sterility is a two-line system resulting from the presence of *Aegilops crassa* cytoplasm (Murai et al. 2016). Pollen abortion mostly occurs in a photosensitive male-sterile plants under long-day conditions of 15h or longer light periods during the floret differentiation stage. Three recessive photoperiod-sensitive male sterility (PMS) governing genes, *wptms1*, *wptms2*, and *wptms3* have been mapped on wheat chromosome 2B, 5B, and 1BS, respectively (Guo et al. 2006).

In this system, the female parent should be a fertile genotype under short-day period while the male should be sterile under long-days conditions, so that the female line will be maintained through self-pollination under short-days and hybrids will be produced under long-day period using a suitable pollinator (Murai et al. 2002, 2016). This feature of male sterility under long-days has been proposed to be useful in obtaining pure hybrid seeds in large scale (Murai et al. 2008).

I-6-1-6. Thermo-sensitive recessive male sterility (TGMS)

Thermo-sensitive recessive male sterility is a two-line system without a maintainer. Some wheat varieties are known to carry genes responsible for male sterility under reduced temperature conditions. A spontaneous TGMS mutant BS20-T was recovered in a commercially grown wheat variety BS20 in China which is completely sterile when a temperature is below 10 °C and completely fertile in temperatures above 13 °C (Li et al. 2006b). The TGMS is controlled by a recessive gene *tmsBS20T* located on 2BL flanked by SSR loci *Xgwm403* and *Xgwm374* at genetic distances of 2.2 and 4.5 cM, respectively (Ru et al. 2015). Xing et al. (2003) identified a nuclear gene *wtms1*, responsible for thermo-sensitive genic male sterility in wheat. Recently, six microRNAs and one tasiRNA (Transacting-siRNA)

have been identified as cold stress responsive small RNAs linked with male sterility in BS366 wheat (Tang et al. 2012). In addition, A3314 which is a thermo-sensitive cytoplasmic male-sterile (TCMS) wheat has been also developed by harboring the cytoplasm of *A. kotschyi* and male-sterile genes from *T. spelta* (He 2000; He et al. 2003). TGMS is considered a better system as compared to the photoperiod-sensitive male sterility, where days length differences are marginal such as in tropical regions (Virmani and Donald 1996). However, no successful application of TGMS for hybrid wheat breeding is reported previously.

I-6-2. Floral biology

The autogamous nature of wheat is one of the major practical limitations for hybrid seed production. Therefore, optimization of the different floral traits may substantially enhance the outcrossing capacity (Whitford et al. 2013). Numerous studies have addressed the importance of appropriate floral and flowering traits as key determinants for potential hybrid parents (D'Souza, 1970; de Vries 1971; Langer et al. 2014; Muqaddasi et al. 2017a, b; Song et al. 2018; Boeven et al. 2018; El Hanafi et al. 2020). Various phenotyping approaches have been addressed and applied for most of the hybrid related traits to check their variability and discuss their contribution for enhanced selection of the potential parents.

The inflorescence of wheat is composed of ear or called spike too; the main axis (rachis) has a number of spikelets which are made up of bract-like organs, glumes, and florets. Each floret consists of a flowering glume. The lemma and palea envelop the floral reproductive organs including anthers, stigma, ovary, and lodicules. The tip of the lemma may bear a long, medium or short awn or may be entirely awnless. A unique organ called lodicule at the base of the floret opens the floret and exposes the anthers and pistil for pollination during the anthesis. Wheat flowers is largely cleistogamous which means that pollen is shed before or just after flowers start opening (de Vries 1971).

Ideally, both male and female parental lines for hybrid seed production need to possess desirable trait combinations to achieve cross-pollination. The male ideotype should be taller than the female, with long extruded anthers producing large amounts of pollen over an extended time period enabled by differential flowering timing between tillers. In comparison, female ideotype needs to be shorter with wide opening florets and long hairy stigmas that are receptive for extended periods (De Vries 1972; Longin et al. 2012). Obviously, flowering time between the parental lines has to be synchronized. For several of these traits, the presence of genetic

variability (Pickett 1993; Langer et al. 2014) and important differences in broad sense heritability have been reported earlier (De Vries 1974a, b; Singh et al. 2007; Langer et al. 2014, Song et al. 2018, Fritz and Lukaszewski 1989, Komaki and Tsunewaki, 1981; Fábián et al. 2019). This is suggesting that much progress can be made in improving the cross-pollinating ability of parental lines derived from breeding populations.

I-6-2. Heterosis

In literature, heterosis is often expressed as mid-parent heterosis, which is the deviation of the hybrid performance from the mean parents. While best-parent heterosis is defined as deviation of the hybrid performance from the better parent for a specific trait (Bernardo 1992). For the first time, heterosis in wheat was reported for plant height (Freeman 1919). Few years later, Briggle (1963) was among the first to report heterosis for grain yield components in various F1 hybrids and since then research efforts over years have demonstrated yield advantage and stability (Borghi and Perenzin 1990, 1994; Dreisigacker et al. 2005; Kant et al. 2011; Gowda et al. 2012; Mühleisen et al. 2014; Nie et al. 2019; Adhikari et al. 2020b; Easterly et al. 2020). These reports demonstrated that the hybrids developed through CMS could not, always, be exploited for production of hybrid wheat at commercial level. In contrast, chemical hybridization agent has been successfully utilized in the recent years. Recent study based on experimental data in hybrid wheat breeding demonstrated that best-parent heterosis ranged from -70.4 to 54.3% and -26.9 to 29.2% in two successive years using CHA Croisor® 100 of elite winter wheat lines from University of Nebraska–Lincoln (UNL) and Texas A&M University breeding programs.

Despite these hybrid yield advantage, development of practical hybrid seed production system has not reached a large-scale yet. Besides all the limitations mentioned above, most crops for which hybrid breeding is considered promising currently lack clearly defined heterotic groups and patterns (Zhou et al. 2005). Heterotic group comprises related or unrelated genotypes from same or different populations and display similar hybrid performance when crossed with genotypes from other genetically distinct germplasm groups (Melchinger and Gumber 1998). While a heterotic pattern defines a specific pair of heterotic groups showing high heterosis level when crossed. Before establishment of any heterotic group, knowledge about combining ability, hybrid performance, and also genetic diversity existing withing the germplasm is required (Gurung et al. 2009). This information will allow breeders to select the most appropriate parental lines from a diverse germplasm and increase the efficiency of the hybrid seed

production. Xie et al. (2013) have used SSR genotyping data combined with field trials to establish two heterotic groups and four heterotic patterns in IRRI hybrid rice germplasm to develop hybrid rice in the tropics. In the same context, Zhao et al. (2015) developed three steps approaches that have been explored and experimentally verified to identify a promising pattern: (1) the performance of all possible single-cross combinations is determined through genome-wide or metabolite-based hybrid predictions. (2) The predicted hybrid performances are used to identify superior heterotic patterns based on a simulated annealing algorithm. (3) The optimum size of the heterotic pattern is determined balancing the expected short- and long-term selection.

I-6-3. Combining ability

Combining ability in a specific cross is defined as the parents ability to combine with each other during the hybridization. In other words, the potential of the parents with superior traits combinations may not always transmit desirable traits to their hybrid progenies. Therefore, knowledge of this genetic parameter is of special importance as it helps in anticipating improvement in productivity via hybridization and selection and helps also in elucidating the nature and magnitude of different types of gene action governing the expression of quantitative characters (Pal and Prodhan 1994). For these raisons, it has been widely adopted in plant breeding to compare the performance of lines in hybrid combination (Sprague and Tatum 1942; Singh et al. 1974; Ahangar et al. 2008; Adhikari et al. 2020b).

The concept of general combining ability (GCA) and specific combining ability (SCA) were first defined by Sprague and Tatum (1942). GCA is the average performance of lines in a series of hybrid combinations, whereas SCA is the deviation in performance of certain hybrid combinations as compared to what would be expected based on the GCA of the parents involved. From statistical point of view, the GCA effect is a good indicator of relative value of a parental line in terms of frequency of favorable genes and of its genetic divergence which will allow the selection of the best performing parents to be used in hybrid breeding programs. SCA effect of two populations expresses the differences of frequencies between two populations and their divergence as compared to the diallel parents.

Low GCA value, positive or negative, shows that the mean of crossed two parents does not vary largely from the general mean of the crosses. In contrast, high GCA value indicates that the parental mean is superior or inferior to the general mean. This indicates a strong evidence

of favorable gene flow from parents to offspring at high frequency and represents information about the concentration of predominantly additive genes (Caixeta Franco et al. 2001). In addition, high GCA estimate indicates, larger adaptability, higher heritability and less environmental effect. While high SCA estimate indicates that both parents involved in that cross are good general combiners. However, high SCA may also result from a cross including good × poor general combiner parents which may be attributed to favourable additive effects of the good general combiner parent and epistatic effects of poor general combiner, which fulfils the favourable plant attribute (Dey et al. 2013). High SCA estimate manifested by low × low crosses may be due to dominance × dominance type of non-allelic gene interaction producing over dominance (Wassimi et al. 1986).

The ratio of the GCA and SCA variance components determines the gene action involves in the expression of the trait of interest. A relatively larger GCA/SCA variance ratio demonstrates importance of additive genetic effects and a lower ratio indicates predominance of dominance and/or epistatic gene effects (Christie and Shattuck 2010). GCA and SCA effects for individual lines are calculated only if the overall analysis shows that mean squares for GCA and SCA are significant (Fasahat et al. 2016).

With the advances in biometrical genetics, several techniques have been developed for estimating combining ability estimates (e.g., diallel, partial diallel, and line × tester analyses). Diallel analysis scheme was used widely to identify parental lines with high GCA and combinations with high SCA (Comstock and Robinson 1948) or to obtain genetic information of hybrids and their parents for further classification in heterotic patterns (Cabral Mendes et al. 2015). The most frequently used methods in diallel analysis are Griffing's diallel procedures. Four different diallel methods were proposed to be used in plants (Griffing et al. 1956). 1) Method 1 (full diallel): parents, F1 and reciprocals, 2) Method 2 (half diallel): parents and F1's, 3) Method 3: F1's and reciprocals, 4) Method 4: F1's. These diallel methods of Griffing are generally used for one year or one location trials. However, multi-environment trials are suggested to produce more reliable genetic information on the tested material (Zhang and Kang, 1997).

Chapter II: Phenotypic evaluation of elite spring bread wheat genotypes for hybrid potential traits

Abstract

Wheat breeding has significantly contributed to enhancing yield potential and stability. However, one of the most promising technologies for further sustainable yield increases, hybrid wheat, is still limited due to lack of well-established heterotic groups and insufficient heterosis to compensate cost of hybrid seed production. Thus, optimizing the appropriate parents and combinations of floral and flowering traits is important to enhance outcrossing ability. Phenotyping methods for floral and flowering traits were applied in this study. Wide phenotypic variation was observed for all the traits assessed. High heritability coupled with high genetic advance for anther extrusion, pollen mass, duration of floret opening, and openness of florets were observed. Ideally, visual anther extrusion and pollen shedding appear as promising traits allowing indirect selection of other associated floral traits, such as pollen mass and duration and degree of floret opening. Adopting these two traits in further selection will allow scoring of large numbers of genotypes in a very short time.

Keywords Female ideotype, Flowering traits, Hybrid seed production, Hybrid wheat, Male ideotype

II-1. Introduction

Wheat (*Triticum aestivum* L.) is one of the main staple crops in the world and demand for wheat is almost synonymous with demand for food. Worldwide, wheat is the primary source of calories for millions of people, accounting for around 28% of cereals used as food (FAOSTAT 2014). Globally, wheat is grown on more than 220 million hectares with annual production likely to reach 750 million tons (FAOSTAT 2018). Despite remarkable progress in wheat productivity, greater efforts need to be made to cope with an ever-increasing world population growth that will reach 9.8 billion by 2050. Global wheat production needs to increase by 2% annually to meet escalating demands.

Over the years, wheat breeding has significantly contributed to enhancing yield potential and stability. Comparing the 1961-1965 and 2013-2017 periods, average yield rose from 1.18 to 3.15 t/ha, and wheat production increased from 247 to 741 million tons (FAOSTAT 2019). However, opportunities for further sustained increases are limited due to the limited genetic variability in wheat germplasm, insufficient exploitation of heterosis and the gap between the technology and farm achievement (Venske et al. 2019). To bridge this gap and to make wheat cultivation more attractive, there is a need to explore new technologies with potential to break yield barriers and substantially enhance the level of productivity (Jordaan 1996). In this context, the exploitation of heterosis through hybrid wheat is one possibility that could capture significant yield benefits.

Hybrid wheat has shown superior grain potential for enhanced yield performance and stability compared to pure-line varieties (Mühleisen et al. 2014). Longin et al. (2013) reported average best parent heterosis levels ranging from -15.7 to 25.7% for grain yield of elite hybrids compared to their respective higher yielding parent (average of 12.45 t ha^{-1}).

Several attempts have been undertaken to establish suitable seed production systems for hybrid wheat. However, these were only partially successful. The constraints included inadequate levels of heterosis, inefficient seed production systems, and lack of defined heterotic groups of germplasm to serve as parents (Zhou et al. 2005). A major hindrance was also the stringent autogamous nature of wheat. Thus, optimization of floral and flowering traits could contribute to increased production of hybrid seed. Several traits such as flowering time, plant height, anther extrusion, pollen mass and degree and duration of floret opening were seen as essential for maximum pollination (De Vries 1971; Whitford et al. 2013). Moreover, the respective male

and female parental lines need to possess specific trait combinations. The male ideotype was taller than the female, with long extruded anthers producing large amounts of pollen over an extended time period enabled by differential flowering timing between tillers. The female ideotype was envisaged as shorter with wide opening florets and long hairy stigmas that were receptive for extended periods (De Vries 1972; Longin et al. 2012). Obviously, flowering time has to be synchronized.

Identification of potential parental lines with these features is challenging and phenotyping of most floral and flowering traits is difficult and time consuming. Hence, it is important to use suitable approaches to expedite selection for enhanced outcrossing capacity. The present study was undertaken to evaluate diverse wheat germplasm for appropriate floral and flowering traits and to investigate potential of genomic approaches for use in hybrid wheat programs. In particular, our objectives were to (i) evaluate genetic variability in bread wheat for male and female traits with potential relevance for hybrid wheat breeding programs, (ii) assess correlation, heritability and genetic advance for hybrid related traits, and (iii) identify time- and cost-effective proxies for good outcrossing ability.

II-2. Materials and methods:

II-2.1. Plant materials

A panel of 400 genotypes, including mainly elite lines from the ICARDA bread wheat breeding program, 15 commercial checks and 63 synthetics and synthetic-derived lines was assembled to study the genotypic variability in hybrid wheat-related traits.

II-2.2. Phenotyping

The whole set of 400 genotypes was phenotyped under plastic house conditions at ICARDA Rabat Guich, Morocco (33 °97'N,6 °86W,57 m) in 2015/16 season and a subset of 196 genotypes were selected based on phenotypic performance for potential hybrid traits (Annex 1). This subset was grown under field conditions in Marchouch station, Morocco (33°36'N, 6°43'W, 394 m) during the 2016-2017 and 2017-2018 cropping seasons. The genotypes were grown in $3m^2$ plot following an augmented design. The experiments were fertilized appropriately, and weeds were controlled according to standard cultural practices.

The following traits we assessed in this study:

Days to heading (DH): number of days from planting until when 75% of the spikes have emerged (Zadoks 67) from the flag leaf sheath (Zadoks et al. 1974).

Days to maturity (DM): known as physiological maturity, recorded when 50% of the spikes were ripe and turned yellow (Zadoks 87).

Flowering start (FLS): Recorded when the anther extrusion started in an observation plot.

Flowering peak (FLP): Recorded when 50% of the spikes of a plot had visible anthers.

Flowering end (FLE): Recorded when the total spikes showed visible anthers.

Flowering duration (FLD): Derived as difference between flowering start and flowering end.

Plant height (PLH): Measured from the ground to the top of the terminal spikes excluding awns.

Spikelets per spike (SPS): Calculated as the average number of spikelets in five randomly selected spikes.

Spike length (SPL): Measured from the base of the spike to the tip of the terminal spikelets excluding awns on five randomly selected spikes.

Tillers per plant (TLP): Only fertile tillers were counted at maturity stage on five randomly selected plants.

Pollen mass (PM): Determined as the amount of pollen released by a genotype, it was assessed following Langer et al. (2014). Ten randomly selected spikes were placed into paper bags 2 days before flowering (Figure II- 1-a). After flowering, the pollen bags were collected by harvesting the spikes and dried in the oven for 72 hours at 65ºC. After drying, the content of the bags is sieved (100 µm mesh) and the pollen retrieved is weighed using a precision balance.

Pollen viability (PV): As per Joppa et al. (1966), 3 randomly selected spikes were collected at Zadoks growth stage 60 (Beginning of flowering) and fixed in 3:1 solution of 95% ethanol: acetic acid. Anthers from central spikelets were used for pollen staining. Each determination is based on a count of 100 pollen grains. The anthers were placed on a microscope slide with three drops of iodine potassium iodide (IKI) solution prepared by dissolving 1g potassium iodide KI and 0.5g iodine in 100ml distilled water, gently crushed to extract the pollen and the debris is removed. Unstained or partially stained pollen grains were rated as unviable. The dark stained pollen grains were considered viable (Figure II- 1-b).

Anther length (AL): at flowering time, the recording was carried out using image analysis software ImageJ (http://imagej.nih.gov/ij/index.html) as the average of three anthers belonging to a lateral floret of the three central spikelets of a spike.

Anther extrusion (AE): Scored as described by Langer et al. (2014) on five randomly selected spikes per genotype harvested 5-7 days after flowering peak and averaged. We counted the number of the anthers retained in the two lateral florets of eight central spikelets of each spike. Since the lateral wheat florets contain three anthers resulting in a maximum of 48 anthers per counted spike (i.e. 3 anthers per floret, 2 florets per spikelet and 8 spikelets per spike) and the number of extruded anthers can be derived by subtracting the number of counted anthers retained in the florets from 48. For an easier comparison, we converted this into the percentage of extruded anthers.

Pollen shedding (PSH): Visually scored during the flowering period only on newly extruded anthers. Using a pencil, we jolted the spikes, and the amount of shed pollen were scored on a scale from 1 to 9 (1 = no pollen shedding and 9 = maximum pollen shedding).

Stigma length (SL): Measured and recorded as average length two stigmas of one lateral floret from three central spikelets of a spike using image analysis software ImageJ (http://imagej.nih.gov/ij/index.html).

Openness of floret (OPF): Measured in degree on two open florets using divider and protractor or using digital angel ruler and then averaged (Figure II- 1-c). The degree of floret opening was taken as the separation angle between the glumes of first two florets of a spikelet.

Duration of floret opening (DFO): Recorded in minutes from the time of opening to closing of florets using digital video recording.

Grain yield per spike (GYS): Measured as the average value/No of 5 randomly selected spikes.

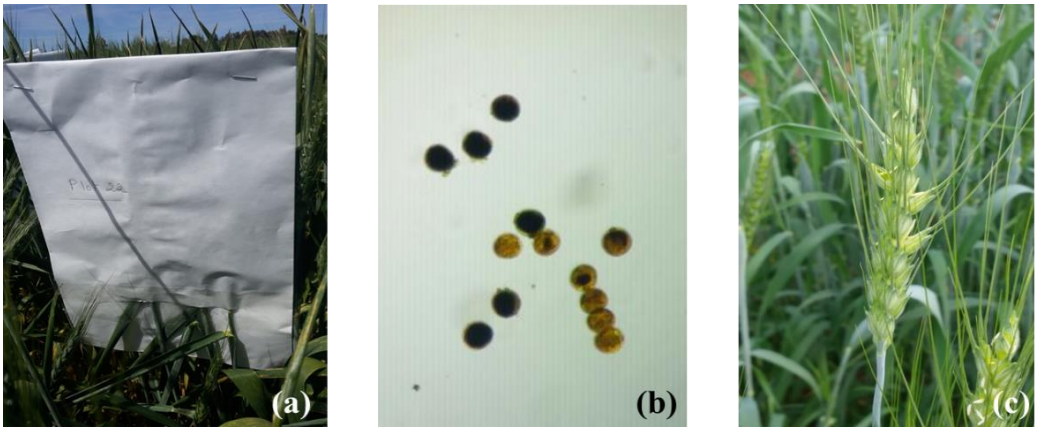


Figure II- 1: Phenotypic evaluation of the traits: (a) Pollen mass assessment, (b) Pollen viability test, (c) openness of the florets

II-2.3. Statistical analyses

Statistical analyses were performed on a yearly basis as well as across years. A linear mixed model using restricted maximum likelihood (REML) method (Patterson and Thompson 1971) was used for data analysis. For yearly analyses, genotypes and checks were considered as fixed whereas blocks were considered as random effects. Best linear unbiased estimates (BLUEs) were calculated for genotypes for all traits specifically to get the estimated means in order to compute the between trait correlations. The same analysis was done using genotypes as random to get the genotypic variance components and hence to compute heritability and the genotypic coefficient of variation. For the across year analysis, years, checks and their interaction were fitted as fixed effects and genotypes and genotype by year interaction were random to get the genotypic and genotype by year interaction variance components. Best linear unbiased predictors (BLUPs) were calculated for genotype by year interactions and used for ranking genotypes and selection of the best genotypes for further experiments.

Pearson correlation coefficients and their corresponding p-values were calculated between traits in each year. Repeatability among biological replicates was calculated from the expression $\sigma^2 g$ / ($\sigma^2 g$ + $\sigma^2 e$ / nr). Broad sense heritability of the evaluated traits was calculated from the expression $\sigma^2 g$ / ($\sigma^2 g$ + $\sigma^2 g.e$ / ne + $\sigma^2 e$ / ne.nr), where $\sigma^2 g$ represented the genetic variance, $\sigma^2 e$ the error variance, ne was the number of environments and nr the number of replicates. All statistical analyses were done and models were fitted in ASReml v3.0-1 (Butler et al. 2007) in R v3.3.1 (R Core Team 2016).

Phenotypic and genotypic coefficients of variation were estimated according to the method suggested by Burton and DeVane (1953) as follows:

The phenotypic and the genotypic coefficients of variation were estimated according to the method suggested by Burton and DeVane (1953) as follow:

$$\text{Phenotypic coefficient of variation (PCV)} = \frac{\sqrt{\sigma^2 \text{p}}}{\bar{\text{x}}} \times 100$$

$$\text{Genotypic coefficient of variation (GCV)} = \frac{\sqrt{\sigma^2 g}}{\bar{\text{x}}} \times 100$$

where: $\sigma^2 p$ was the phenotypic variance; $\sigma^2 g$ was the genotypic variance and $\bar{\text{x}}$ was the sample mean.

The genetic advance (GA) and percentage of the mean (GAM) assuming selection intensity at 5% was estimated as per formula given by Allard (1960)

$$\text{GA} = \text{K} \times \sigma^2 p \times \text{H}$$

$$\text{GAM} = \frac{GA}{\bar{\text{x}}} \times 100$$

Where: K = standardized selection differential (at 5% selection intensity K = 2.063); and H is broad sense heritability

II-2.4. Genotypic analysis

The panel was genotyped by whole genome scanning using the 15K Single Nucleotide Polymorphism (SNP) marker array (TraitGenetics Gmbh). SNP raw data were filtered according to marker criteria; minor allele frequency (MAF) >5% and missing data ≤10%. This resulted in 10,477 SNPs for this study. Cluster analysis was performed by the unweighted pair group method with arithmetic mean (UPGMA) method to find discrete groups based on linkage criteria between genotypes.

II-3. Results

The results of the mixed model analysis showed large phenotypic and genotypic variations as well as important differences in broad sense heritability for all flowering and floral traits evaluated for the 200-genotype subset (Table II- 2). Heritabilities ranged from low to high and genotypic variances were significantly different from zero (P <0.001) for all traits across the three data sets.

Floral traits showed a wide range of variability across years. Genotypes SOMAMA-9/ICARDA-SRRL-2 and QADANFER-11/REBWAH-11 among others showed very good performance for floral traits favoring outcrossing. FLAG-1 was the check having highest values for anther extrusion and anther length but low values for the other floral traits. Among the

synthetic and synthetic-derived lines, STY-US/CELTA//PALS/3/SRN_5/4/AE.SQUARROSA (431) was the only genotype with good performance for duration of floret opening, pollen mass and anther extrusion with values of 100 min, 38.42 mg and 79.17%, respectively. Most of the synthetic and synthetic-derived genotypes had long anthers but produced lower pollen mass compared with the elite lines used in this study. For example, synthetics DOY1/AE. SQUARROSA (1026) and SORA/AE. SQUARROSA (208) had large anthers (>5 mm) but shed small amounts of pollen (<14 mg).

The trait distributions of the BLUES followed approximate normal distributions except for heading date, flowering start and flowering peak (Figure II- 2). Absolute values of phenotypic correlations among the 20 traits ranged between -0.40 and 0.92 (Table II- 3). There were high correlations among pollen mass, openness of the floret, duration of floret opening, pollen shedding, anther extrusion and visual anther extrusion ranging from 0.65 to 0.90 ($P < 0.001$). The highest correlation for pollen mass was with anther extrusion ($r = 0.90$). The correlation between anther extrusion and visually scored anther extrusion was highly significant ($r = 0.75$, $P < 0.001$). Spikelet number per spike was near-independent of pollen shedding and pollen mass ($r = -0.18^*$ and $r = -0.15^*$, respectively) but moderately positively correlated with spike length ($r = 0.47$, $P < 0.001$). Anther length was weakly correlated with plant height ($r = 0.16$).

Table II- 1: Summary statistics for the evaluated traits across the three years

	Year 1						Year 2						Year 3					
Trait	**Min**	**Max**	**Mean**	**H**	**Vg**	**Ve**	**Min**	**Max**	**Mean**	**H**	**Vg**	**Ve**	**Min**	**Max**	**Mean**	**H**	**Vg**	**Ve**
FLS													101	122	111.35	0.63	14.30	8.42
FLP													107	128	118.08	0.60	16.14	10.59
FLE													110.24	133.05	124.66	0.54	10.87	9.37
FLD													6.00	21	13.31	0.23	2.10	7.22
DH													93	114	105.49	0.75	11.82	3.93
DM													138	173	164.53	0.68	13.30	6.32
PLH													57	120	95.42	0.74	28.76	10.06
TLP	2.2	9.89	4.71	0.43	1.84	2.4	2	7.6	3.75	0.41	0.54	0.78	107	128	4.30	0.10	0.13	1.15
SPS	14.6	28.6	19.76	0.68	5.41	2.59	15.8	30.8	21.86	0.58	5.1	3.75	17.07	25.00	21.23	0.33	1.40	2.79
SPL	8.04	15.7	11.66	0.61	1.53	1	9.28	15.35	12.1	0.49	1.13	1.18	8.93	15.70	11.99	0.43	0.92	1.22
GYP	0.72	3.6	2.28	0.36	0.16	0.28	1.13	3.22	2.26	0.4	0.15	0.22	1.57	4.82	3.30	0.46	0.24	0.28
SPC	1.29	2.48	1.71	0.42	0.03	0.04	1.13	2.54	1.82	0.45	0.04	0.05	1.42	2.38	1.79	0.21	0.01	0.05
OPF	9.05	45.6	26.57	0.82	50.17	11.11	14.5	39	24.29	0.76	24.94	7.88	13.26	40.82	24.89	0.74	25.45	8.86
DFO	15	109	57.44	0.85	559.07	98.94	17	101.5	56.1	0.78	405.64	112.95	7.36	107.17	55.44	0.67	259.55	130.21
PM	1.9	51.72	19.69	0.86	86.99	13.65	1.54	42.81	22.3	0.94	78.24	5.41	6.37	42.96	23.61	0.81	40.38	9.77
VAE													0.85	9.14	5.35	0.73	2.90	1.06
PV													27.73	104.88	92.35	0.63	34.06	20.30
PSH													2.46	9.75	6.32	0.72	1.87	0.71
AE	3.33	95.42	44.6	0.65	387.83	209.56	6.25	87.08	42.49	0.74	379.97	130.44	0.82	93.84	50.12	0.60	322.49	214.05
AL	2.76	4.95	3.82	0.82	0.15	0.03	3.17	4.72	3.94	0.43	0.05	0.07	2.52	4.65	3.62	0.76	0.15	0.05
SL	2.1	5.03	3.48	0.77	0.28	0.08	1.98	5.05	3.64	0.61	0.21	0.13	1.78	5.04	2.99	0.67	0.24	0.12

H: Heritability, Vg: genotypic variance, Ve: error variance, FLS: flowering start, FLP: flowering peak, FLE: flowering end, FLD: flowering duration, DH: days to heading, DM: days to maturity, PLH: plant height, SPC: spike compactness, VAE: visual anther extrusion, PSH: pollen shedding, TLP: tillers per plant, SPKLSP: spikelets per spike, SPL: spike length, GYS: grain yield per spike, OPF: openness of the florets, DFO: duration of floret opening, PM: pollen mass, AE: anther extrusion, AL: anther length, SL: stigma length.

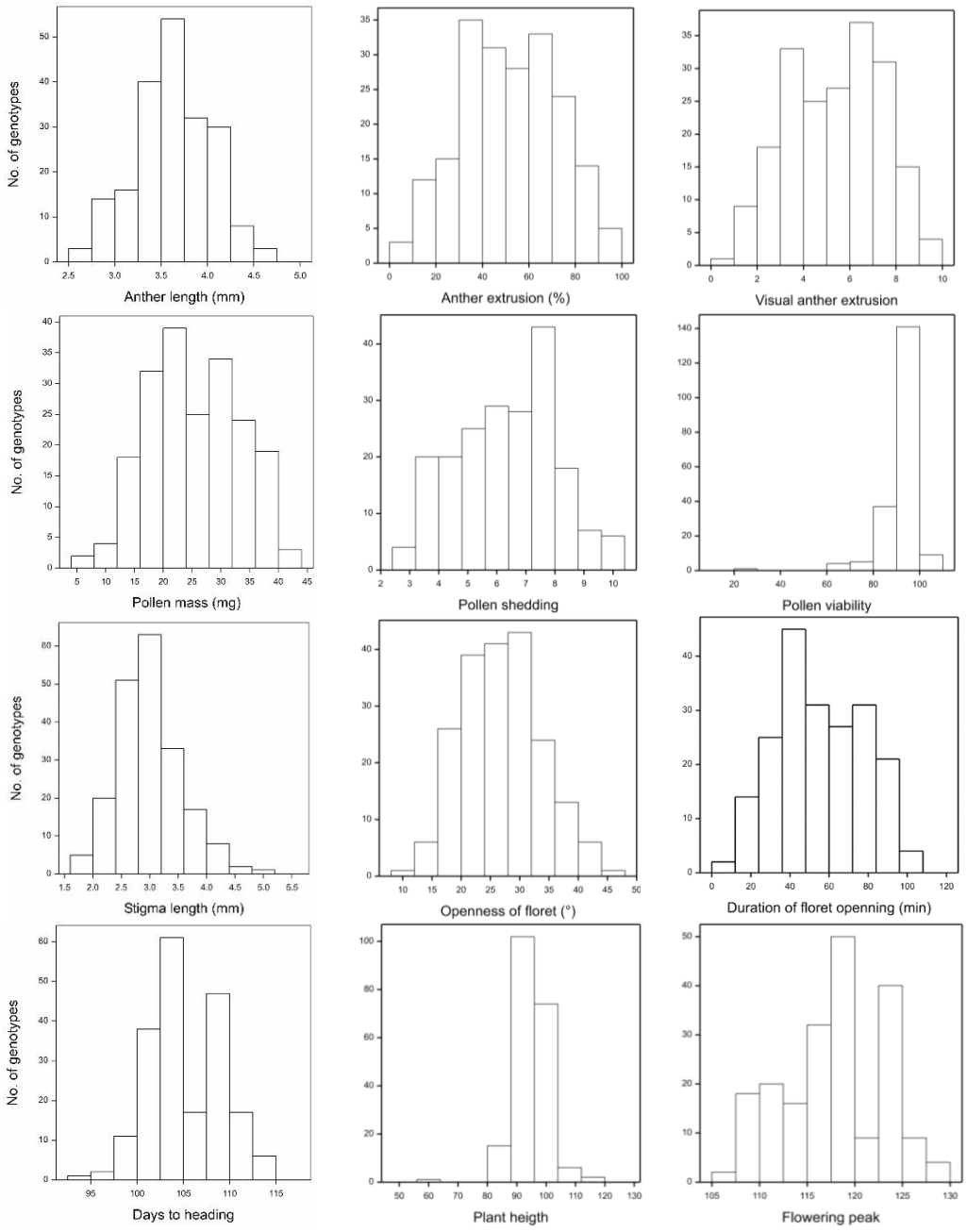


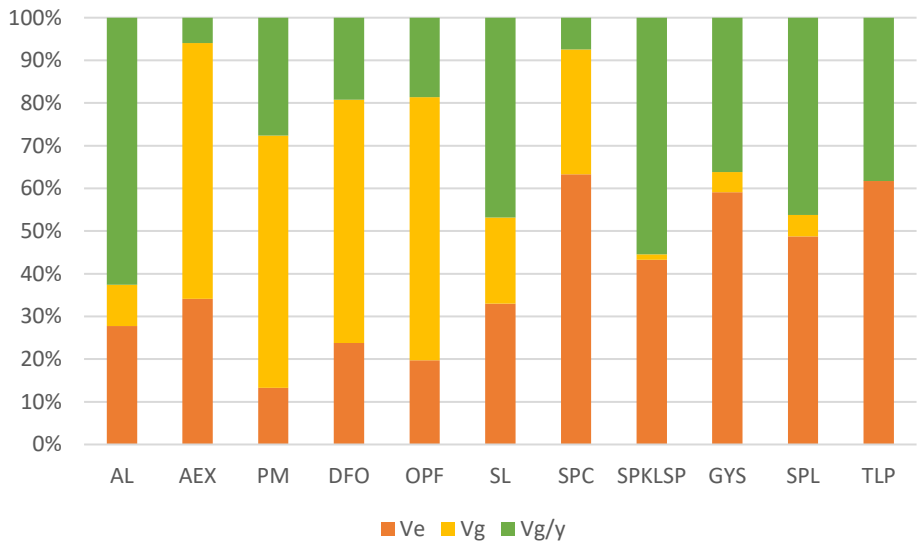
Figure II- 2: Histograms of the phenotypic values of some evaluated traits

Among the flowering traits, days to heading showed strong correlation between days to maturity, flowering start, flowering peak and flowering end with the highest value between flowering start and flowering peak (r = 0.92).

The across year analysis has shown high heritability of the important floral traits except for anther length. The highest heritability (0.93) was observed for anther extrusion. However, anther length and spike length showed low to medium heritability across year. The percentage of estimated genotypic variances and the variances due to genotype-by-year interaction was

significant for most of the floral and flowering traits except for spike compactness (Figure II-3). Genetic variation dominated the total variation for anther extrusion (59.94%), pollen mass (59.09%), duration of floret opening (57.01%) and floret openness (61.64%). However, genotype-by-year variation had the greatest effect on anther (62.56%) and stigma length (46.81%). The genotypic variance for agronomic traits accounted for a maximum 5% of the total variation.

The phenotypic and genotypic coefficients of variability ranged from 4.01 and 44.24 and from 1.46 and 41.92, respectively. The highest coefficient of variability for both phenotypic and genotypic levels was recorded for anther extrusion. Highest values were also found for pollen mass, duration and degree of floret opening (Table II- 3). Similarly, the highest genetic advance was for anther extrusion (GA = 38.09). Genetic advance estimated at 5% selection intensity was high for anther extrusion, pollen mass, duration and degree of floret opening.



Traits abbreviation is shown in table II- 1. Ve: error variance, Vg: genotypic variance, Vg/y: genotype by year variance

Figure II- 3: Percentage of variances explained by each component for some traits investigated

Table II- 2: Pearson correlation coefficient and their levels of significant between traits

	AL	AE	DFO	DH	DM	FLD	FLE	FLP	FLS	GYP	OPF	PLH	PM	PSH	PV	SL	SPC	SPS	SPL	TLP
AE	0.04																			
DFO	0.01	0.86****																		
DH	0.1	0.03	0.02																	
DM	0.07	-0.04	-0.06	0.83****																
FLD	0.08	-0.13	-0.12	-0.21**	-0.17*															
FLE	0.14	-0.1	-0.1	0.75****	0.67****	0.24***														
FLP	0.12	-0.02	0	0.84****	0.72****	-0.11	0.89****													
FLS	0.09	0	-0.01	0.85****	0.75****	-0.40****	0.78****	0.92****												
GYP	0.04	-0.04	-0.08	-0.04	-0.08	-0.02	-0.1	-0.07	-0.06											
OPF	-0.07	0.85****	0.80****	0	-0.08	-0.13	-0.14	-0.05	-0.03	0.02										
PLH	0.16*	0.03	0.07	0.14*	0.1	0	0.17*	0.21**	0.16*	0.18*	-0.01									
PM	0.09	0.90****	0.84****	0.02	-0.04	-0.1	-0.08	-0.01	0	-0.01	0.82****	-0.01								
PSH	-0.08	0.75****	0.68****	0.03	0	-0.15*	-0.14	-0.03	-0.02	0.02	0.65****	0	0.67****							
PV	0.11	-0.03	0	0.06	0.09	-0.12	-0.03	0	0.07	0.12	-0.01	0.07	0	0.02						
SL	0.30****	-0.1	-0.1	0.01	0.03	-0.07	0.01	0.01	0.06	-0.02	-0.07	0.05	-0.04	-0.07	0.09					
SPC	0.03	-0.11	-0.13	0.19**	0.15*	-0.03	0.15*	0.17*	0.17*	-0.05	-0.07	-0.03	-0.11	-0.04	0.02	0.05				
SPS	0	-0.14*	-0.12	0.22**	0.19**	-0.01	0.27***	0.25***	0.23***	0.01	-0.13	0.12	-0.15*	-0.18*	-0.08	0.08	0.29****			
SPL	-0.06	0.03	0.06	-0.01	0.01	0.04	0.05	0.03	0.01	0.03	0.01	0.15*	0	-0.05	-0.06	0.06	-0.66****	0.47****		
TLP	-0.01	0.08	-0.01	0.04	0.09	-0.06	-0.06	-0.03	-0.01	-0.19**	-0.03	-0.14*	0.05	0.12	0.01	0.07	0.11	-0.01	-0.1	
VAE	-0.11	0.75****	0.61****	0.05	0	-0.14*	-0.11	-0.03	-0.01	0.08	0.64****	0.04	0.68****	0.81****	-0.02	-0.03	-0.1	-0.12	0.02	0.08

*, **, ***, ****Significant at the 0.05, 0.01, 0.001, 0.0001 probability level. Traits abbreviation is shown in table II- 1.

Table II- 3: Estimation of genetic parameters for some hybrid related traits

Trait	Min	Max	Mean	H	Coefficient of variability		GA	GAM
					Phenotypic	Genotypic		
AL	3.24	4.43	3.79	0.31	4.01	3.49	0.17	4.58
AEX	5.41	90.03	43.19	0.93	44.24	41.92	38.09	88.20
PM	6.23	41.69	20.49	0.84	34.33	32.55	13.28	64.83
DFO	17.12	101.72	53.29	0.85	34.29	31.19	34.67	65.06
OPF	12.65	41.47	23.74	0.87	23.53	21.85	10.73	45.20
SL	2.53	4.40	3.35	0.54	8.95	7.93	0.45	13.51
SPC	1.43	2.33	1.77	0.81	9.89	8.26	0.33	18.40
SPKLSP	17.47	24.53	20.93	0.06	4.01	1.46	0.42	2.00
SPL	9.78	13.89	11.98	0.21	10.28	5.49	0.27	10.12
GYS	1.73	3.78	2.63	0.23	4.94	2.88	0.56	4.70

Traits abbreviation is shown in table II-1, H: Heritability, GA: Genetic advance, GAM: Genetic advance as % of mean

II-4. Discussion

Although hybrids are well established for allogamous crops such as maize, hybrid wheat has not been successful due to relatively low levels of hybrid vigor and lack of an efficient hybrid seed system. Floral biology will play an important role in increasing out-crossing. Several studies have described the floral and flowering biology of wheat. Although male and female ideotypes for efficient hybrid seed production are well defined, slow progress has been made in key trait improvement. In this study, we applied several approaches to evaluate floral and flowering traits and discuss their contribution in improving pollination capacity.

II-4.1. Variation in floral and flowering traits

A high outcrossing ability is most likely influenced by a combination of floral traits such as anther extrusion, pollen shedding, anther size, openness of the floret, and stigma length (Singh et al. 2010). The approaches suggested by Langer et al. (2014) to assess pollen mass and anther extrusion are generally used as proxies to determine the amount of pollen released and percentage of extruded anthers. Anther extrusion can be visually and repetitively assessed under optimum weather conditions, but assessments could be disrupted by wind or rain. In the present study, anther extrusion assessment was carried out using counted and visual scoring methods and the results showed that both were successful in identifying highly extruding genotypes. Given the high correlation between anther extrusion and visual anther extrusion, visual anther extrusion is a promising method for initial selection of male parents for hybrid seed production.

It is not surprising that anther extrusion depends on opening florets and the duration of opening. A line showing these traits should serve as a potential pollen donor as well as a more receptive female parent. As in the present study, previous research showed that a high percentage of anther extrusion was directly related to high numbers of open florets (Rajki 1960, 1962; Singh and Joshi 2003). In addition to anther extrusion, visual anther extrusion also showed a close relationship with floret openness and duration of floret opening.

Another important determinant for outcrossing is the quantity of shed pollen. The wide variation in pollen shedding in this study was in concordance with previous studies. D'Souza (1970) reported differences of 5–80% between genotypes for the amount of pollen shed. However, such differences will dependent on traits like anther extrusion, openness of the floret and duration of floret opening. Generally, there was profuse pollen shedding outside the florets when there was adequate floret widening and high anther extrusion. Our results were in agreement with previous studies (De Vries 1974a, b; Singh et al. 2007; Langer et al. 2014).

Pollen viability is also an important factor that enhances cross-pollination. The longevity of pollen grains is less than 3 h (Fritz and Lukaszewski 1989). Regardless of low viability values, most lines showed high variability. The low values might be due to moisture loss during transportation from the field to the laboratory.

Anther length, used as proxy for anther size, is also a key spike trait that contribute to enhance outcrossing in wheat. We found ranges of 2.52-4.95 mm over the three years. Song et al. (2018) showed anther length to be 2.7-6 mm in a set of 305 lines. Anther size was associated with pollen grain number in wheat/rye addition lines (Athwal and Kimber 1970; De Vries 1974a; Nguyen et al. 2015). Other studies showed that anther size per se had no impact on the amount of pollen shed outside the floret (Beri and Anand 1971). We also found no correlation between the anther length and pollen shedding or any other trait except for stigma length and plant height. The lack of correlation between the two characters suggests that genes controlling anther length differ from those controlling the other floral traits.

Open florets in hybrid seed production is crucial for both male and female parents. Lines with wide floret opening for an extended duration should be prone to greater outcrossing. Few studies have addressed female traits due to their intensive work and time requirements. In this study, we observed a wide range of variability in the degree of floret opening (9.05-45.6° as separation angles) and duration (8.61-109 minutes).

Selection for stigma size was shown to have positive effects for cross-pollination and seed set (Virmani and Edwards 1983; Blouet et al. 1999). Long fully extruded and receptive stigmas for extended periods should be advantageous. Wide ranges in stigma length have been published (2.13-5.2 mm) (Komaki and Tsunewaki 1981; Fábián et al. 2019) as well as 1.78 to 5.05 mm being recorded in this study.

An optimized difference in plant height and synchronized flowering between the male and female parents are prerequisite for an efficient hybrid wheat seed production system. Non-uniformity of flowering causes low seed set. The pollen shed by taller male parents can disperse longer distances and gradually fall to reach the shorter female parents. The flowering period is expected to be determined by genotype and weather conditions. For hybrid production, a prolonged flowering time should be advantageous.

Phenotyping specific traits for hybrid production is hard and time-consuming as floral and flowering traits are strongly dependent to environmental conditions. High temperatures accelerate the flowering process and shorten the flowering period, narrowing the time window for pollination.

II-4.2. Genetic advance

Improvement of a given trait is through the available genetic variation and its heritability. Estimated broad-sense heritability alone is not very helpful because it includes the effect of both additive and non-additive genetic variation. Prediction of genetic advance is therefore useful to indicate the expected genetic progress from selection for a particular trait and helps to determine gene action affecting that trait (Sravan et al. 2012). Hazel and Lush (1942) defined the genetic advance as the improvement in average phenotypic or genetic value due to selection within a population in each breeding cycles. Genetic advance as percent of the mean was classified as low for values from 0 to 10%, 10-20% for moderate and 20% and above for high values (Johnson et al. 1955). Since high heritability does not necessarily indicate high genetic gain, heritability coupled with genetic advance is a more reliable guide for selection of superior genotypes at early generation (Akinwale et al. 2011).

The genetic advances as percentages of means ranged from 4.58% to 88.20% for floral traits. Predicted genetic advances in anther extrusion, duration of floret opening, pollen mass, and floret openness were high at 88.20%, 65.06%, 64.83% and 45.20%, respectively. Moderate GAM was estimated for stigma length (13.51%) while that for anther length was low (4.58%).

Singh (2006) predicted high genetic advances as percentage of mean values for the same floral traits. The agronomic traits underwent moderate genetic advances when compared to their mean values.

Traits like anther extrusion, pollen mass, openness of the floret and duration of floret opening showed high GAM coupled with high heritability estimates. This indicates the preponderance of additive gene action in determination of these traits and that a large proportion of the phenotypic variance was genotypic and effective selection could be made for such traits based on phenotypic expression. However, high heritability along with low to moderate genetic advance as percent of mean, i.e. spike compactness, was indicative of non-additive gene action and that selection might be less successful. Low heritability and predicted genetic advance for anther length was observed due to non-additive gene action and high environmental influence as reported by Akinwale et al. (2011).

The estimates of phenotypic variance were only slightly higher than genotypic variance for some floral traits indicating a low influence of environmental factors. Thus, selection based on phenotype could be successful for these traits. Anther extrusion, duration of floret opening, pollen mass, and floret openness showing high genotypic and phenotypic coefficients of variation along with high heritability and predicted genetic advance may be considered important traits in breeding for high hybrid seed production.

II-4.3. Associations among traits

The results of selection for hybrid parent attributes depend on the heritability and genetic variation of floral and flowering traits. Positive associations between traits could simplify parental selection. Most floral and flowering traits show positive correlations. Anther extrusion, a key trait for improving pollen dispersal, was highly correlated with released pollen mass and pollen shedding. Similar associations were reported by Langer et al. (2014). Given the high correlation between pollen shedding and both pollen mas and anther extrusion, pollen shedding is an easily scored trait that could be taken as proxy for selection based on the respective correlated traits. Several studies showed that genotypes with high anther extrusion are more likely to have resistance to Fusarium head blight (FHB) (Buerstmayr and Buerstmayr 2015; Xu et al. 2019). Therefore, it will also be interesting to check correlations between FHB and other evaluated floral traits.

Whether longer anthers influence pollen mass remains unclear. We assessed anther length, but no significant correlation was found. However, Langer et al. (2014) reported that anther size was positively correlated with pollen mass and anther extrusion. Boeven et al. (2018) reported that both counted and visual anther extrusion as well as filament length were correlated with hybrid seed set. In other hand, AL was found to be weakly associated with SL (r = 0.29) in the present study.

An extended period of open flowers should favor outcrossing. The degree and the duration of floret opening were highly associated (r = 0.85) over two years. OPF and DFO were both correlated with other traits including pollen mass, pollen shedding, and visual and counted anther extrusion. Singh et al. (2007) reported weak but significant associations between OPF and DFO (r = 0.30) and a high correlation between DFO and AEX (r = 0.89).

Langer et al. (2014) reported a moderate correlation between anther extrusion and plant height and therefore suggested that the correlation between these traits was worthy of further investigation. Boeven et al. (2016) later reported that reduced height genes had a negative effect on male floral traits. However, our results did not confirm this effect as we found only a weak association between anther length and plant height.

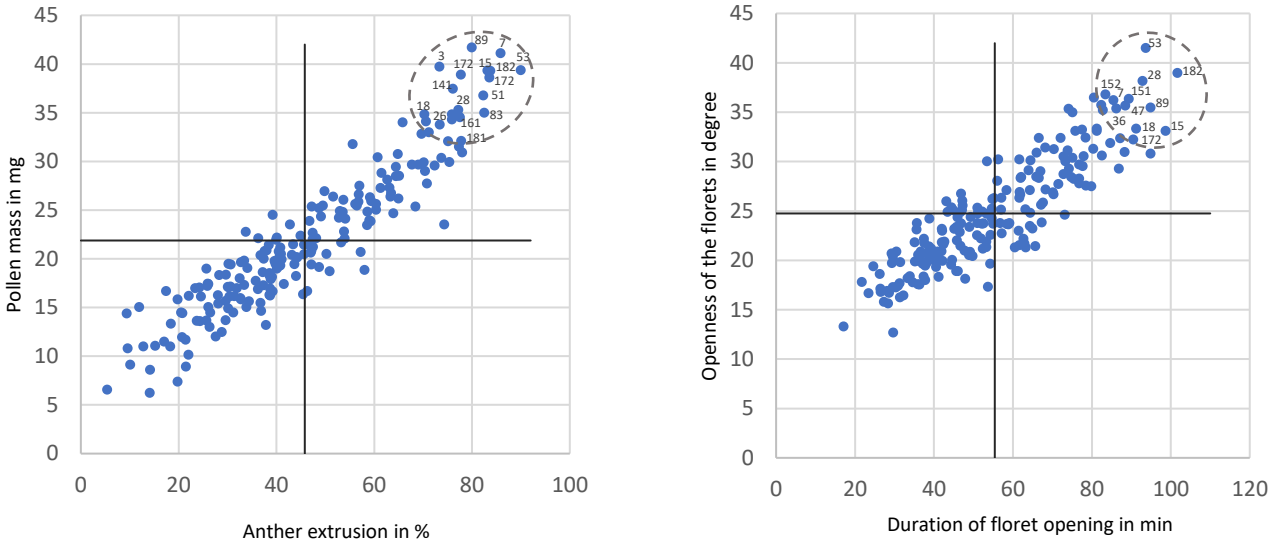
Floral traits in relation to flowering traits showed only weak negative correlations between flowering duration and pollen shedding and visual anther extrusion. Langer et al. (2014) reported a high negative correlation between AEX and DH. It would be interesting to determine whether the early flowering is associated with greater anther extrusion and pollen mass in further studies.

AEX previously showed a moderately negative correlation (r = -0.48) with numbers of spikelets per spike (Langer et al. 2014). Other weak negative correlations were between spikelets per spike and both pollen mass and pollen shedding. But no associations between tillers per plant, spike length, spike compactness and the floral traits were measured in this study.

The floret structure within spikes was reported to influence flowering type characteristics with tighter spikelets tending to have narrower separation angles (Obermayer 1916). However, no significant relationship was observed between the spike compactness and floret gap in this study.

II-4.4. Clustering of germplasm

We made a cluster analysis of panel members based on phenotypic values (Figure II- 4). We identified genotypes that combined high pollen mass and anther extrusion as promising traits for male parents and openness of the florets and duration of floret opening for female parents. This allowed us to confirm that most genotypes exhibiting high male potential carried features of good females as well. The selected genotypes were already used in hybrid wheat trials to study the combining ability and level of heterosis of hybrids using a chemical hybridizing agent to sterilize the female lines.



(a) (b)

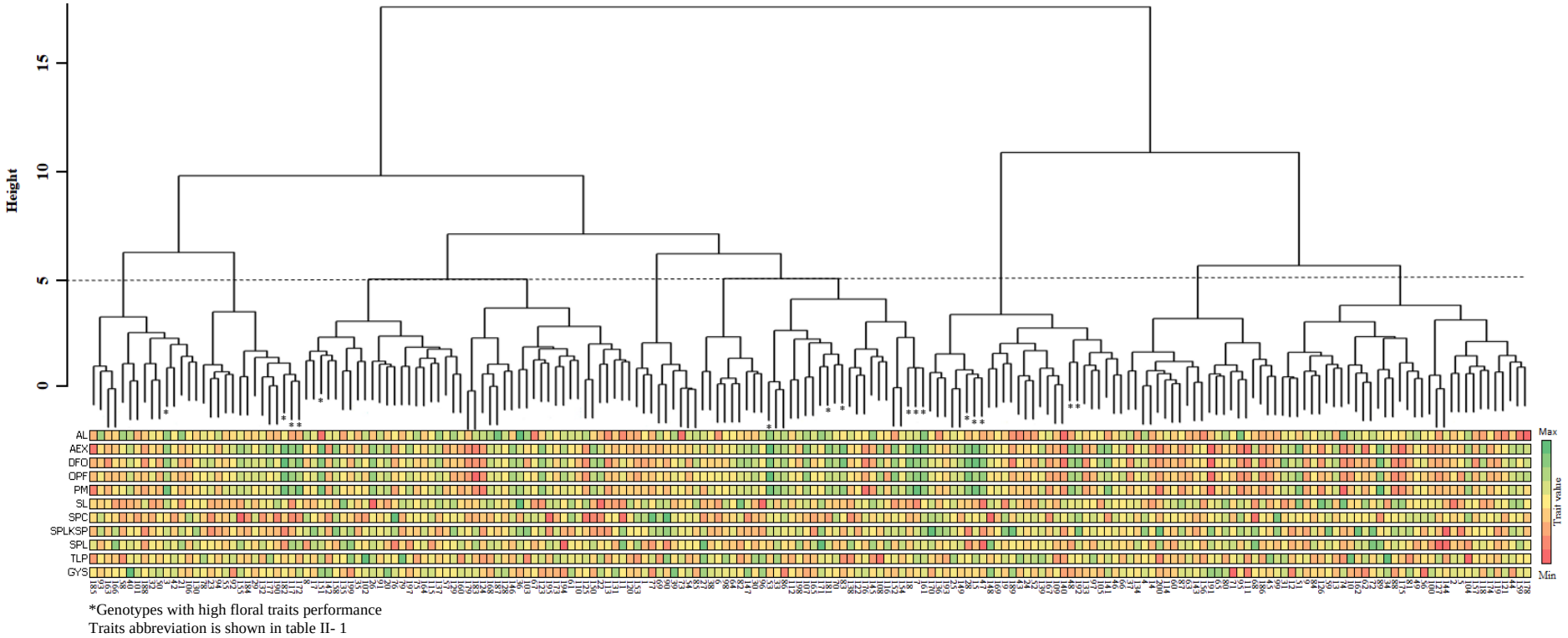
Figure II- 4: (a) Best male candidates with high rate of anther extrusion and pollen mass (b) Best female candidates with high rate of duration of floral opening and openness of the florets.

We also tried to determine the molecular relatedness of the characterized genotypes in relation to observed phenotypic values. From the cluster plot (Figure II- 5) eight main clusters were identified at branch level 5. Except for QAFZAH-21/ICARDA-SRRL-9, lines derived from crosses involving QAFZAH and QIMMA in the first cluster showed a weak performance in floral traits. Six different lines derived from the cross QADANFER-11/REBWAH-11 grouped in the second cluster had high potential for both male and female parent traits. QAMAR-6, in the third cluster, was the best check having high values of anther extrusion (83.6%), pollen mass (38.6 mg) duration of floral opening (89.3 min), and floret openness (39°). The fifth and sixth clusters included the most diverse genotypes in terms of potential parents. SOMAMA-9/ICARDA-SRRL-2 and FLAG-1 showed high potential as male/female for hybrid seed production. Some genotypes were closely related but differed in hybrid parent potential. Hence

pedigree was not an acceptable guide for prediction of potential hybrid parents. However, we could identify different groups of genotypes with favorable trait combinations for both male and female parents with high rates of extruded anthers, likely to release more pollen and with florets that remained open for extended time periods. The identification of these groups of genotypes appears interesting and can be classified as one heterotic pattern. It will therefore be interesting to investigate the heterotic potential among the selected genotypes to demonstrate high levels of heterosis. The identification of heterotic loci and exploring their expression levels will help to map stable QTLs and simplify the hybrid wheat breeding process. When the heterotic loci and genes are clearly known, it will be possible to characterize the genotypes of the key loci across the collection of parental lines and predict the potential hybrid combinations with strong heterosis performances. Genome-wide association mapping (GWAS) can be also an alternative to detect genomic regions harboring genes/markers for hybrid traits and thereby substantially reduce the time for selection.

III. 5. Conclusion

The self-pollinating nature of wheat makes hybrid wheat development a major challenge. Considerable progress has been made in defining phenotyping approaches in order to identify parents for hybrid breeding. Visual anther extrusion and profuse pollen shedding are promising traits to predict associated target floral traits. While the genetic architecture of male floral traits has been intensively studied, female traits are far less dissected. We therefore recommend shorter lines with prolonged opening of florets and stigma receptivity as potential female parents. The superior potential male and female parents identified in this study must be converted into cytoplasmic male sterile and/or restorer parents for hybrid seed production. The phenotypic variability within the present screened panel is adequate and allowed us the identification of best performing male and female genotypes tested for cross-pollination in hybrid wheat crossing blocks.



*Genotypes with high floral traits performance
Traits abbreviation is shown in table II- 1

Figure II- 5: Cluster-plot revealing the genetic relationships among the 196 genotypes and heat plot for some of the evaluated traits

Chapter III: Genome-Wide Association and Prediction of Male and Female Floral Hybrid Potential Traits in Elite Spring Bread Wheat Genotypes

Abstract

Hybrid wheat breeding is one of the most promising technologies for further sustainable yield increases. However, the cleistogamous nature of wheat displays a major bottleneck for a successful hybrid breeding program. Thus, an optimized breeding strategy by developing appropriate parental lines with favorable floral trait combinations is the best way to enhance the outcrossing ability. This study, therefore, aimed to dissect the genetic basis of various floral traits using genome-wide association study (GWAS) and to assess the potential of genome-wide prediction (GP) for anther extrusion (AE), visual anther extrusion (VAE), pollen mass (PM), pollen shedding (PSH), pollen viability (PV), anther length (AL), openness of the flower (OPF), duration of floret opening (DFO) and stigma length. To this end, we employed 196 ICARDA spring bread wheat lines evaluated for three years and genotyped with 10,477 polymorphic SNP. In total, 70 significant markers were identified associated to the various assessed traits at FDR $\leq$ 0.05 contributing a minor to large proportion of the phenotypic variance (8–26.9%), affecting the traits either positively or negatively. GWAS revealed multi-marker-based associations among AE, VAE, PM, OPF and DFO, most likely linked markers, suggesting a potential genomic region controlling the genetic association of these complex traits. Of these markers, Kukri_rep_c103359_233 and wsnp_Ex_rep_c107911_91350930 deserve particular attention. The consistently significant markers with large effect could be useful for marker-assisted selection. Genomic selection revealed medium to high prediction accuracy ranging between 52% and 92% for the assessed traits with the least and maximum value observed for stigma length and visual anther extrusion, respectively. This indicates the feasibility to implement genomic selection to predict the performance of hybrid floral traits with high reliability.

III-1. Introduction

Bread wheat (*Triticum aestivum* L.) is the second most important cereal cultivated crop worldwide with 766 million tons produced in 2019 (FAOSTAT 2019). However, global climate change intensifies the effect of biotic and abiotic stresses on wheat sustainability and production. Increased wheat productivity is the ultimate objective of the breeder to meet

escalating future demands. Therefore, new strategies and innovative breeding technologies might be adopted to substantially contribute to the required increase in wheat production.

Hybrid wheat is a promising technology to increase yields worldwide. However, less than 1% of the global wheat area is planted with hybrids, mainly due to the lack of cost-effective hybrid seed process and low heterosis (Kempe et al. 2014), although significant progress has been made to improve a competitive hybrid wheat breeding program. The exploitation of high-level heterosis through different hybrid systems appears more promising than ever. Easterly et al. (2020) reported mid-parent heterosis for grain yield of up to 24% for hybrids developed using chemical hybridization agent (CHA), demonstrating that F1 hybrid cultivars are able to outperform inbred varieties and ensure greater economic yield. However, without baseline studies to identify parental combinations for an efficient cross-pollination system, hybrid wheat will not be attractive commercially. Moreover, to guarantee reasonable F1 hybrid seed yield, the cleistogamous nature of wheat has to be addressed. For this reason, optimizing the appropriate parents with favorable trait combinations is essential for high outcrossing ability irrespective of the employed hybridization system. Conversely, male genotypes with high level of anther extrusion for appropriate pollen release during anthesis should serve as potential male donors for receptive females with open florets for extended time periods El Hanafi et al. (2020). Moreover, plant height showed its contribution in improving wheat cross-pollination ability (Murai et al. 2002; Boeven et al. 2016). It was reported previously that *Rht-B1b* and *Rht-D1b* dwarfing alleles lead to low anther extrusion that subsequently affect male floral traits (Lu et al. 2013; He et al. 2016). Therefore, given these facts, breeding hybrids in such manner optimizes the exploitation of heterosis and hybrid performance. However, the intensive work and time requirements of selecting the important male and female trait combinations make the identification of hybrid parental lines more challenging. Hence, phenotypic selection might be assisted by employing molecular markers tightly linked with major QTLs for the trait of interest that could greatly accelerate and simplify the selection of wheat floral traits. However, in cases where the trait is complex and mainly governed by many loci with small effects, genomic selection or (GS) genome-wide prediction (GP) can be an alternative approach to predict the genomic estimated breeding values (GEBVs) for all the genotypes of breeding population (Crossa et al. 2017). These GEBVs allow breeders to select superior genotypes that would be suitable either as parent or for next generation advancement of the breeding program. To perform GS, a population that combines a phenotypic and genotypic data, referred to as the

training population (TP), is used to train a model that takes genotypic information from a candidate population of untested individuals. This second set of individuals is considered as a validation set to estimate the prediction accuracy of the model used. GS has been extensively used in animal and plant breeding to predict traits using the molecular markers and pedigree information (Crossa et al. 2017; Hickey et al. 2017). To date, little is known of the genetic architecture of floral traits or the potential use of genomics-assisted breeding that holds promise for enhancing selection for the quantitative traits. Few studies have reported the prediction accuracy of some male floral traits (anther extrusion and pollen mass) using different models (Boeven et al. 2016; Muqaddasi et al. 2017b; Adhikari et al. 2020a). In parallel, some other studies deployed genome-wide association studies (GWAS) to detect target loci associated with anther extrusion, pollen mass and anther length (Boeven et al. 2016; Muqaddasi et al. 2017a; b, 2019; Song et al. 2018). While the genetic architecture of male floral traits has been fairly well studied, female traits are far less understood. Our aim was therefore to identify the genetic basis of various male and female hybrid potential traits using 196 diverse wheat genotypes by: (1) perform-ing a genome-wide association study to identify loci associated with hybrid male and female potential traits; (2) evaluating the effect of *Rht-B1* and *Rht-D1* on the floral traits; and (3) investigating the potential of genome-wide prediction (GP) for the evaluated floral traits.

III-2. Materials and Methods

III-2.1. Plant Materials and Phenotyping

This study was based on a diverse set of 196 elite genotypes from ICARDA bread wheat breeding program (Annex 1). The panel and the trial protocol were previously described in detail by El Hanafi et al. (2020). The genotypes were first evaluated under plastic house conditions at ICARDA Rabat Guich, Morocco (33°97′ N, 6°86 W', 57 masl) in 2016 and then were grown in 3 m^2 plots in an augmented design for two more years during the 2017 and 2018 cropping seasons at Marchouch Station, Morocco (33°36′ N, 6°43′ W, 394 masl).

During the growth period, various floral traits were assessed in all environments, as described by El Hanafi et al. (2020): (1) Visual anther extrusion (VAE) was recorded on a scale from 1 to 9 (1 = no anthers extruded, 9 = maximum anther extrusion). (2) Anther extrusion (AE) was based on the count of the remained anthers inside the florets of the spikes harvested 5–7 days post flowering peak. (3) Pollen mass (PM) was recorded to estimate the released pollen from

10 randomly selected spikes. (4) Pollen shedding (PSH) was visually scored on a scale from 1 to scale (1 = no pollen shedding and 9 = maximum pollen shedding). (5) Pollen viability (PV) was determined by the I-KI method based on a count of 100 pollen grains (Joppa et al. 1966). (6) Openness of the floret (OPF) was recorded as separation angle between the glumes of first two florets of a spikelet. (7) Duration of floret opening (DFO) was visually recorded in minutes from the time of opening to closing of florets by a digital video. (8) Anther length (AL) was scored at flowering stage as the average of three anthers belonging to a lateral floret of the three central spikelets of a spike using image analysis software ImageJ. (9) Stigma length (SL) was determined using as average length of two stigmas of one lateral floret from three central spikelets of a spike using image analysis software ImageJ.

III-2.2. Genotyping by SNP Markers

Genomic DNA from two-week-old bulked leaf samples were frozen in liquid nitrogen and stored at −80 °C before DNA extraction. The DNA was carried out according to Ogbonnaya et al. (2001), after which 10 µL of a 100 ng µL−1 DNA of each sample were sent to Trait Genetics, Germany, as a commercial service provider for whole genome scan using Single nucleotide polymorphism (SNP) markers. In total, 15,000 SNP markers were used to genotype the 196 wheat genotypes; 10,477 SNP markers resulted after discarding SNPs with minor allele frequency of <5% and missing values cut off (10%).

III-2.3. Population Structure and Linkage Disequilibrium

The genetic structure of the 196 genotypes was assessed with 102 unlinked SNP markers distributed across the wheat genome with at least two loci on each wheat chromosome using the STRUCTURE software (Pritchard et al. 2000). A detailed analysis of the population structure and discriminant analysis of principal components (DAPC) can be found in the work of Tadesse et al. (2019).

In total, 10,477 out of 12,725 SNPs, retained after removal of those with MAF < 5% and missing values over 10%, were used to study linkage disequilibrium (LD). TASSEL 5.0 was used to estimate LD as squared allele frequency correlation estimates (R^2) and measure the significance of R^2 at p-values for each pair of loci on different chromosomes (interchromosomal LD) and within the same chromosome (intrachromosomal LD) (Bradbury et al. 2007).

III-2.4. Association Mapping

Genome-wide association studies (GWAS) were utilized to find target loci associated with higher anther extrusion, pollen mass, maximum pollen shed outside the anthers, long and viable pollen grains, wide florets opening and hairy stigmas receptive for extended periods.

Marker trait association analysis was performed using mixed linear model (MLM) with TASSEL 5.0 software (Bradbury et al. 2007), applying both kinship matrix (K) and population structure (Q) matrices as covariates. Individuals with >10% missing SNP calls and markers with >10% missing and <5% minor allele frequency (MAF) were removed. To eliminate linear dependency between columns of the Q matrix, the last column was removed prior to GWAS. To check whether the model we implemented was sufficiently stringent to control the population stratification and spurious associations, quantile–quantile (qq) plots were drawn to compare the distribution of observed versus expected p-values of all the SNPs at −log10 scale (Yu et al. 2006).

For the detection of significant marker trait associations (MTAs), we applied a significance threshold of false discovery rate (FDR) ≤ 0.05. The −log10 (p) values were displayed in the form of Manhattan plots using R library "CMplot" package in R 3.3.1.

III-2.5. Genomic Prediction

The genomic prediction (GP) was performed based on a two-stage analysis with weights. A genomic relationship matrix G was generated using 10,478 markers resulting from filtering and following the methodology described by Endelman et al. (2012) using rrBLUP (Endelman 2011) package in R (R Core Team, 2019). Genomic heritability (h^2) was computed as the ratio between the genetic variance due to markers over the sum of the genetic variance plus the error variance. This was done using the complete dataset to estimate variance components (additive and residuals) and hence the genomic heritability. Genomic best linear unbiased prediction (G-BLUP) was used to perform genome wide predictions. Models were performed using ASReml-R version 3.0 (Butler, 2009).

The evaluation of the different GP models for each trait was done by calculating the predicted ability (PA) and the prediction accuracy (Pacc) of the genomic estimated breeding values (GEBV) by using 10-fold cross-validation with 20% of the genotypes as validation set and the remaining 80% as training set for the models. PA was estimated as the correlation between the adjusted phenotypic value and the GEBV from the GP model. In addition, Pacc of GEBV is

the correlation between the true genomic breeding value and the predicted breeding value, which was estimated according to Equation (1):

$$\text{Pacc} = \frac{\text{PA}}{\sqrt{\text{h}^2}}$$

III-3. Results

We observed a wide phenotypic variation for the assessed floral traits among the 196 diverse bread wheat genotypes (Table II-3). The distribution of the evaluated traits showed normal distributions, and the estimated variabilities, correlations and heritabilities were reported in chapter II (El Hanafi et al. 2020) (Figure II-2; Tables II- 2, 3). We showed that absolute values of phenotypic correlations among the evaluated floral traits ranged be-tween 0.3 and 0.90 ($p < 0.001$) with the highest correlation recorded between anther extrusion and pollen mass (Table II- 2) (El Hanafi et al. 2020). The resulting BLUEs were used to run GWAS and genomic prediction.

III-3.1. Population Structure and Linkage Disequilibrium

Population structure (Q) was investigated by Bayesian-based structure analysis using 102 non-redundant markers across the whole genomes. The population structure of the 196 genotypes showed three subpopulations. A similar clustering result was obtained using discriminant analysis of principal components (DAPC) and principal component analysis (PCA). A detailed analysis was described by Tadesse et al. (2019).

In total, 10,477 polymorphic SNP markers out of 12,725, retained after removing SNPs with >10% missing data and MAF < 5%, were used to determine LD between all pairs of markers. The LDs for locus pairs and the scatter plot of LD (R^2) as a function of the intermarker distance (Mbp) for all genotypes were described by El Hanafi et al. (2021b) (Annex 2) and Tadesse et al. (2019) The plot of the LD estimates (R^2) as a function of the inter-marker genetic distance (Mbp) within the same chromosome for all genotypes indicated a clear LD decay with genetic distance. The baseline intersected with the spline curve at 6 Mbp based in the average R^2 = 0.23, as shown by El Hanafi et al. (2021b).

III-3.2. Marker Trait Associations

Extensive SNP genotyping was used to analyze the 196 ICARDA spring bread wheat genotypes. Out of the 12,725 markers obtained, 10,477 SNP markers were used to identify

markers associated to the floral traits using Q + K MLM method. Genome-wide association mapping revealed 70 significant markers at FDR ≤ 0.05. For the male floral traits, 17 markers were associated with anther extrusion (AE), 18 with visual anther extrusion (VAE), 14 with pollen mass (PM), 3 with anther length (AL), 1 with pollen shedding (PSH) and 1 with pollen viability (PV) (Tables III- 1 and 2 and Figure III- 1). For the female potential traits, 11, 3 and 2 MTAs were detected for openness of the floret (OPF), duration of floret opening (DFO) and stigma length (SL), respectively (Tables III- 1 and 2 and Figure III- 1). The percentage of the phenotypic variance ranged from 8% to 26.93%. Marker wsnp_Ex_c23235_32471767 on chromosome 1B explained the highest phenotypic variation of R^2 = 26.93% for anther extrusion. Most of the significant markers showed an association with at least three different traits (Table III- 1). *Kuk-ri_rep_c103359_233* and *wsnp_Ex_rep_c107911_91350930* showed associations with AE, VAE, PM, OPF and DFO. Seven markers (*wsnp_Ex_c13109_20725640, wsnp_Ex_c23235_32471767, wsnp_Ex_rep_c107911_91350866, wsnp_Ex_c21198_30327016, wsnp_Ku_rep_c89089_82009060, IAAV3802 and Ex_c11930_509*) were significantly associated with AE, VAE, PM and OPF. MTA analysis based on semi-dwarfing genes *Rht-1* (*Rht-B1* and *Rht-D1*) detected an association of Rht-D1b with both AE and VAE, which explained 20.57% and 17.34% of the phenotypic variation, respectively. There was no significant association with *Rht-B1*. A small negative effect was observed for the dwarfing allele *Rht-D1b* on AE and VAE of −3.12 and −0.59, respectively (Figure III- 1 and Tables III- 1 and 2)

Table III- 1: Significant common markers associated with anther extrusion, visual anther extrusion, pollen mass, openness of the floret and duration of floral opening at FDR ≤ 0.05.

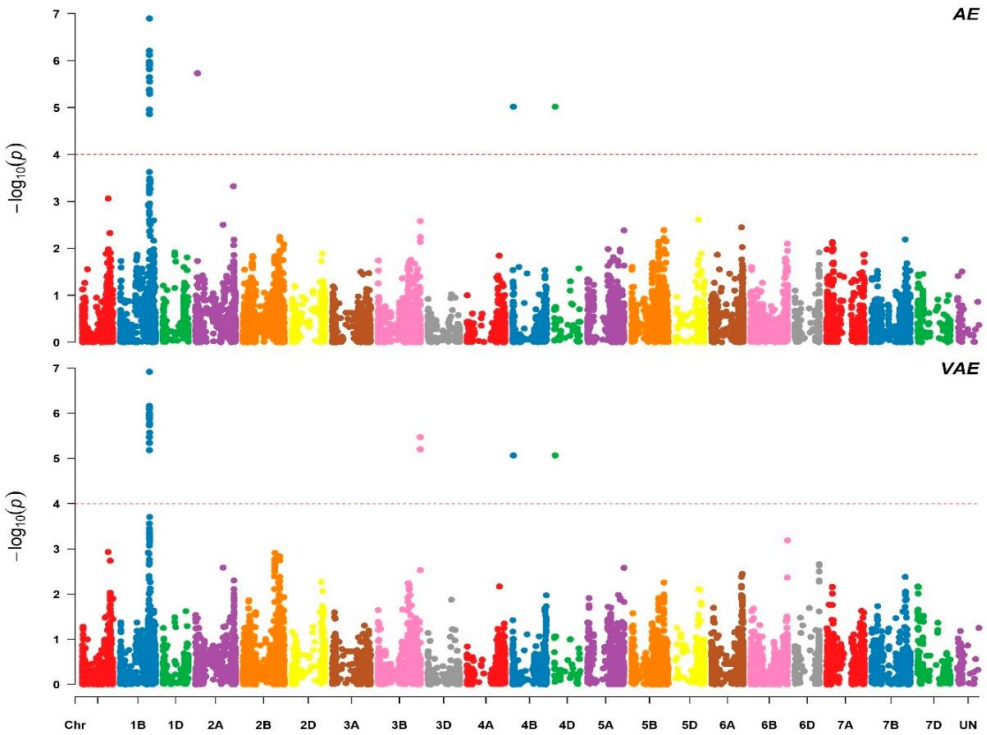
Marker	Chr	Physical position (Mbp)[a]	AE			VAE			PM			OPF			DFO		
			LOD[b]	Effect	%R²[c]	LOD	Effect	%R²	LOD	Effect	%R²	LOD	Effect	%R²	LOD	Effect	%R²
Rht-D1b	4D	-	5.02	0.18	20.58	5.07	0.05	17.34									
wsnp_Ex_rep_c103167_88182254	2A	33.04	5.73	-11.40	26.08				5.86	-3.03	23.76	5.13	-3.03	24.07	5.20	-10.1	18.7
Tdurum_contig30517_578	1B	566.84	5.37	12.88	25.34	5.18	1.22	20.95	5.01	3.54	21.98						
Tdurum_contig30517_310	1B	566.84	5.65	13.51	25.87	5.34	1.26	21.16				5.30	3.75	24.24			
BS00062724_51	1B	567.71	6.13	14.17	26.21	6.10	1.38	22.39	5.64	3.69	22.79						
Kukri_rep_c103359_233	1B	568.52	6.90	13.57	26.28	5.88	1.32	22.33	5.65	3.64	22.99	6.34	3.64	24.38	6.04	11.37	18.68
wsnp_Ex_c13109_20725640	1B	568.53	4.86	-14.10	26.53	5.96	-1.37	22.52	5.67	-3.60	23.24	6.16	-3.60	24.00			
wsnp_Ex_c23235_32471767	1B	568.57	6.21	14.35	26.92	6.16	1.39	22.93	5.70	3.74	23.28	5.44	3.74	24.53			
Excalibur_c10496_651	1B	568.97	5.38	-12.80	24.75	5.74	-1.33	21.64	5.17	-3.01	21.88						
wsnp_Ex_c23230_32466568	1B	568.97	4.96	12.68	25.56	5.48	1.23	21.62	5.30	3.37	22.39						
wsnp_Ex_rep_c107911_91350866	1B	570.28	5.97	13.73	26.52	6.92	1.33	22.44	5.66	3.63	23.03	5.31	3.63	24.24			
wsnp_Ex_rep_c107911_91350930	1B	570.28	5.90	13.57	26.28	5.88	1.32	22.33	5.65	3.64	22.99	5.34	3.64	24.38	5.52	11.36	18.67
wsnp_Ex_c21198_30327016	1B	570.29	5.98	13.79	26.47	6.00	1.35	22.66	5.52	3.61	22.98	5.27	3.61	24.24			
wsnp_Ku_rep_c89089_82009060	1B	570.30	5.82	-13.50	25.37	5.93	-1.34	21.90				5.23	-3.60	23.48			
IAAV3802	1B	571.06	5.56	-13.30	25.42	4.79	-1.30	21.73	5.31	-3.52	22.14	5.41	-3.52	23.57			
wsnp_Ku_c13043_20902807	1B	571.06	5.29	12.56	24.50	5.57	1.29	21.16	5.03	3.02	21.49						
Ex_c11930_509	1B	571.18	5.93	-3.52	24.10	5.98	-1.36	22.67	5.62	-3.52	23.22	5.11	-3.52	24.10			
Excalibur_c20309_539	3B	819.86				5.47	1.55	21.16									
Kukri_rep_c110544_248	3B	819.89				5.20	1.54	21.09									

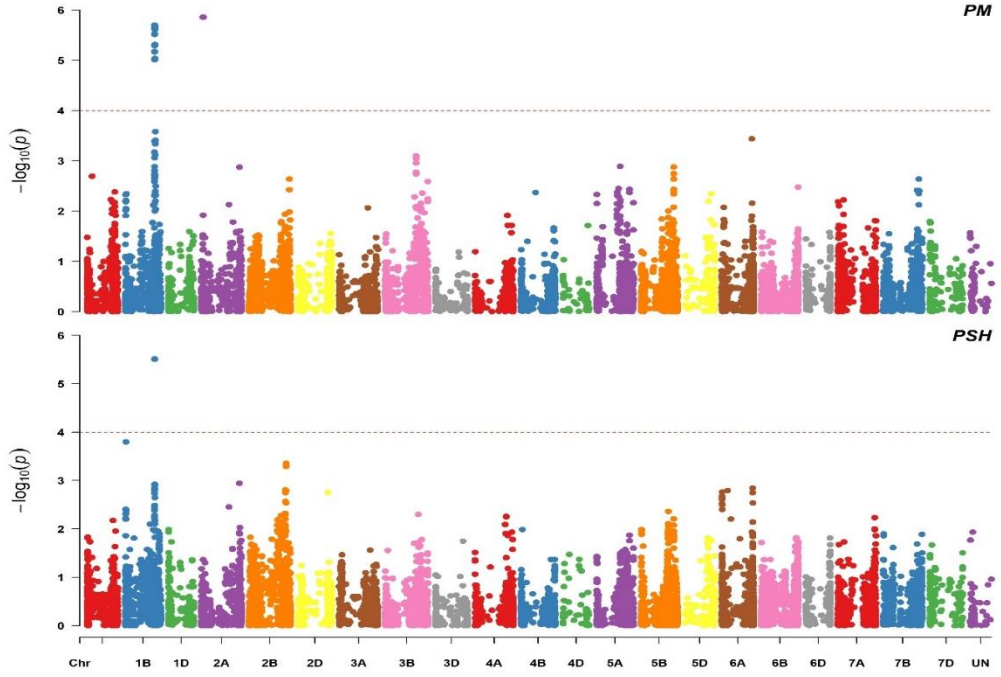
[a] Physical position based on the IWGSC RefSeq. 1.0; [b] LOD = -Log10(P) (logarithm of the odds); [c] R^2: phenotypic variation; AE: anther extrusion, VAE: visual anther extrusion, PM: pollen mass, OPF: openness of the floret, DFO: duration floral opening

Table III- 2: Significant markers associated with pollen shedding, stigma length, anther length and pollen viability at FDR ≤ 0.05.

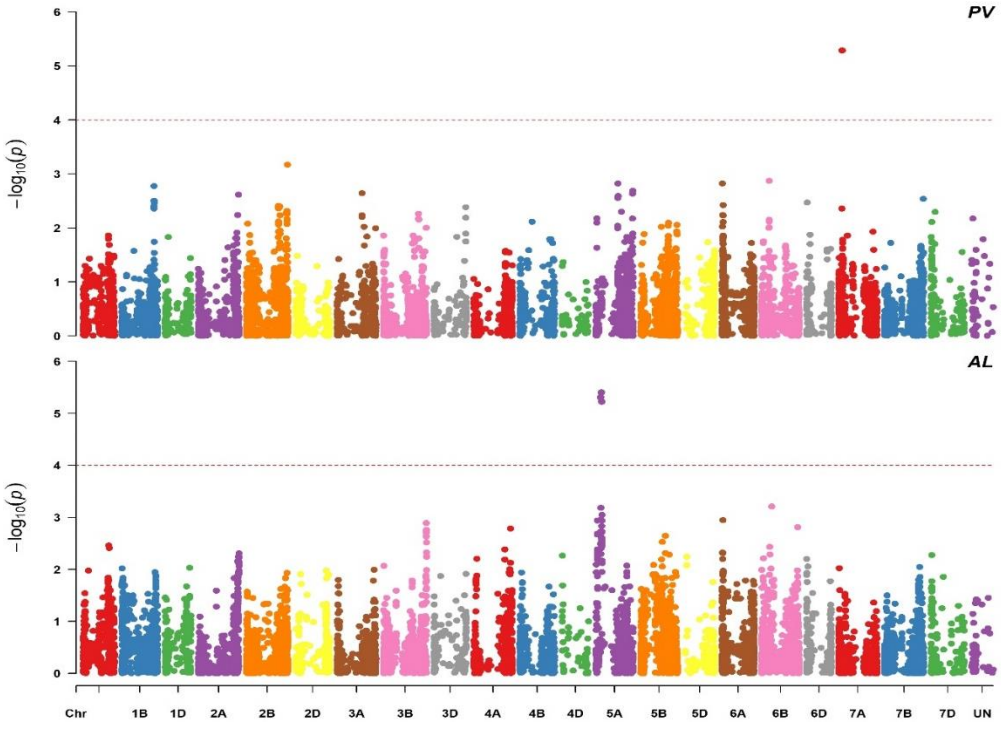
Marker	**Trait**	**Chr**	**Physical Position (Mbp)** [a]	**−Log10(*p*)**	**Effect**	**%R²** [b]
Excalibur_c10496_651	PSH	1B	568.97	5.51	−1.04	22.10
BS00063511_51	SL	1D	485.71	5.78	0.39	8.01
Kukri_c62142_683	SL	2A	692.64	5.90	0.24	9.55
BS00064387_51	AL	5A	87.40	5.30	0.20	17.86
wsnp_Ra_c10053_16636851	AL	5A	99.02	4.40	0.20	17.86
wsnp_Ku_c328_679106	AL	5A	104.23	5.22	0.20	17.86
Excalibur_rep_c109881_701	PV	7A	65.97	5.29	5.40	19.35

[a] Physical position based on the IWGSC RefSeq. 1.0; [b] R^2, phenotypic variation; PSH, pollen shedding; SL, stigma length; AL, anther length; PV, pollen viability




PM
PSH
−log10(p)
Chr
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
UN

(**b**)


PV
AL
−log10(p)
Chr
1B
1D
2A
2B
2D
3A
3B
3D
4A
4B
4D
5A
5B
5D
6A
6B
6D
7A
7B
7D
UN

(**c**)

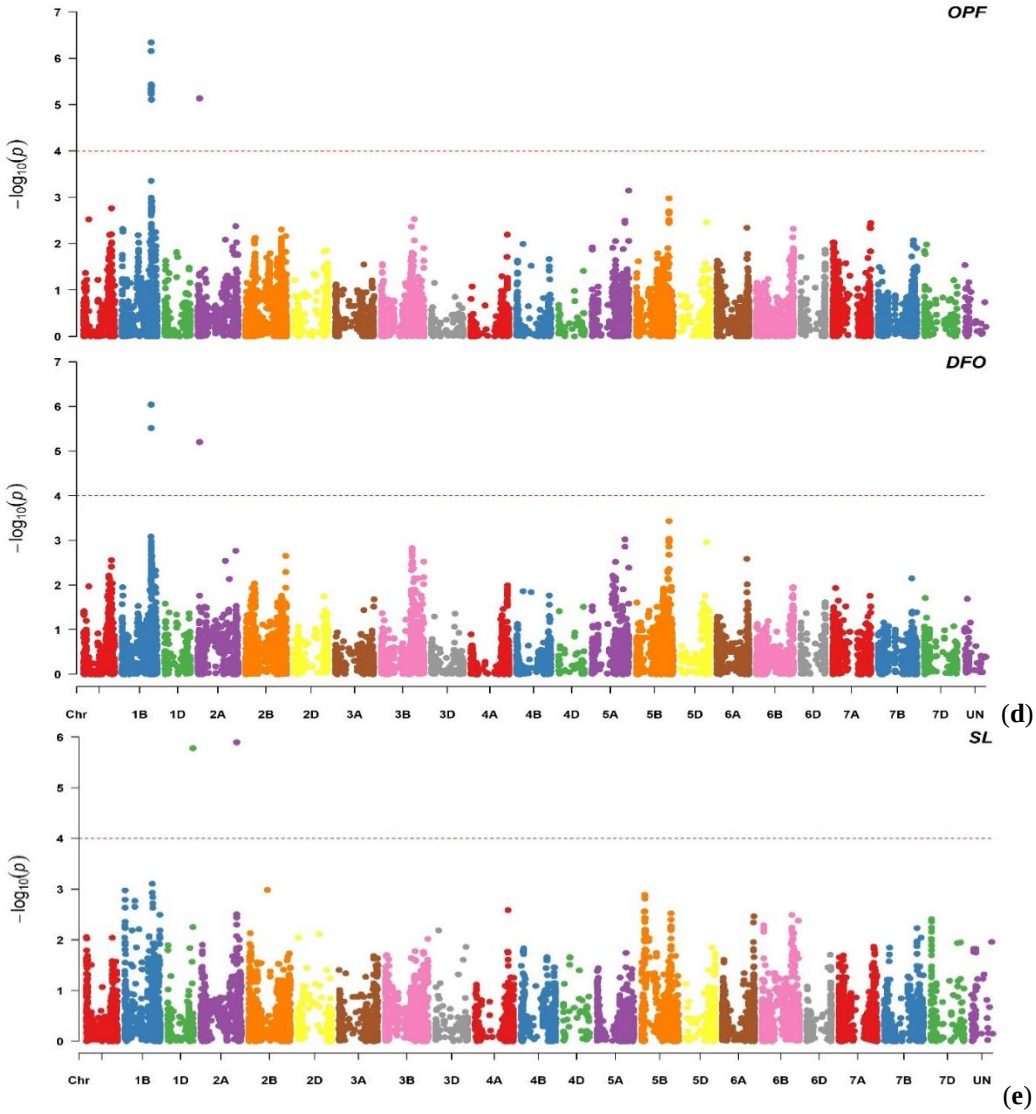


Figure III- 1: Manhattan plots of genome-wide association analysis for the evaluated floral traits: (**a**) AE and VAE; (**b**) PM and PSH; (**c**) PV and AL; (**d**) OPF and DFO; and (**e**) SL. p values are shown on a −log10 scale and the significant markers are above the threshold FDR ≤ 0.05. Trait abbreviations are given in Tables III- 1 and 2.

We investigated the relationship between the number of favorable alleles and the performance of the genotypes. Of the 17 significant markers associated with AE, 11 have a favorable effect (an increasing effect on AE) while 6 have an unfavorable effect (decreasing effect on AE). Sixty-six genotypes harbored the maximum favorable allele and five the maximum unfavorable one (Annex 3). The best performing genotype carrying the maximum number of favorable alleles was SOMAMA-9/ICARDA-SRRL-2 with the high AE value of 90.03%. We investigated the relevance of the addition of every allele in predicting AE performance by correlating the cumulative number of favorable and unfavorable alleles on the AE-BLUEs values. There was significant cor-relation between the AE-BLUEs values and their respective favorable and unfavorable alleles with $R^2 = 0.16$ and $R^2 = 0.18$ ($p < 0.001$), respectively.

Alleles per marker for VAE varied between 24 and 139. *Rht-D1b* was the marker with the highest number of observed alleles. SE-RI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15 and SOMAMA-9/ICARDA-SRRL-2, with the best VAE performances of 9.45 and 9.3, respectively, were the genotypes having the maximum favorable alleles (12). The linear regressions of the number of favorable and unfavorable alleles per genotypes versus the VAE-BLUEs values showed significant association with R^2 = 1.6 and R^2 = 1.5 ($p < 0.001$), respectively (Annex 4).

GWAS identified 14 significant markers associated with PM at FDR < 0.05, which were all commonly present with those associated with AE (Table III- 1). Ten markers had favorable additive effects, while five were unfavorable (Annex 5). The highest number of observations per marker was associated with those having unfavorable effect. The significant markers explained phenotypic variances (R^2) ranging from 21.29% to 25%. Again, SOMAMA-9/ICARDA-SRRL-2 appears among the best genotypes having the highest number of favorable alleles (six) and showing good pollen mass performance (41.11 mg). The linear regressions of the number of favorable and unfavorable alleles per genotypes versus the PM-BLUEs values showed significant associations with R^2 = 1.7 and R^2 = 1.6 ($p < 0.001$), respectively.

GWAS identified 11 significant markers associated with OPF on chromosomes 1B (10) and 2A (1), of which 12, 11 and 12 were common with AE, VAE and PM, respectively (Table III-1). The significant markers explained phenotypic variances (R^2) ranging from 23.48% to 24.53%. Eighty-six genotypes had the maximum cumulative number of favorable allele effect (six) (Annex 6). SOMAMA-9/ICARDA-SRRL-2 is the best genotype in terms of high OPF phenotypic values and carrying the highest number of favorable alleles. Linear regression on the relationship between the OPF-BLUEs values and the number favorable and unfavorable alleles showed significant associations with R^2 = 1.13 and R^2 = 1.19 ($p < 0.001$), respectively.

Besides the female floral traits, GWAS detected three significant markers associated to duration of floret opening (DFO) with phenotypic variance of 18.7% each located in 1B (two) and 2A (one) (Table III- 1). The same markers were present in association with AE, PM and OPF. SOMAMA-9/ICARDA-SRRL-2 and HAAMA-17/ANGI-2 are among the genotypes having the best OPF performance and carrying the highest number of favorable alleles (Annex 7).

III-3.3. Genomic Prediction of the Potential Floral Traits

We evaluated genome-wide prediction of the potential floral traits with relevance for hybrid breeding in wheat. The accuracies of genome-wide prediction were estimated by using tenfold cross-validation based on the same set of phenotypic and genomic data used for association mapping analysis. Low to moderate genomic heritability (h^2) was observed for the evaluated floral traits with the highest value (0.30) recorded for anther length while stigma length showed the lowest heritability (0.08) (Table III- 3). The prediction ability ranged between 0.45 for anther extrusion and 0.14 for stigma length across years. The prediction accuracy of the model for hybrid wheat floral traits were high for all evaluated floral traits, ranging between 0.92 for visual anther extrusion and 0.52 for stigma length.

Table III- 3: Genomic heritability, prediction ability and prediction accuracy of the assessed floral traits.

Trait	h^2	PA	Pacc
VAE	0.21	0.43	0.92
PSH	0.24	0.42	0.86
AEX	0.28	0.45	0.85
DFO	0.2	0.38	0.85
PM	0.24	0.41	0.83
OPF	0.27	0.39	0.76
AL	0.3	0.4	0.73
SL	0.08	0.14	0.52

PA, prediction ability; Pacc, prediction accuracy; AE, anther extrusion; VAE, visual anther extrusion; PM, pollen mass; OPF, openness of the floret; DFO, duration floral opening; PSH, pollen shedding; SL, stigma length; AL, anther length; PV, pollen viability.

III-4. Discussion

Hybrid wheat breeding technology has great potential to raise wheat production, particularly in the context of global climate change and future food security. The maximum heterosis achieved can assess the usefulness and efficiency of a hybrid wheat program. However, the stringent autogamous nature of wheat presents a bottleneck for suitable seed production system. The ability of enhanced outcrossing might be achieved by choosing specific parental lines for best hybrid combination. Selection for potential hybrid wheat traits such as anther extrusion, pollen mass, degree and duration of floret opening is challenging and time consuming. Molecular tools are widely considered to hold promise for the dissection of genomic regions harboring genes/markers for hybrid traits. In this study, we evaluated the potential of genomic approaches in identifying genes/QTL that could be effectively used to fine-tune the floral traits and greatly simply redesigning wheat flower.

III-4.1. Marker Traits Associations

While hybrid wheat programs have operated for several decades and phenotyping floral characteristics traits have been fairly studied, little is known of the genetic mechanisms controlling male and female flower development. Marker–trait associations (MTAs) were identified for the potential floral hybrid wheat traits by using a mixed linear model considering both kinship (K) and population structure (Q) matrices as covariates. In total, 70 markers were associated with various evaluated traits. For the floral associated markers, 17 and 18 MTAs were linked to AE and VAE, respectively, of which 16 markers were in common. The phenotypic variance explained by these markers ranged 17.33–26.92%, which is greater than

what was reported by Muqaddasi et al. (2016) and Boeven et al. (2016). The MTAs associated with AE and VAE located on chromosome 1B are in tight LD with each other and located at close distances (566–571 Mbp), making this genomic region very important and warranting further investigation. The consistently significant markers carrying the maximum favorable alleles confer high performance for that specific trait, indicating the importance of accumulating favorable alleles to improve the potential floral traits key success for hybrid wheat production.

The comparison of marker intervals with previously reported studies is challenging because most used different marker systems (AFLP, DArT and SSR markers) and genetic linkage maps (RIL, DH and F2 populations) (Skinnes et al. 2010; Lu et al. 2013; Buerstmayr and Buerstmayr, 2015; Okada et al. 2019). Few recent association mapping studies have been published using DArT and GBS markers (Muqaddasi et al. 2016, 2017b; Ollier et al. 2020) or SNP markers (Boeven et al. 2016a; Muqaddasi et al. 2017a; Adhikari et al. 2020a). The marker *wsnp_Ex_c5101_9053178* on chromosome 1B found associated with AE and VAE was reported previously by Boeven et al. (2016). Similarly, the two markers *Excalibur_c20309_539* and *Kukri_rep_c110544_248* (3B) associated with VAE were previously reported by Muqaddasi et al. (2017a). Other than those, all markers identified were different despite the high heritability of the trait; this might be explained by the strong dependence of anther extrusion on the genetic background and/or other related floral traits such as flower gapping and spikelet spacing.

The complex nature of anther extrusion indicates the trait is governed by many loci with small to large effects and explains the considerable level of phenotypic variance. A similar conclusion has been drawn in many studies (Skinnes et al. 2010; Lu et al. 2013; Langer et al. 2014a; Buerstmayr and Buerstmayr, 2015; Muqaddasi et al. 2016; Boeven et al. 2016).

Semi-dwarfing allele *Rht-D1b* showed an association with both AE and VAE, which explained, respectively, 20.57% and 17.33% of the phenotypic variance. The candidate gene *Rht-B1* was not significantly associated with any of the evaluated floral traits, probably due to the very low number of lines carrying the mutant type of *Rht-B1*, or it might also be attributed to the different mechanism of action of the semi-dwarfing gene under the influence of the surrounding genetic backgrounds (Muqaddasi et al. 2016, 2017a). On average, lines carrying the dwarfing allele *Rht-D1b* had a reduced AE by 1.45% and showed reduced VAE by almost two scoring points (Figure III- 2). In line with previous studies (Langer et al. 2014; Boeven et al. 2016), our results

thus provide strong evidence for the negative effect of *Rht-D1b* on anther extrusion. However, it is important to note that the observed effect might be attributed not only to the allelic variation of the gene itself but also to significant epistatic interaction with other closely linked genes in the locus (Miedaner et al. 2011; Li et al. 2012). Some other GWAS studies did not reveal an association of AE or VAE with *Rht-B1* alleles (Muqaddasi et al. 2016, 2017a; b; Boeven et al. 2016; Adhikari et al. 2020a). Parallelly, He et al. (2016) did not show the effect of *Rht-D1* on anther extrusion in a population of 131 RILs. Nevertheless, high AE wheat accessions carrying semi-dwarfing alleles of *Rht-B1b* or *Rht-D1b* do exist (Würschum et al. 2018).

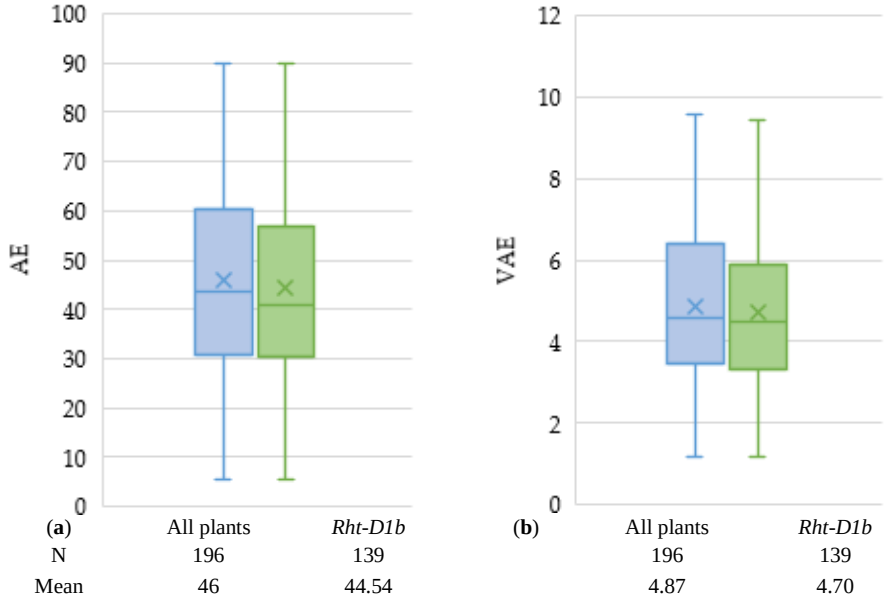


Figure III- 2: Box plots showing AE (**a**) and VAE (**b**) for genotypes carrying dwarfing gene allele Rht-D1b. N represents the total number of genotypes for the whole set (bleu) and the genotypes carrying *Rht-D1b* (green) and the mean values associated with each group. AE, anther extrusion; VAE, visual anther extrusion.

It has been demonstrated that dwarfing genes affect not only the height but also some other floral associated traits (Boeven et al. 2016). We showed weak correlation between plant height and anther length using the same panel in El Hanafi et al. (2020), but we did not observe any association between *Rht* genes and anther length. In the same context, Okada et al. (2019) found that *Rht-1* had significant association with anther length, anther extrusion, plant height and spike length. Moreover, Cheng et al. (2004) reported that *Rht-D1* encodes DELLA proteins that act as repressors of plant growth in the presence of gibberellin acid (GAc). Semi dwarfing alleles have gibberellin-insensitivity resulting in reduced plant height and low anther extrusion at the same time. This shows that *Rht* genes are involved in multiple floral traits and, therefore, can be used as candidate genes for improved anther extrusion. However, it would be interesting to test additional plant height genes for better understanding of their effect on anther extrusion and other floral traits, and it might subsequently offer choices to take full advantage of the

existing dwarf genetic resources and to use them in designing superior hybrids in breeding programs.

More recently, anther extrusion has been the focus of several linkage mapping for fusarium head blight (FHB) resistance since high extruded anthers showed tight association with lower FHB infection (Lu et al. 2013; Kubo et al. 2013; Buerstmayr and Buerstmayr, 2015; He et al. 2016; Xu et al. 2019; Ollier et al. 2020). Several studies found that the peaks of FHB QTL were constantly located at the *Rht-D1* locus on 4DS, which is in concordance with our findings. Similarly, the two markers *Excalibur_c20309_539* and *Kukri_rep_c110544_248* on chromosome 3B at very close distances were associated with VAE and located in the *QFhs.ndsu-3B* region, which is a major QTL for FHB resistance (Liu and Anderson, 2003). Considering the close relationship of VAE and FHB resistance, these markers could be proposed as promising morphological markers for FHB resistance and vice versa.

Pollen mass, another important male floral trait, was shown to be taken as proxy for an initial selection of superior genotypes in early generations (Langer et al. 2014). However, considering its intensive work and time requirements, we therefore focused in this study on understanding its genetic basis in order to identify associated markers and thereby pave the way for a systematic exploitation of heterosis in wheat. Our genome-wide association mapping revealed 14 MTAs associated with pollen mass with a small effect observed and accounting for maximum 23.76% of the phenotypic variance. Among these markers, 14 and 13 markers were implicated as sites for AE and VAE, respectively. A recent association mapping study for PM in winter bread wheat detected six QTL for pollen mass, among which *Rht-D1* was the most strongly associated locus (Boeven et al. 2016). Conversely, our study did not show similarities between the markers previously detected or an association of PM with *Rht* alleles. Therefore, the absence of a major marker and small effect markers suggests the complex genetic architecture of pollen mass.

Pollen shedding, taken as a proxy, appears to be a promising trait, allowing indirect selection of the best performing male and female associated traits (El Hanafi et al. 2020). Being an important determinant for outcrossing, profuse pollen shedding outside the florets is important when there is a high rate of extruded anthers. This has been demonstrated by the high correlation between released pollen mass and pollen shedding (Langer et al. 2014; El Hanafi et al. 2020). However, GWAS did reveal one marker *Excalibur_c10496_651* on 1B associated to PSH commonly identified for PM, AE and VAE. The small number of markers with no

major gene associated to PSH more likely suggests a quantitative inheritance and thus a complex genetic architecture underlying the trait pollen shedding.

The importance of pollen viability as a factor in enhancing the out-crossing ability in wheat has long been recognized. However, to our knowledge, assessment of the viability of wheat pollen has been done using staining methods, in vitro germination or impedance flow cytometry (Impe et al. 2020). However, except these studies, the genetic basis of pollen viability has not been investigated in great detail up to now. Therefore, we performed GWAS, aiming to find tightly linked markers to pollen viability. *Excalibur_rep_c109881_701* (7A) was the only marker showing an association with pollen viability and contributing 19.35% of the observed phenotypic variation. The small number of associated markers might be in part due to gaps in the genome with poor marker coverage or the complex genetic architecture underlying this trait.

Adequate cross pollination attributes are essential for hybrid development. Many reports have emphasized the importance of wheat anthers and stigma size as favorable pollinator traits in grain setting, a crucial factor determining the success of hybrid wheat programs (de Vries 1974; Langer et al. 2014; Guo et al. 2015). Work focusing on the genetic control of anther and stigma length in wheat is very limited. Therefore, identification of genomic regions associated with AL through genome-wide association study would help in this regard. In the current study, MLM analysis revealed three markers associated with AL on 5A and two other markers associated with stigma length on 1D and 2A. To date, few genes have been identified associated effectively in increasing wheat AL, while the genetic basis of stigma length is still unknown. Hence, it is currently difficult to align and compare the identified markers. Song et al. (2018) revealed seven major markers that were associated with AL which did not overlap with any of the identified markers. It has also been observed that the addition of rye chromosome 4R to wheat increases anther length by 16% (Nguyen et al. 2015). This finding can greatly enhance pollination in wheat. Besides, further studies are required to dissect the novel genes related to anther and stigma length, which can increase our understanding of the underlying genetic mechanisms of AL and SL that represent key traits for the successful production of hybrid wheat seeds.

The genetic architecture of female traits is far less dissected. Previous studies have hinted at wide variability among female hybrid traits such as the degree and the duration of floret opening. However, it is often challenging to address these traits due to the intensive work and

time requirements. Therefore, we opted to detect the genomic regions harboring markers associated to openness of the florets and duration of floret opening to substantially select the superior female parents. GWAS revealed 11 and 3 MTAs associated with OPF and DFO, respectively. Among these markers, *wsnp_Ex_rep_c103167_88182254* (2A) was reported to be associated with OPF, DFO, PM and AE. *Kukri_rep_c103359_233* and *wsnp_Ex_rep_c107911_91350930* on chromosome 1B were potentially linked to OPF, DFO, PM, AE and VAE, suggesting a potential genetic association that controls these complex traits.

Moreover, it has been demonstrated that wheat plants with narrow flower opening (cleistogamous) and/or short duration of floret opening will have a lower incidence of FHB by reducing the area and period in which Fusarium spores can enter the floret and initiate infection (Gilsinger et al. 2005; Kubo et al. 2010, 2013). Therefore, the genomic regions of the markers associated to OPF and DFO merit further research and thus the validation of the markers associated to FHB is particularly valuable and may allow indirect selection for these two female traits or vice versa.

III-4.2. Genome-Wide Prediction of Hybrid Floral Traits

The potential of genomic selection for hybrid floral traits have previously only been conducted for anther extrusion and pollen mass (Boeven et al. 2016; Muqaddasi et al. 2017b; Adhikari et al. 2020a). Boeven et al. (2016) evaluated different schemes to perform genomic selection using 209 winter wheat lines and reported prediction abilities of 0.3, 0.6 and 0.4 for VAE, counted AE and PM, respectively, using rrBLUP model. Inclusion of weighted effect *Rht* loci via based on weighted ridged regression BLUP (wrrBLUP) increased the prediction abilities to 0.5, 0.7 and 0.57 for VAE, counted AE and PM, respectively. In Muqaddasi et al. (2017b), prediction accuracy was 0.62 for VAE using rrBLUP medel. Recently, Adhikari et al. (2020) performed genomic prediction for AE using 603 advanced lines from the CIMMYT hybrid breeding program applying seven models of increasing complexity and using three cross-validation scenarios. High prediction accuracy was observed for AE and VAE with 0.45 and 0.73, respectively, where all lines in one environment were fully predictable by the other environments. However, prediction accuracy of the main effect model comparable to the previous studies was in the range of 0.42–0.44 for counted AE and 0.33–0.46 for VAE. In the current study, using tenfold cross-validation, high prediction accuracy was recorded for AE, VAE and PM with 0.85, 0.92 and 0.83, respectively, demonstrating the feasibility of using genomic selection for quick identification of potential hybrid parents. Pollen shedding, anther

length, openness of the floret and duration of floret opening have also shown high prediction accuracy with 0.86, 0.73, 0.85 and 0.76, respectively, indicating that these traits can be predicted with high reliability. Stigma length showed on the one hand moderate prediction accuracy of 0.52, indicating that genomic selection can be useful to preselect promising female lines with acceptable accuracy, and on the other hand very low genetic heritability. However, subsequent field/lab test might be needed to verify the suitability of the preselected lines for hybrid wheat production. The low to moderate predictive ability and genomic heritability recorded in the current study might be due to the small population size, the genetic architecture of the trait of interest and/or the impact of adding fixed effects (genes/QTL) (Sarinelli et al. 2019).

III-5. Conclusions

Considering the high heritabilities coupled with high genetic advance, conventional methods based on direct or visual selection of phenotypes have contributed significantly to improving floral traits with the relevance for hybrid breeding. However, the assessment of these traits is challenging and time-consuming. To cope with this constraint, we employed genome-wide association study to reveal the genetic basis of various male and female hybrid potential traits. Many markers with small to large effects were detected, indicating the genetic complexity of the evaluated traits. Within this context, the significant markers with medium to large effects can be applied in hybrid breeding via marker-assisted selection. However, in the case of minor genes/QTL associated to the trait of interest, phenotypic selection remains most cost-effective and promises a high selection response. Nevertheless, these minor effect genes might be included with the aim of improving the related trait. In parallel, genome-wide prediction appears promising to predict most of the hybrid floral traits with high accuracy for the highly quantitative traits that are controlled by small- to large-effect genes/QTLs.

Chapter IV: Hybrid seed set in relation with floral traits and estimation of heterosis and combining abilities for yield and its components in wheat

Abstract

Breeding hybrids with maximum heterosis requires an efficient cross-pollination and an improved male sterility system. Renewed efforts have been done to dissect the phenotypic variation and genetic basis of hybrid floral traits, although the potential of tailoring the appropriate flower design on seed setting is less known. To this end, elite wheat genotypes were crossed using a chemical hybridizing agent at different doses. 23 hybrids were developed from a partial diallel design and planted in alpha lattice design with their parents at two locations in Morocco for two years to evaluate for yield components heterosis and combining abilities. The 13.5 l ha-1 dose induced maximum level of sterility (95%) and seed set showed large phenotypic variation and high heritability. Parallelly, seed set showed tight correlation with pollen mass (0.97), visual anther extrusion (0.94) and pollen shedding (0.91) ($p < 0.001$) allowing direct selection of the associated traits. Using the combined data, mid-parent heterosis ranges were -7.64 – 14.55% for Biomass (BM), -8.34 – 12.51% for thousand kernel weight (TKW) and -5.29 – 26.65% for grain yield (YLD). While best-parent heterosis showed ranges of -11.18 – 7.20%, -11.35 – 11.26% and -8.27 – 24.04% for BM, TKW and YLD, respectively. The magnitude of general combining ability (GCA) variance was greater than the specific combining ability (SCA) variance suggesting the preponderance of additive gene action for BM, TKW and YLD. The favorable GCA estimates showed a simple attitude to predict additive effects contributing to high heterosis and thus could be an effective approach to be used for selection of excellent parents in early generations.

IV-1. Introduction

As of 2020, the world population is over 7.8 billion and projected to increase by more than 25% to reach 9.9 billion in 2050 IISD (2020). This ever-growing population is leading to concerns about the increasing demand for food. In order to achieve sustainable agricultural development for food security, it is important to breed resilient high-yielding crops that are suited to suboptimal growing conditions. Hybrid wheat represents a promising approach that improves yield potential, yield stability across diverse environments and consequently increases global wheat productivity (Gupta et al. 2019). However, hybrid wheat occupies nearly 1% of total world wheat area and produced mainly in Europe, China and India (Singh et al. 2015). Central Europe, particularly in France, Hungary, and Germany, represents about half of the world's hybrid wheat production area with two leading hybrid seed producer companies ASUR Plant Breeding SAS, (previously SAATEN UNION) and Nordsaat Saatzuchtgesellschaft mbH (Longin et al. 2012). All hybrids in Europe are currently produced using chemical hybridization agents (CHAs), most commonly Croisor® 100 (Sintofen; former Dupont-Hybrinova, Saaten-Union Recherche, France). In China, photoperiodic sensitivity and cytoplasmic male sterility (CMS) systems are being successfully used for grain production (Longin et al. 2012; Whitford et al. 2013). Wheat hybrids in India are developed based on cytoplasmic male sterility (CMS) systems derived from *Triticum timopheevii* and CHA approach (Singh et al. 2010; Gupta et al. 2019).

For long term success of hybrid breeding programs, adequate exploitation of heterosis, cost-effective hybridization system, high seed density, and development of high-yielding heterotic groups and patterns must be established (Dreisigacker et al. 2005; Longin et al. 2012, 2013; Selva et al. 2020). Heterosis or hybrid vigor is the superiority of a hybrid in performance over its corresponding parental inbred lines (mid- or best parent heterosis). Recently, significant effort has been done to develop successful hybrids through different approaches for more cost-effective seed production (Kempe et al. 2014; Würschum et al. 2017; Tucker et al. 2017). Studies based on experimental data in hybrid wheat breeding demonstrated that best-parent heterosis ranged from -70.4 to 54.3% and -26.9 to 29.2% in two successive years using CHA Croisor® 100 of elite winter wheat lines from University of Nebraska–Lincoln (UNL) and Texas A&M University breeding programs while mid-parent heterosis for grain yield varied from -15.33% to 14.13% of 112 hybrids produced using CMS system (Dreisigacker et al. 2005; Adhikari et al. 2020b).

Despite these hybrid yield advantage, development of practical hybrid seed production system has not reached yet large-scale hybrid wheat production. This can, in part, be attributed to the high cost of hybrid wheat seed. Therefore, maximizing seed set per spike is an effective approach to tremendously improve the cost-efficiency of hybrid seed production in self-pollinating crops such as wheat irrespective of the hybridization system. For this matter, floral biology will play an important role in increasing the out-crossing ability. Knowledge of the genetic basis of the important floral traits and exploring the genetic variability of the parental pairs might offer new perspectives to restore the fertility control system (Whitford et al. 2013; Selva et al. 2020).

Numerous studies have addressed the importance of appropriate floral and flowering traits as key determinants for potential hybrid parents. Traits like anther extrusion, visual anther extrusion, pollen mass, degree and duration of flower opening, stigma receptivity, anther length, number and longevity of pollen grains showed a large genotypic variation and moderate to high heritabilities and thus might be integrated for successful fertility control system (D'Souza 1970; de Vries 1971; Langer et al. 2014; Muqaddasi et al. 2017; Song et al. 2018; Boeven et al. 2018; El Hanafi et al. 2020). Considerable progress has been made to identify proxies allowing direct and indirect selection of the associated hybrid potential traits through using new precision phenotypic approaches and advanced high-density genomic tools (genome wide association studies and genomic prediction) (Langer et al. 2014; Muqaddasi et al. 2016, 2017; Boeven et al. 2016, 2018; El Hanafi et al. 2020; Adhikari et al. 2020a). Consequently, anther extrusion and profuse pollen shedding shown to be promising traits to predict related correlated traits such as pollen mass, openness and duration of floral opening (Langer et al. 2014; El Hanafi et al. 2020). In the same context, selection of the parents with best parental trait combinations is key determinant of the heterosis level (van Ginkel and Ortiz 2018). However, potential male and female parents with superior floral and agronomic trait combinations may not always transmit desirable traits to their hybrid progenies. To approach this issue, combining ability, as important genetic parameter, has been widely adopted in plant breeding to compare the performances of lines in hybrid combination (Sprague and Tatum 1942; Comstock et al. 1949; Singh et al. 1974; Ahangar et al. 2008; Adhikari et al. 2020b).

The concept of general combining ability (GCA) and specific combining ability (SCA) were first defined by (Sprague and Tatum 1942). GCA indicates the average performance of a parent in different hybrid combinations, whereas SCA is the deviation in performance of certain hybrid combinations as compared to what would be expected based on the GCA of the parents

involved. While the GCA is an important indirect criterion in selecting inbred parents which means that the phenotypic selection could be based on the GCA (Longin et al. 2013), SCA might be used to identify a specific cross combination for exploitation through heterosis breeding (Kumari et al. 2015).

Diallel analysis scheme was used widely to identify parental lines with high GCA and combinations with high SCA (Comstock and Robinson 1948) or to obtain genetic information of hybrids and their parents for further classification in heterotic patterns (Cabral Mendes et al. 2015). To establish heterotic groups, selecting high-yielding parental lines with appropriate traits combinations could help in clustering the germplasm in different heterotic groups based on trait-per-se performance. In this regard, phenotypic and genotypic assessment of 196 genotypes for various floral and flowering traits was done previously in (El Hanafi et al. 2020, 2021a). Our objectives in this study were (1) investigate the efficiency of the CHA Croisor ®100 on the selected female in a crossing blocks using three different doses; (2) assess hybrid seed set of the successful hybrids produced using the appropriate rate; (3) investigate the genetic variance and heritability of hybrid seed set and its correlation with male traits; (4) evaluate hybrids performance and obtain estimates of the expressed percentage of mid-parent (MPH) and best-parent heterosis (BPH) levels; (5) and determine patterns of GCA and SCA.

IV-2. Materials and methods

IV-2.1. Plant materials and field experiments

Total of 18 elite bread wheat (*Triticum aestivum* L.) parents were selected from ICARDA bread wheat breeding program based on their performance for specific hybrid potential traits demonstrated in El Hanafi et al. (2020) and for their tolerance to drought and yellow rust resistance (Annex 2) (El Hanafi et al. 2021b). Male lines were taller than the females, with long extruded anthers producing large amounts of pollen, while the female lines were shorter with wide and extended floral opening and prolonged stigma receptivity. The parents were all selected for grain yield and adaptation in different environments and their flowering nick was well synchronized. Seven male lines and eleven female testers were used for this study (Annex 8). Experimental hybrids were made using Croisor®100 (active ingredient sintofen; 1-(4-chlorophenyl)-5-(2-methoxyethoxy)-4-oxo-1,4-dihydrocinnoline-3-carboxylic acid) (Asur Plant Breeding) sprayed on the female plots. We used 3 different rates 11.5, 12.5 and 13.5 l ha^{-1} to test the efficiency of the chemical. The chemical was sprayed According to the manufacturer's recommendations, that is before flowering at stage 34 of the Zadoks scale

(Zadoks et al. 1974) mixed with Heliosol at a rate of 0.3 ml m^{-2} as an adjuvant agent. The CHA performs as a male gametocide and the sterility in the female treated plots was verified by bagging individual spikes. CHAs are an easy and rapid hybrid production system that induce male sterility by a simple spray at the correct stage. Each crossing block consisted of different females surrounded by a unique male pollinator. To avoid pollen flow from any nearby plots, isolation walls were placed around every crossing block with early and tall barley (*Hordeum vulgare L.*).

To evaluate hybrid seed set and its relationship with other male floral and agronomic traits, the male lines were sown in 2 long strips of 5 m with 0.3 m spacing next to 3 female rows (Figure IV- 1). Field experiments were conducted during 2017-2018 and 2018-2019 growing seasons at Merchouch Station, Morocco (33°36'N, 6°43'W, 394 m a.s.l.). All experiments were managed according to standard cultural practices. During the flowering stage, different traits were assessed: (1) Visual anther extrusion (VAE) was visually scored on a scale from 1 to 9 (1 = no anthers extruded, 9 = maximum anther extrusion). (2) Pollen mass (PM) in mg was recorded to estimate the released pollen from 10 randomly selected spikes. (3) Pollen shedding (PSH) was visually scored on a scale from 1 to 9 (1 = no pollen shedding and 9 = maximum pollen shedding). (4) Anther length (AL) in mm was recorded as mean of nine anthers collected at flowering stage using image analysis software ImageJ (https://imagej.nih.gov/ij/index.html). (5) Seed set was the average of seed count harvested from 10 randomly selected female heads. (6) days to heading (DH) was recorded as number of days at 75% spike emergence (Zadoks GS 67) (Zadoks et al. 1974). (7) Plant height was measured in cm from ground to spike tip without awns. (8) Spikelets per spike (SPS) was counted as mean spikelets per 5 random spikes. (10) Grain yield per spike (GYS) was the mean weight of 5 random spikes. VAE, PM, PSH, AL, DH, and PLH were taken from the male plots at the flowering stage, while seed set, SPS and GYS were collected from female plots at post-flowering stage.

Figure IV- 1. Experimental design to test the efficiency of the CHA used and female seed set

The produced hybrid seed from each production year (2018-2019) along with their parents were sown in two yield trials following an alpha lattice design with two replications in $3m^2$ plots. The trails were conducted in semi-arid region at Merchouch Station, Morocco (33°36'N, 6°43'W, 394 m a.s.l.) and in dry arid location in Sidi El Aydi station, Morocco (33°12'N, -7°62'W, 236 m a.s.l.) during the 2018-2019 and 2019-2020 cropping seasons. Days to heading (DH) and maturity (DM) in Julian days, plant height (PLH), number of spikelets per spike (SPS), spike length (SPL), biomass (BM), thousand kernel weight (TKW) and grain yield (YLD) were recorded on a plot basis. SPS and SPL were collected accordingly on five randomly selected spikes for each plot.

IV-2.2. Statistical analyses

A linear mixed model using restricted maximum likelihood (REML) method (Patterson and Thompson 1971) was used to analyze the first data generated after the testcross by the chemical. Best linear unbiased estimates (BLUEs) were calculated for genotypes for all traits following mixed model (1):

$$(1) \qquad y_{kmn} = g_k + l_m + b_{nm} + gl_{km} + ek_{mn}$$

Where y_{kmn} is the phenotypic observation of the kth genotype in the nth block of the mth location, gk the effect of the kth genotype, lm the effect of the mth location, bnm the effect of the nth block nested within the mth location, and ekmn the residual plot error associated with ykmn. Genotypes were considered as fixed to get the estimated means in order to compute the correlations between the traits. While genotypes were considered as random to estimate the

genotypic variance components and hence to compute heritability. Broad sense heritability (H^2) of the evaluated traits was calculated from the expression (2):

$$(2) \qquad \sigma^2 g / (\sigma^2 g + \sigma^2 g.e / \text{ne} + \sigma^2 e / \text{ne.nr})$$

Where $\sigma^2 g$ represented the genetic variance, $\sigma^2 e$ the error variance, ne was the number of environments and nr the number of replicates. All models were fitted using ASReml v3.0-1 (Butler et al. 2007) in R v3.3.1.

To evaluate the produced F_1 hybrids performances versus their parents, statistical analyses were performed on a yearly basis as well as across years and environments. A linear mixed model using restricted maximum likelihood (REML) method was used for data analysis (Patterson and Thompson, 1971). For yearly analyses, genotypes were considered as fixed whereas blocks were considered as random effects. BLUEs for each genotype were calculated for each genotype and each trait and hence used to compute traits correlations. Genotypes were taken as random to calculate the BLUPs and hence to get the genotypic variance components and then to compute heritability and the genotypic coefficient of variation. For the across years and environments analyses, years, environments and their interaction were taken as fixed effects and the genotype by year and environment interaction were random to get the genotypic and genotype by year interaction variance components. Broad sense heritability was computed following the equation (2).

Pearson correlation and its corresponding p-values was calculated between each trait in each year. Repeatability (h^2) was calculated from the expression (5)

$$(5) \qquad \sigma^2 g / (\sigma^2 g + \sigma^2 g + \sigma^2 e / \text{nr})$$

For each parent combination, mid parent heterosis (MPH) and best parent heterosis (BPH) for each location and environment as well as for the combined dataset were calculated as follows:

$$(6) \qquad \text{MPH} = (F_1 - \text{MP}) / \text{MP} \times 100\%$$

$$(7) \qquad \text{BPH} = (F_1 - \text{BP}) / \text{BP} \times 100\%$$

Where F_1 was the value of the hybrids produced; MP was the mean parent value; and BP represents the highest performing parent.

To evaluate the overall heterosis across locations and years, the BLUEs were used, while BLUPs were considered to estimate the combined GCA and SCA using the following model and using year and locations as replications.

$$(8) \qquad y_{ijk} = \mu + g_i + g_j + s_{ij} + \varepsilon_{ijk}$$

In which y was the phenotypic performance for the hybrid between the male and female genotypes i and j at k^{th} environment, μ was the overall mean, g_i and g_j are the GCA effects of the i^{th} and j^{th} parents respectively, s_{ij} is the effect of the SCA for the combination ij and ε_{ijk} was the residual random error for the observation in the k^{th} block and with the parentals i and j. All variance components were determined by the restricted maximum likelihood method using the software ASReml-R version 3.0 (Butler et al. 2007).

IV-3. Results

IV-3.1. Efficiency of the chemical hybridizing agent

The effectiveness of any CHA is environmental and genotype dependent. Therefore, it was necessary to carry out tests in order to determine the appropriate rate that works under Moroccan conditions where spring bread wheat is mainly grown. The application of the three different chemical doses 11.5, 12.5 and 13.5 l ha^{-1} based on the manufactory recommendation were sprayed for their ability to induce sterility. Application dates at early booting stage (immature heads at 15 to 20 mm in the stem) differed based on environmental conditions in each year. The staging was frequently and individually verified to meet the optimal time window for CHA treatment. Variation for gapping date and different reaction to the chemical were observed. 12.5 l ha^{-1} dose was not sufficient to induce male sterility in the treated female lines (only few sterilized plants produced seed) while 11.5 l ha^{-1} was not effective at all. However, almost a complete male sterility and slight phytotoxic effects were observed using the 13.5 l ha^{-1} rate (Figure IV- 2a). In addition, sterility control was verified by the number of the seed in the bagged female heads, sufficient gapping and light green color observed as result of the effective dose, white-yellowish anthers that do not dehisce nor shed pollen, and presence of waxiness on the leaf surfaces of true F_1 hybrids (Figure IV- 2b, c). As result, we dropped the unsuccessful two rates (11.5 l ha^{-1} and 12.5 l ha^{-1}) and we opted for 13.5 l ha^{-1} in the second-year experiment.

(a) (b) (c)

Figure IV- 2. Reaction of the CHA on female plants; (a) phytotoxicity of the chemical; (b) gapping and light green color of a treated female; and (c) white-yellowish anthers

IV-3.2. Relationship between seed set and both floral and flowering traits

Furthermore, we evaluated the flowering and floral traits of the males used and we tested hybrid seed set on the females as well. We observed a large phenotypic variation for the assessed traits (Table IV- 1 and Annex 9). For the effective 13.5 l ha^{-1} dose, we recorded a range of 24.5 and 47.05 seed per head. Significant genotypic and genotype-by-year interaction variances were observed for all traits. However, the genotype-by-year interaction variance was lower than the genotypic variance for all traits except for anther length. Heritabilities were medium to high and ranged between 0.54 for spikelets per spike and 0.94 for pollen mass (Table IV- 1). The cross P1/P18 revealed the highest value of seed set per spike (47.05) and showed the highest visual anther extrusion and pollen mass with 9 and 39.58 mg, respectively (Annex 9). Males contributing to the best outperforming parental combinations were basically selected for their highest values for visual anther extrusion, pollen shedding and pollen mass traits. Parents P1 (QAMAR-6) and P10 (SOMAMA-9/ICARDA-SRRL-2) were the two best males contributing to the best hybrids with high seed set. The best female contributors were P18 (VEE/PJN//2*KAUZ/3/SHUHA-4/FOW-2), P6 (SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15) and P2 (KAUZ//ALTAR 84/AOS/3/TNMU/MILAN/4/MILAN//PSN/BOW) which were among the parents having the high number of spikelets spikes.

Table IV- 1: Estimation of genetic parameters for the evaluated traits at Merchouch station (2019-2020), Morocco

Parameter	HD	PLH	SPS	GYS	PSH	VAE	PM	AL	Seed set (seed/spike)
Min	99	96.5	18.3	1.336	6	6	26.545	3.5	24.5
Max	109	100	21.35	2.208	9	9	39.58	4.05	47.05
Mean	104.38	98.19	19.61	1.82	7.65	7.73	33.42	3.81	35.68
LSD	3.28	3.76	0.95	0.36	0.93	1.07	4.01	0.35	7.97
σ^2_g	6.27**	10.63**	2.22***	0.53**	0.68***	1.04***	21.31***	0.63*	19.23**
$\sigma^2_{g\times y}$	0.93**	2.32*	1.15**	0.23**	1.85***	0.36***	14.43**	0.94*	23.05**
$\sigma^2 e$	0.43	0.47	1.20	0.17	0.27	0.36	4.84	0.53	69.51
H^2	0.55	0.74	0.54	0.48	0.93	0.92	0.94	0.56	0.63

HD: heading date, PLH: plant height, SPS: spikelets per spike, GYS: grain yield per spike, PSH: pollen shedding, VAE: visual anther extrusion, PM: pollen mass, AL: anther length

Min, mean, max of BLUEs; least significant difference (LSD); σ^2_g = genotypic variance; $\sigma^2_{g\times y}$ = genotype-by-location interaction variance; $\sigma^2 e$ = error variance; and heritability estimates (h^2)

*, **, *** Significantly different from zero at the 0.05, 0.01, and 0.001 level of probability, respectively

The phenotypic correlations among the evaluated traits ranged between 0.11 and 0.98 with the strongest correlation between grain yield per spike and seed set (Table IV- 2). Seed set, as important determinant of successful hybrid production, was significantly correlated with visual anther extrusion, pollen mass, pollen shedding and anther length with 0.94, 0.97, 0.91 and 0.45 respectively. There were also high correlations between seed set and both spikelets per spike and plant height with 0.79 and 0.63, respectively. The assessed male floral traits showed significant correlations with each other, with the highest value observed between pollen mass and pollen shedding (r = 0.96), as well as with some of the evaluated agronomic traits.

Table IV- 2: Phenotypic correlation of seed set with other agronomic and male floral traits among the 18 parents of the combined data set across the two years

Parameter	DH	PLH	SPS	GSP	PM	PSH	VAE	AL
PLH	-0.47*							
SPS	0.11	0.63**						
GSP	0.21	0.15	0.66***					
PM	0.28	0.25	0.81****	0.94****				
PSH	0.35	0.3	0.87****	0.86****	0.96****			
VAE	0.32	0.25	0.82****	0.90****	0.95****	0.95****		
AL	0.67***	-0.14	0.32	0.48*	0.55**	0.60**	0.55**	
Seed set	0.18	0.29	0.79****	0.98****	0.97****	0.91****	0.94****	0.46*

*, **, ***, **** Significantly different from zero at the 0.05, 0.01, 0.001, and 0.0001 level of probability, respectively
Trait abbreviations are listed in Table IV- 1.

IV-3.3. Hybrid performance and heterosis

To evaluate the performance of the produced F_1 hybrids using CHA Croisor 100, the resulting seed from the crosses were planted in each of the following years. The germination rate was good indicating that the progeny from the chemical hybridization was fully functional. We observed a phenotypic variability for all the traits in each year and environment and across years and environments (Table IV- 3) and the heritability ranged from low to high with maximum value observed for yield in the 2019 trial in Sidi El Aydi. Correlation between the traits identified relationships of grain yield with the yield components for the overall data set. High positive effects of biomass (r = 0.77), thousand kernel weight (r = 0.75) and number of productive tillers per plant (0.69) on grain yield, whereas moderate positive effect of spikelets per spike on grain yield was recorded with 0.55 coefficient of correlation (Table IV- 4).

Table IV- 3: Performance of the parents and the hybrids and the statistics summary for biomass, thousand kernel weight and grain yield across years and environments

Parents / F_1 hybrids	Biomass (t ha^{-1})					TKW (g)					Yield (t ha^{-1})				
	E1Y1	E1Y2	E2Y1	E2Y2	Overall	E1Y1	E1Y2	E2Y1	E2Y2	Overall	E1Y1	E1Y2	E2Y1	E2Y2	Overall
Parents															
P1	10.15	11.08	6.01	5.48	8.60	36.78	40.38	30.99	27.37	36.49	4.53	**5**	3.29	4.10	4.25
P2	9.83	9.78	5.71	4.27	7.42	37.35	**43.58**	30.36	25.73	34.1	4.44	4.77	3.76	3.08	4.08
P3	**12.37**	**12.21**	5.27	5.66	8.89	**40.6**	42.66	25.99	30.86	35	**4.84**	4.72	2.95	3.83	3.93
P4	9.78	10.72	5.13	4.47	7.77	35.19	40.67	28.65	30.51	34.71	4.3	4.74	3.37	3.59	4.26
P5	11.28	10.22	5.04	5.26	8.04	**38.22**	42.15	30.54	32.87	35.72	4.63	4.66	3.58	3.82	4.19
P6	**11.8**	8.99	5.25	4.67	7.65	**38.78**	37.38	34.14	28.03	34.7	**4.9**	4.72	3.14	3.44	4.07
P7	9.89	8.45	5.79	5.26	7.35	32.81	38.48	33.28	31.17	34.56	4.04	3.9	3.61	4.04	3.88
P8	**11.56**	10.63	5.17	5.39	8.22	36.43	40.7	33.45	29.73	34.83	4.81	**5.17**	3.58	3.78	**4.39**
P9	9	**12.06**	**6.39**	6.17	**8.95**	35.64	40.39	**39.66**	**35.82**	**38.77**	4	4.85	3.76	4.09	4.24
P10	10.21	**12.01**	6.24	5.59	**8.97**	36.85	40.71	**35.46**	34.34	**37.62**	4.56	4.73	3.61	4.16	4.25
P11	10.11	9.81	**6.34**	4.66	7.79	36.89	41.27	34.46	35.47	36.93	4.55	4.94	3.38	3.72	4.17
P12	10.55	8.58	5.10	5.89	7.58	37.12	36.79	**37.51**	**39.37**	**37.8**	4.7	4.17	3.67	3.95	4.11
P13	10.11	11.63	5.56	4.48	8.01	36.3	**46.01**	31.26	31.65	35.97	4.39	**5.09**	**4.01**	3.77	**4.32**
P14	9.45	11.61	5.76	5.98	8.12	33.42	42.76	34.25	32.95	35.95	3.87	4.87	**3.94**	4.03	3.92
P15	9.54	8.2	5.06	**7.48**	7.51	32.18	37.47	29.89	35.23	33.98	4.07	4.27	3.51	**4.25**	3.97
P16	10.09	10.48	5.26	**7.41**	8.32	36.41	**48.29**	31.95	31.82	36.99	4.35	4.24	**3.9**	4.12	4.14
P17	11.5	11.21	**6.27**	**6.94**	**9**	36.1	40.16	32.49	30.47	34.53	4.54	4.93	3.11	**4.39**	4.23
P18	11.56	10.87	5.14	6.26	8.5	37.15	40.44	31.97	**35.91**	36.42	**4.83**	4.54	3.28	**4.42**	**4.29**
F_1 hybrids															
P1/P2	9.15	11.59	5.31	**7.33**	8.35	35.42	44.20	30.51	34.39	36.13	4.38	5.00	3.24	4.21	4.21
P1/P6	10.88	10.78	6.58	5.82	8.57	**41.30**	45.01	34.51	35.88	38.84	4.79	4.82	3.62	4.72	4.52
P1/P8	11.60	10.88	6.64	7.08	8.79	38.69	43.67	32.50	35.51	37.80	4.84	4.91	3.03	**4.85**	4.37
P1/P18	10.15	11.15	5.41	6.54	8.32	37.07	42.73	30.74	33.62	36.11	4.51	4.82	3.80	4.49	4.38
P3/P2	9.86	9.72	5.64	7.10	8.02	35.36	42.15	28.00	35.39	36.16	3.97	4.91	3.42	3.54	4.04
P3/P6	**12.22**	12.33	4.98	7.15	9.08	39.59	**46.44**	26.51	30.37	35.48	4.38	4.84	3.25	3.61	4.06
P3/P8	10.46	9.36	4.68	6.26	7.90	37.59	38.94	27.36	31.27	34.55	4.52	4.02	3.93	4.41	4.17
P3/P18	10.85	10.84	5.74	6.60	8.51	39.07	37.73	30.34	32.70	35.03	**4.74**	4.62	3.85	4.41	4.39
P4/P7	10.84	9.91	5.97	4.93	7.14	37.73	39.79	36.85	36.30	37.04	4.92	4.96	3.77	4.15	4.65
P4/P11	8.86	10.57	5.39	6.06	7.75	35.64	43.19	33.41	34.29	36.62	4.81	5.01	3.60	3.83	4.29
P4/P17	10.37	**12.86**	5.15	4.61	8.11	39.46	42.81	35.41	35.20	38.62	4.63	5.70	3.85	4.16	4.73
P9/P2	**12.04**	12.83	6.50	7.23	**9.28**	39.22	43.02	35.20	**40.88**	39.36	**5.23**	5.25	**4.37**	**4.88**	**5.13**

P9/P6	10.24	11.40	**7.27**	7.01	8.90	40.75	44.32	**40.10**	39.93	**41.19**	4.86	5.74	**4.63**	**5.04**	**5.27**
P9/P8	11.31	**13.43**	6.18	5.75	8.69	**40.79**	45.07	37.32	39.19	**40.59**	4.84	**5.84**	**4.18**	4.36	**4.98**
P9/P18	11.47	**12.95**	5.82	6.37	**9.13**	**42.14**	44.89	38.41	31.78	39.44	5.18	**5.87**	3.82	4.17	4.70
P10/P5	12.25	11.96	6.23	5.71	9.06	40.56	42.98	36.99	36.69	39.81	5.26	5.68	4.00	4.36	4.84
P10/P12	10.92	11.97	**6.78**	**8.23**	**9.48**	37.91	43.79	**39.00**	**40.36**	40.14	4.96	5.52	3.87	4.45	4.72
P10/P14	9.77	12.41	6.46	6.99	8.94	37.09	**48.14**	38.55	39.55	**40.78**	4.84	**6.12**	3.90	4.74	4.65
P10/P15	**10.77**	11.84	**6.90**	6.17	8.93	38.41	42.79	**39.45**	**40.96**	40.28	**4.92**	5.43	3.44	4.26	4.58
P13/P7	10.69	9.08	5.80	5.66	7.90	38.72	38.65	32.19	31.97	35.16	4.55	3.91	3.77	3.81	4.07
P13/P11	10.52	11.06	6.61	5.90	8.58	39.30	**47.01**	32.01	34.38	37.84	4.15	5.34	3.40	3.92	4.19
P16/P7	10.60	9.72	5.28	**7.25**	8.15	33.44	36.42	29.60	30.96	32.79	3.84	3.91	3.49	3.70	3.81
P16/P11	10.17	10.75	6.28	5.82	8.32	36.46	41.39	36.20	38.38	37.92	4.08	4.38	3.50	3.76	4.04
σ^2_g	0.13	0.25*	0.39	0.53*	12.25*	9.62*	10.6*	3.51*	8.92*	2.63*	0.21*	0.07	0.16*	0.18*	0.82**
$\sigma^2_{L\ x\ E}$	NA	NA	NA	NA	0.20**	NA	NA	NA	NA	4.26**	NA	NA	NA	NA	0.14*
σ^2_e	1.28	0.59	1.90	0.72	1.20	5.77	12.81	9.01	12.74	9.76	0.37	0.15	0.25	0.13	0.26
h^2	0.24	0.34	0.29	0.60	0.37	0.29	0.62	0.44	0.58	0.47	0.38	0.49	0.57	0.73	0.53

σ^2_g genotypic variance, σ^2_e error variance, $\sigma^2_{L\ x\ E}$ location x environment variance, h^2 repeatability E1: Merchouch Location, E2: Sidi El Aydi. TKW: Thousand kernel weight
Bold values are the top three highest genotypes for that specific trait

Table IV- 4: Phenotypic correlations among traits in 23 F_1 hybrids and their corresponding parents over the two years data

Traits	DH	PLH	SPS	TLP	BM	TKW	YLD
PLH	0.83***						
SPL	-0.01	-0.18					
SPS	-0.2	0.07	0.12				
TLP	0.15	0.11	-0.07	0.41**			
BM	0.29	0.18	-0.09	0.48**	0.6***		
TKW	0.08	0.13	0.2	0.38*	0.55***	0.56***	
YLD	0.03	0.06	-0.04	0.45***	0.49***	0.77***	0.75***

*, **, *** Significantly different from zero at the 0.05, 0.01, and 0.001 level of probability, respectively. DH: days to heading, PLH: plant height, SPL: spike length, SPS: spikelets per spike, TLP: tillers per spike, BM: biomass, TKW: thousand kernel weight, YLD: grain yield

All hybrids exhibited either positive or negative heterosis over the mid- (relative heterosis) and best-parent (heterobeltiosis) in each and across environments (Figure IV- 3, annexes 9 & 10). Since BM, TKW and YLD were the only significant traits in each and across years and environments, heterosis and combining abilities were discussed in detail for these three traits only.

Hybrid biomass ranged between 8.86 - 12.25 t ha^{-1}, 9.08 - 13.43 t ha^{-1}, 4.86 - 7.27 t ha^{-1}, 4.61 - 8.23 t ha^{-1}, 7.14-9.48 t ha^{-1} in MER-2019, MER-2020, SEA-2019, SEA-2020 and across years and environments. The majority of the hybrids falling in the 10 t ha^{-1} to 13.43 t ha^{-1} in Merchouch environment with the highest value observed for the cross P9/P8 in 2020 trial in Merchouch. The highest mid-parent heterosis (14.55 %) for overall mean biomass (9.48 t ha^{-1}) was recorded for the cross P10/P12 which performs very well in Merchouch and Sidi El Aydi during the two years experiments (Annex 10). The P10 parent (SOMAMA-9/ICARDA-SRRL-2) involved in three other crosses (P5, P14 and P15) displayed significant contribution in terms of biomass performance and hence could be categorized as high combiner. The two crosses P16/P7 and P16/P11 showed mostly the least biomass in each year and in every environment as well as across years and environments (Table IV- 3). Significant relative heterosis ranged from -7.64 to 14.55 % for the combined data across years and environment with the highest heterosis recorded for the cross P10/P12. Compared with the single environments, maximum mid-parent heterosis was recorded in MER-2019, MER-2020, SEA-2019, SEA-2020 with 27.88, 16.04, 24.95 and 50.24 %, respectively. Best-parent heterosis ranged between -20.29 - 22.48 %, -24.58 - 12.58 % for the first and second year in Merchouch, respectively. While ranges -17.96 - 13.77 % and -33.53 - 39.74 % were recorded in the first and second year in Sidi El Aydi, respectively. Relatively low heterosis over the best-parent was calculated for the combined data with the lowest (-11.18 %) and highest (7.21%) values for the two hybrid combinations P3/P8 and P13/P11, respectively.

Thousand kernel weight ranged between 33.44 - 42.14 g, 36.42 - 48.14 g, 26.51 - 40.10 g, 30.37 - 40.96 g, 32.79 - 41.19 g with the best performing hybrids recorded for P9/P18, P10/P14, P9/P6, P10/P15, P9/P6 in MER-Y1, MER-Y2, SEA-Y1, SEA-Y2 and across the four environments, respectively (Table IV- 3). P9 (ANGI-2/HUBARA-3) and P10 (SOMAMA-9/ICARDA-SRRL-2) showed to be good combiners and had high TKW values in and across environments (Table IV-3). For the two years data, the trials in Merchouch and Sidi Aydi had wide variation of mid- and best-parent heterosis, reaching up 32.83 % in the second-year trial in Sidi El Aydi (Annex 11). The best hybrid combinations showed consistently better mid- and

best parents heterosis in each and across environments and thus, MPH and BPH trends were almost similar. Hybrids with P3 parental line (QADANFER-11/REBWAH-11) showed the lowest values for TKW for each and across environments as compared with the rest of the crosses except the hybrid P3/P6 in Merchouch for the second-year trial (Table IV- 3). Similarly, the cross P16/P7, showing low biomass, has also lower TKW in all environments.

Hybrid grain yield in Merchouch in 2019 ranged from 3.84-5.26 t ha^{-1} with the highest yield observed for the cross between P10 and P5 (Table IV- 3). In contrast, the 2020 trial in Merchouch showed more yield variation than 2019 trial with maximum yield (6.12 tha^{-1}) recorded for the hybrid P10/P14. Among the 23 hybrids produced in Merchouch, 15 and 13 hybrids showed positive significant heterosis in 2019 and 2020 trials, respectively. MPH in 2019 at Merchouch ranged from -14.42 and 23.76%, whereas in 2020, it ranged from -22.66 and 22.31% (Figure IV- 3). The crosses P9/P2 and P10/P14 showed the highest BPH for grain yield with 17.60 and 18.36% in 2019 and 2020 at Merchouch, respectively. For the trials in Sidi Aydi, highest relative heterosis was shown by P9/P6 (33.86%) in 2019 and P9/P2 (36.02%) in 2020. Hybrids P10/P14 (18.36%) in 2019 and P9/P6 (22.71%) in 2020 exhibited the highest BPH in Sidi El Aydi. For the combined data, grain yield for the produced hybrids ranged between 3.81 and 5.26 t ha^{-1} with maximum value expressed by P9/P6 (Table IV- 3). Same cross identified to be the most promising combination with the highest percent of mid- (26.65%) and best-parent heterosis (24.04%) (Figure IV- 3).

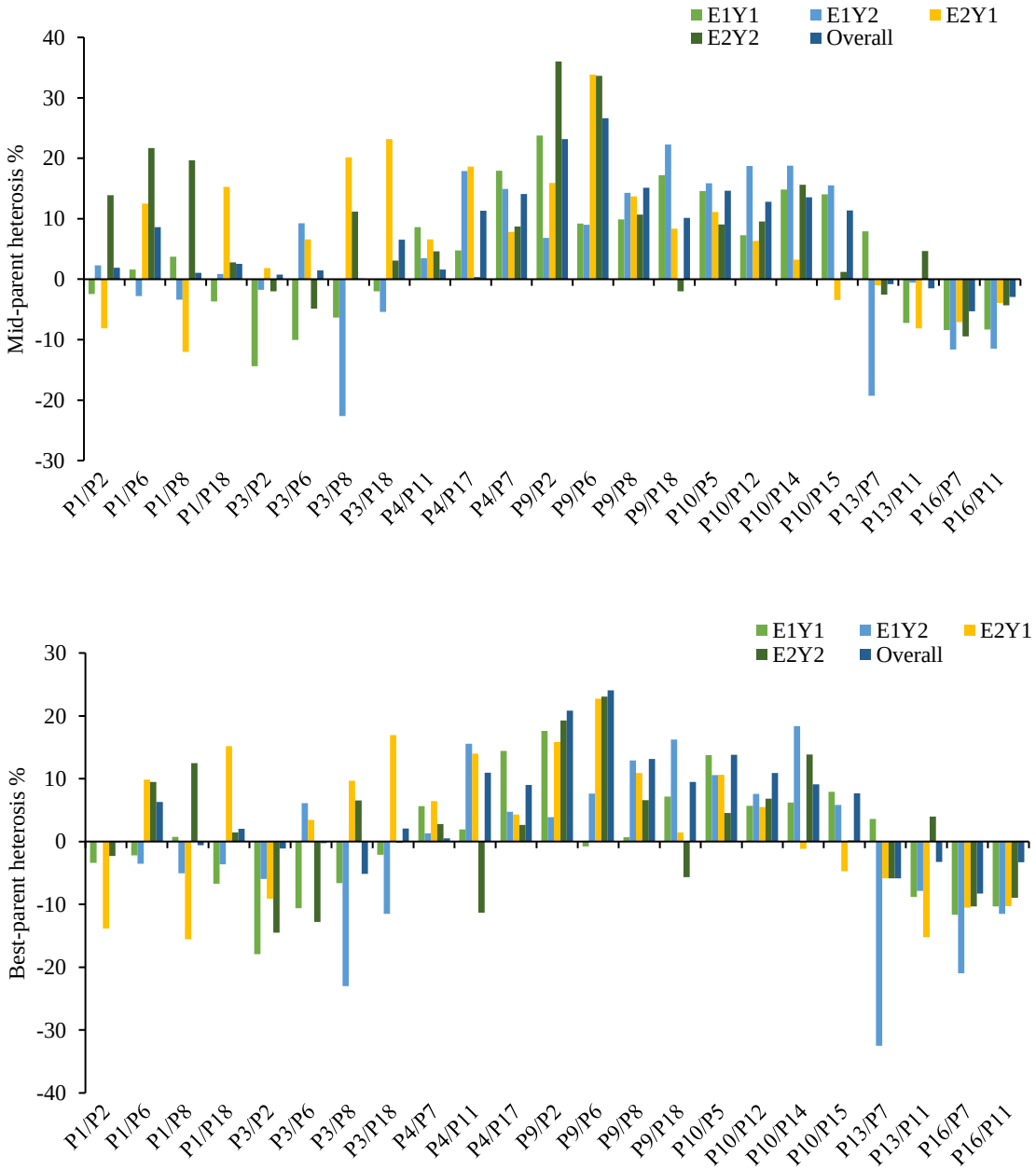


Figure IV- 3: Mid- and best-parent heterosis for grain yield in Merchouch and Sidi El Aydi during 2019 and 2020 seasons.

E represents the environment and Y is year; E1: Merchouch; E2: Sidi El Aydi; Y1:2019 and Y2:2020

IV-3.4. Combining ability

Combining ability analysis based on Griffing's method 3 was carried out for the 23 hybrids derived from different parental combinations and the variances calculated are presented in Table IV- 5. The genetic variances of the parental lines were considerably larger than the variance due to GCA of the parents for all traits. Similarly, the environment variance for the parents analyzed separately was much higher than the variance due to GCA of the parents x environment for the evaluated traits. The error variances of the parental lines and hybrids were

almost comparable. However, it is important to note that these estimates may vary if we had a larger number of parental lines and therefore must be taken with caution. The analysis of variance showed also significant effect for GCA estimates for the evaluated traits except for SPS, SPL and TLP while the effects of the SCA were not significant except for yield. The ratio of GCA to SCA was greater than 1 for TLP, BM, TKW and YLD.

Table IV- 5: Analysis of variance for the combining ability of traits for parents and their corresponding F_1 hybrids

Variance component	DH	PLH	SPL	SPS	TLP	BM	TKW	YLD
Parents analyzed separately								
σ^2_g	5.45*	4.40*	0.60	12.54*	0.91	0.30*	0.22*	0.16*
σ^2_L	6.78	17.62	0.31	0.19	0.07	0.28	4.12	0.08
σ^2e	1.38	12.88	60.84	91.29	90.22	1.95	11.23	0.93
H^2	0.39	0.45	0.74	0.82	0.78	0.31	0.35	0.42
Hybrids analyzed separately								
$\sigma^2_{GCA\text{-}parent}$	0.19*	2.64*	0.03	0.07	0.05	0.08*	0.09*	0.07*
σ^2_{SCA}	0	0	0	0	0.02	0.015*	0.08*	0.011*
$\sigma^2_{GCA\text{-}parent\ x\ L}$	0.09	0	0	0.02	0	0.07	0.04	0.02
σ^2e	3.31	11.45	53.61	101.17	82.46	2.91	9.27	1.06
$\sigma^2_{GCA}/\ \sigma^2_{SCA}$	-	-	-	-	2.50	5.33	1.13	6.36
H^2	0.29	0.52	0.31	0.72	0.69	0.48	0.53	0.68

DH: days to heading; PLH: plant height; SPL: spike length; SPS: spikelets per spike; TLP: tillers per spike; BM: biomass; TKW: thousand kernel weight; YLD: grain yield. σ2g: genotypic variance; σ2L: location variance; σ2e error variance; H: heritability; σ2GCA: general combining ability variance; σ2SCA: specific combining ability variance; σ2GCA-parent x L: general combining ability x Location variance

BLUPs values across environments calculated from the combining ability analysis provided an estimate for GCA values for each parental genotype (Table IV- 6). Out of the 18 parents used, only few had a GCA estimate significantly different from zero ($P < 0.05$) for each trait. The parents P10 (SOMAMA-9/ICARDA-SRRL-2) and P12 (ATTILA*2/PBW65//PFAU/MILAN) were identified with high and significant GCA values for all traits except for TLP. While the two parents P5 (KAUZ//ALTAR 84/AOS 3/KAUZ/3/SHUHA-4//NS732/HER/4/QAFZAH-33) and P9 (ANGI-2/HUBARA-3) had significant GCA for BM, TKW and YLD. Hybrids involving these two potential parents performed well and showed high MPH and BPH. Parent P9 had the highest positive GCA score (560 kg ha^{-1}) for grain yield while P16 (ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9) was the poorest GCA with a value of -540 kg ha^{-1} (Table IV- 6).

Table IV- 6: Estimates of GCA for the parental lines and SCA for the hybrids

Parents / hybrids	DH	PLH	SPL	SPS	TLP	BM	TKW	YLD
GCA Parents								
P1	5.45	4.40	0.60	12.54	0.91	0.30	0.22	0.16
P2	-0.85	-4.42**	0.01	-0.38	0.17	0.01	-0.70	-0.05
P3	-0.74	-3.46*	-0.08	-0.04	0.01	-0.13	-2.39*	-0.30*
P4	-0.87*	1.53	-0.39	-0.32	-0.23	-0.85*	-0.27	0.29*
P5	-1.29	1.38	0.05	0.14	0.42*	0.55*	2.11*	0.38*
P6	-0.33	-0.24	-0.28	0.21	0.21	0.34	0.81	0.15
P7	-0.58	1.37	-0.19	-0.16	-0.26*	-0.78*	-2.70*	-0.29
P8	-1.57*	2.04*	0.25	-0.23	-0.09	-0.05	-0.05	0.04
P9	-0.83	0.59	0.44	0.06	0.14	0.49*	2.45*	0.56*
P10	1.51**	2.83*	-0.37*	0.46*	0.11	0.59*	2.56*	0.23*
P11	0.58	-1.31	0.15	-0.32	-0.11	-0.30	-0.24	-0.29*
P12	3.97***	5.04**	-0.59*	0.54*	-0.07	0.97*	2.44*	0.36*
P13	1.27*	-1.78	-0.04	-0.11	0.10	-0.27	-1.20	-0.33*
P14	1.01*	3.35*	-0.22	1.21*	0.26	0.42*	3.08**	0.28*
P15	2.35**	1.54	-0.70*	-0.05	-0.19	0.41	2.58*	0.12
P16	0.07	-0.23	0.12	-0.19	-0.18	-0.28	-2.34*	-0.54*
P17	0.08	0.38	-0.86*	-0.12	0.27*	-0.41	0.92	0.27*
P18	0.70	-1.35	0.86	0.31	-0.15	0.14	-0.84	0.03
SE	0.33	0.55	0.10	0.09	0.05	0.11	0.44	0.07
SCA cross combination								
P1/P2	1.14	2.94	0.51	-0.72	-0.29	-0.27	-2.17	-0.47
P1/P6	0.29	-2.50	-0.08	-0.10	0.01	-0.30	-0.30	-0.22
P1/P8	0.91	2.11	0.57	0.08	-0.14	0.30	-0.49	-0.26
P1/P18	0.02	0.59	0.19	0.09	-0.09	-0.36	-1.39	-0.23
P3/P2	-1.58	-7.50*	-0.38	0.48	0.15	-0.64	-3.23*	-0.67*
P3/P6	1.85	-3.01	-0.19	0.09	-0.08	0.10	-5.41*	-0.86*
P3/P8	-3.29	-8.42*	-0.53	-0.53	-0.14	-0.69	-5.49*	-0.63*
P3/P18	0.52	-7.47*	1.37	0.37	-0.27	0.01	-5.89*	-0.35
P4/P7	-1.53	4.00	-0.38	-0.17	-0.59	-1.44	1.77*	0.57*
P4/P11	-2.88	3.19	-0.65	-0.84	-0.47	-1.32	-1.11	0.21
P4/P17	-0.87	1.53	-0.39	-0.32	-0.23	-0.85	-0.27	0.09
P9/P2	-1.10	1.59	0.48	0.17	0.24	1.24*	4.81*	1.27*
P9/P6	-3.67	2.54	0.88	-0.06	0.17	0.53	5.14*	1.21*
P9/P8	0.84	3.34	0.57	0.38	0.39	0.72	5.39*	1.02*
P9/P18	2.53	3.09	0.75	0.66	-0.44	0.12	-0.01	-0.27
P10/P5	1.08	8.64*	-0.33	0.97	0.36	1.12*	5.37*	0.66*

P10/P12	1.51	2.83	-0.37	0.46	0.11	0.59	2.56*	0.23*
P10/P14	1.51	2.83	-0.37	0.46	0.11	0.59	2.56*	0.23*
P10/P15	1.51	2.83	-0.37	0.46	0.11	0.59	2.56*	0.23*
P13/P7	2.59	-3.94	-0.19	-0.17	0.50	-0.10	-1.03	-0.44
P13/P11	2.49	-3.24	0.08	0.20	0.28	0.09	-0.82	-0.32
P16/P7	-0.58	-0.54	0.26	-0.28	-0.21	0.13	-4.55*	-0.91*
P16/P11	0.86	-0.43	0.26	0.01	-0.12	-0.18	-1.88	-0.67*
SE	0.38	0.88	0.11	0.09	0.06	0.14	0.73	0.13

DH: days to heading; PLH: plant height; SPL: spike length; SPS: spikelets per spike; TLP: tillers per spike; BM: biomass; TKW: thousand kernel weight; YLD: grain yield; GCA: general combining ability variance; SCA: specific combining ability variance SE: standard deviation

The specific combining ability effects for the evaluated traits using the combined data set in each parental combination are presented in table IV- 6. From the total 23 crosses, only five P3/P2, P3/P8, P3/P8, P3/P18 and P10/P5 showed a significance SCA for plant height ($P < 0.05$). SCA effects for days to heading, spike length, spikelets per spike and tillers par plant were not significant. While P9/P2 and P10/P5 demonstrated significant positive SCA for biomass with maximum value shown 1.24 for P9/P2. Unlike the above traits, fourteen cross combinations out of twenty-three showed significant negative or positive SCA values for thousand grain yield ($P < 0.05$). The highest SCA was estimated for the hybrid P9/P8 (+5.39), while the lowest value of SCA was obtained for the hybrid P3/P18 (-5.89). Similarly, most of the cross combinations with significant effect SCA for TKW were found present for yield as well. The crosses P9/P2 and P9/P6 ranked the top two best combinations with +1.27 and +1.21 SCA values, respectively. In contrast, the hybrid P16/P7 showed the highest negative SCA effect for yield with -0.91. Taking all the cross combinations into consideration, P9 and P10 appeared to be the best parents since six out of seven crosses expressed significant positive SCA effects.

IV-4. Discussion

Efforts to investigate the potential of hybrid breeding in bread wheat have been carried out in this study. Understanding the genetic basis of the floral and flowering traits to enforce the outcrossing ability may lead to an effective hybrid system and thus, achieve large-scale hybrid production. Successful hybrid wheat breeding programs rely on an optimized breeding strategy that combines several prerequisites for maximum effective pollination and seed set. Identification of parents with potential hybrid traits, application of effective CHA with proper dose and timing, determining the combining abilities and heterosis are key factors that determine the success of hybrid wheat breeding.

IV-4.1. CHA efficiency

In this study, we have used parental lines identified previously in El Hanafi et al. (2020) with favorable floral traits to assess the impact of male floral traits on female seed set in designed crossing blocks. First the efficacy of the gametocide Croisor ®100 was tested using three different doses applied at early booting stage. This CHA is widely used particularly in Central Europe and has proven its commercial value for hybrid seed production. Frequent verifications were made for well-timed application. The sterility control mechanisms applied have proven the efficiency of the chemical and adequate gapping was observed. Male sterility was induced on almost 95% of the female plants using the 13.5 l ha^{-1} rate. Afterwards, we collected seed data to determine the success level of the CHA-treated female plants. The different parental combinations showed significant phenotypic variation of hybrid seed set and the other flowering and floral evaluated traits. The 13.5 l ha^{-1} dose had an average of 35 seeds on the mother lines which supplied enough seed for hybrid performance trials.

IV-4.2. Variation in cross-pollination traits

The significant genotypic variances recorded for the important floral traits such as pollen mass, visual anther extrusion, pollen mass and anther length suggest the potential of exploiting the variation present in the genetic material used. Moreover, the high heritability recorded for all traits indicates that there was sufficient variation in the population used and low influence of the environmental factors. This also suggest that the genotypes might be efficient for hybrid seed production in several environment. Similar variation and heritabilities have been observed in previous studies pointing out that these traits can be improved by consecutive hybridization for efficient hybrid breeding program (Langer et al. 2014; Boeven et al. 2018; El Hanafi et al. 2020). Anther extrusion, pollen mass and profuse pollen shedding showed to be promising traits to be used as proxies to predict other harder to measure associated floral traits such as openness of the florets and duration of floral opening of female plants. However, little is known about the relationship with seed set and the floral traits. Boeven et al. (2018) was the only study that showed a correlation between visual anther extrusion and hybrid seed set ($r = 0.76$, $P < 0.001$). Similarly, in this study, seed set was correlated with visual anther extrusion with $r = 0.94$ ($P < 0.001$). Therefore, visual anther extrusion can serve as rapid proxy to estimate the potential of a female line in hybrid breeding program. The present study also addresses for the first time as far as the authors know the effect of other floral traits over seed setting. Thus, high correlations could be found between seed setting and pollen mass, pollen shedding and anther length with $r = 0.97$ ($P < 0.001$), $r = 0.91$ ($P < 0.001$) and $r = 0.46$ ($P < 0.05$), respectively.

Surprisingly, seed set in relationship with the phenology traits, showed non-significant correlation even though we have specifically chosen male lines to flower 1-3 days earlier and to be taller than the female lines. That demonstrates the inaccuracy of the potential of phenology traits in producing high seed set on female lines. Therefore, genomic tools might be incorporated to predict the flowering synchronization in hybrid seed production based on historical weather information (Gillberg et al. 2019).

Our study showed that pollen mass had high correlation with pollen shedding ($r = 0.96$) and visual anther extrusion ($r = 0.95$; $P < 0.001$) as expectedly the maximum extruded anthers will contribute to maximum shed pollen outside the florets (Joppa et al. 1968). By this, we have confirmed the result previously found in (El Hanafi et al. 2020) and (Langer et al. 2014). Moreover, anther length can, in turn, increase the quantity of pollen released from every single anther which is confirmed by collective correlation observed between pollen mass with each of anther length ($r = 0.55$), pollen shedding ($r = 0.60$) and visual anther extrusion ($r = 0.55$). Similar associations were reported by (Langer et al. 2014). In contrast, there was no association between anther length and pollen mass or any other floral trait in a previous experiment carried out in Morocco (El Hanafi et al. 2020).

Spikelets per spike and grain yield per spike have shown to be beneficial for seed production given that SPS would produce more seed. This is especially notable by the high correlation observed between seed set and both SPS and GYS with $r = 0.79$ and $r = 0.98$ ($P < 0.001$), respectively. Additionally, we hypothesized that more SPS would produce and shed more pollen. Unlike some previous studies, high positive significant correlation was observed between SPS and the three male floral traits PM ($r = 0.81$), PSH ($r = 0.87$) and VAE ($r = 0.82$) ($P < 0.001$) (Langer et al. 2014; El Hanafi et al. 2020).

IV-4.3. Hybrid performance and heterosis

Successful hybrid seed production was obtained to test the 23 F_1 hybrid combinations in yield trials across two cropping seasons in Merchouch and Sidi El Aydi. The genotypic variability for days to heading, plant height, spike length and tillers per plant were mostly found non-significant. The significant G × E observed for PLH, SPS, BM, TKW and YLD indicating that for at least some of the hybrid combinations, the cross and its parents, exhibited different levels of phenotypic expression under different environmental conditions. While for the rest of the evaluated traits (DH, SPL, TLP) G × E interaction effects were non-significant indicating the stability of the evaluated hybrids and their parents across the environments.

The main target of hybrid breeding program is to identify potential parents that can be crossed to produce F_1 hybrids with high heterosis level. Previous studies have reported important heterosis and heterobeltiosis in wheat (Li et al. 1997; Akel et al. 2019; Kumar et al. 2020; Adhikari et al. 2020b; Easterly et al. 2020). In this study, several hybrids exhibited significant heterosis and heterobeltiosis for the evaluated traits.

In this study, MPH for biomass ranged from -19.18 to 50.24% in the four environments. While maximum of 13.35% was recorded for the overall data expressed by the hybrid P9/P2. 50.24% was recorded by the hybrid P1/P2 in the 2020 trial in Sidi El Aydi even though the parental pair involved had not the best biomass performance. This is not surprising considering that the cross performed better in extreme drought station (Sidi El Aydi) with supplement irrigation as compared with drought rainfed station (Merchouch). However, negative mid- (-9.34%) and best-parent heterosis (-11.58%) was recorded for the same cross in 2019 trial in Sidi El Aydi attest the instability of this cross across the environments.

Stable positive heterosis over mid-parent for biomass was recorded by 6 hybrids P1/P8, P9/P2, P9/P18, P10/P5, P10/P12 and P13/P11 in each and across year and environment. This might be explained by the use of parental lines which have been well characterized for potential floral traits combined with good agronomic performance. The wide positive range found for biomass heterosis is far better than what found in previous studies with maximums of 0.9% and 5.4% in (Morgan et al. 1989) and (Kindred and Gooding 2005), respectively. BPH ranged from -33.53 to 39.74 % in the four environments with maximum value observed for the hybrid P10/P12 in 2020 SEA trial. The maximum BPH for biomass for the combined data set was low (7.21%) displayed by the hybrid P13/P11.

For thousand kernel weight, wide range of mid- and best parent heterosis was found for the individual experiments and for the combined data. Some hybrids consistently outperformed their parents across all environments. Of the 23 hybrids produced, 10 (P4/P17, P4/P7, P9/P2, P9/P6, P9/P8, P9/P18, P10/P5, P10/P12, P10/P14 and P10/P15) had consistently positive MPH in all environments except the cross P9/P18 in 2020 SEA trial. This appears very promising especially because the parents were basically shown to have dual parentage purpose (male and female). Many studies have reported positive and negatives mid- and best parent heterosis (Singh et al. 2004; Kumar et al. 2011; Thomas et al. 2017). (Singh et al. 2004) reported highest relative heterosis and heterobeltiosis of 28.14 and 24.07%, respectively, in three different environments. While the two other studies reported a maximum of 16.14 % MPH. Generally,

greater MPH or BPH might be attributed to genetically distant parental lines that belong to different heterotic groups (Virmani and Edwards, 1983).

Grain yield heterosis in wheat has been a trait of widespread interest of many researchers as early as 1934 (Engledow and Pal 1934). (Briggle 1963) was among the first to report heterosis in wheat and since then research efforts over years have demonstrated predictable yield advantage and stability (Borghi and Perenzin 1990, 1994; Dreisigacker et al. 2005; Kant et al. 2011; Gowda et al. 2012; Mühleisen et al. 2014; Longin et al. 2015; Nie et al. 2019; Adhikari et al. 2020b; Easterly et al. 2020b). Easterly et al. (2020) evaluated 650 hybrids developed from a full diallel of 26 parents in experimental yield plots and reported mid- and best parent heterosis of maximum 24%. Adhikari et al. (2020b) reported also ranges from -70.4 to 54.3% and -26.9 to 29.2% grown in two cropping seasons in Lincoln, NE, and Greenville, TX-USA. In the current study, MPH ranged from -14.41 to 23.76% and from -22.66 to 22.31 in 2019 and 2020 Merchouch trials, respectively. While, in Sidi El Aydi, MPH tended to be better with ranges of -12.01 to 33.86% and -9.47 to 36.02% in 2019 and 2020, respectively. Hybrids have been shown to out-yield their best parents by 18.36% and 23.07% as highest values found for Merchouch and Sidi El Aydi, respectively. The higher positive MPH and BPH found in Sidi EL Aydi might be attributed to the greatest response to the applied irrigation during the critical time of the vegetation growth. For the combined data across locations, the cross P9/P6, considered as best performing hybrid, have shown to out-yield the mid and the best-parent by more than 1t ha^{-1} each or 26.65 % and 24.04 % BPH, respectively. High yield on individual hybrids is observed in crosses involving high-yielding parents with promising parental floral traits previously evaluated (P4, P9 and P10). Concurrently, some other hybrids did not perform as expected even though both parents were reported to be high yielding, indicating that heterosis depends not only on performance *per se* but also combining ability.

Competitive hybrid wheat production depends crucially on the heterosis level for yield that must be sufficiently high to compensate seed cost. It has been demonstrated in previous study that cost-efficient hybrids would be economically viable only if the range of MPH is between 6 to 34% (Pickett and Galwey 1997). Similarly, Angus (1997) estimated that 5% yield advantage over the best line-bred is required to counterbalance the higher seed cost. Thus, we can conclude that our results were very promising and heterosis level demonstrated the potential of hybrid wheat to boost global wheat productivity and hence, show commercial hybrids to be viable.

IV-4.4. General and specific combining abilities

Several studies have estimated the GCA of each parental genotype and SCA for each hybrid combination (Sprague and Tatum 1942; Dreisigacker et al. 2005; Kumar et al. 2011; Gowda et al. 2012; Rashid et al. 2019; Nie et al. 2019; Adhikari et al. 2020b; Easterly et al. 2020). The analysis of combining ability provides an indication of the type of gene action involved in the expression of the traits. The ratio σ^2GCA/σ^2SCA determines the pre-ponderance of either additive or non-additive type gene action. Higher σ^2GCA to σ^2SCA ratio was observed for TLP, BM, TKW, and YLD indicating the importance of additive gene action for the inheritance of these traits (Beck et al. 1990; Eugenio Gomes Gama et al. 1995). Reif et al. (2005) reported that higher σ^2GCA to σ^2SCA ratio generally indicates the genetic dissimilarity of the parents used. Similar results were observed by (Gowda et al. 2012; Adhikari et al. 2020b).

In this study, the magnitude of the GCA effects was relatively higher in some of the parental lines for certain traits such as BM, TKW and YLD. Seven parents (P4, P5, P9, P10, P12, P14 and P17) had significant GCA effects for yield. Interestingly, of these seven identified parents, five (P5, P9, P10, P12 and P14) were identified as good combiners having significant GCA effects for biomass and thousand kernel weight as well. It is evident that genotypes showing high GCA effects for yield might be attributed to their high GCA for some of yield components such as biomass and thousand kernel weight. In the same context, we have shown these two traits had high direct effect on grain yield with 0.77 and 0.75, respectively. These results are in agreement with earlier findings (Nie et al. 2019; Adhikari et al. 2020b). The parental lines with high GCA effects may be used in future crossing strategies as improved parents for F_1 hybrid production.

Significant positive SCA effects were observed in two crosses P9/P2 and P10/P5 for biomass which both involved high × high general combiners as parents. These two crosses along with six others (P4/P7, P9/P6, P9/P8, P10/P12, P10/P14 and P10/P15) exhibited significant positive SCA effects for TKW and YLD. Of these eight hybrids exhibiting significant SCA effects in desirable direction identified for TKW, four of them (P10/P5, P10/P12, P10/P14 and P10/P15) involved high × high general combiners as parents while four hybrids (P4/P7, P9/P2, P9/P6, and P9/P8) involved high × low general combiners. The negative SCA effects observed in some of hybrids produced might be due to the high and contrasting specialization of the two parents involved for yield formation or due to the presence of unfavorable gene combinations in the parents. The hybrids with high and positive SCA effects are recommended for hybrid breeding.

Because high specific combiners were not only obtained from high × high general combiners as parents but also obtained from the combination of high × low general combiners, high GCA effects of the parents are not necessarily reliable criterion to predict high SCA effects. Rewale et al. (2003) have reported that the out performance of the hybrids having high SCA might be attributed to additive × dominance gene action in case of high × low GCA parental lines or to an epistatic interaction in case of parental pair having low GCA (case that was not reported in this study).

IV-5. Conclusion

Competitive hybrid breeding program on a large scale requires sustainable and robust hybridization system. Development of hybrids through improved floral architecture shown to be valuable to maximize seed set on female plants. The level of the sterility induced in the female lines and the ability to produce higher seed set per head demonstrated the success of CHA application and the beneficial utilization of wheat phenology. The heterosis found in this study appears to be enough to drive hybrid seed production and make it economically feasible. However, the genetic mechanisms of heterosis are not clearly understood. It is therefore necessary to dissect the genes involved in the regulation of heterosis. In such away, the analysis of the gene expression would lead to the genetic basis of the trait in question and heterosis would be conferred by either additive, dominant, or epistatic action of the genes that control the trait. For instance, combining ability analysis also provide information on additive and non-additive variances which aids in selecting the desirable parents and crosses for the exploitation of heterosis. We have shown that the greater general combing ability estimates of the parents enables the prediction of true genetic potential of an individual in subsequent segregating populations. Therefore, GCA is highly recommended to be performed at early generations in order to reduce time and cost in hybrid breeding program. However, selection of hybrids based on high specific combining ability estimates alone would not prove effective since certain cross combinations may provide superior hybrids where some others involving equally promising parents produce subsequent poor progenies. Despite the fact that combining ability method has many advantages, the use of genomic selection to identify single crosses may help in redesigning hybrid breeding pipeline and thus shorten the time to hybrid release.

General discussion

While hybrid breeding has been very successful technology for the outcrossing crops, hybrids for self-pollinating crop such as wheat haven't been extensively applied (Coors and Pandey 1999). Even being technically more challenging, hybrid wheat breeding has been approached and proposed as a potential strategy to boost yield and enhance yield stability. However, less than 1% of the global wheat area is currently cultivated with hybrids. One of the major bottlenecks in breeding and production of hybrid wheat is the lack of a cost-efficient hybrid seed system due to the stringent autogamous nature of wheat. Many studies have elucidated the importance of redesigning the floral biology to increase the outcrossing ability.

Both male and female pair lines must combine different floral and flowering characteristics. Male parent should flower 2-3 days before females, with long extruded anthers that shed large amount of viable pollen. Female parents need to open their florets for an extended period of time with hairy receptive stigmas during the anthesis. Langer et al. (2014) have proposed different approaches to assess male floral traits. We have applied the same approaches along with other phenotypic methods to screen different hybrid potential traits. For male floral traits, anther extrusion (AE), visual anther extrusion (VAE), pollen mass (PM), pollen shedding (PSH), pollen viability (PV) and anther length (AL) have shown a wide phenotypic variability within the germplasm used. Large variation has been observed for the female traits also including openness of the floret (OPF), duration of floret opening (DFO), and stigma length (SL) similarly to what has been reported in previous studies (de Vries 1970, Singh et al. 2007; Langer et al. 2014, Fábián et al. 2019). High heritability coupled with high genetic advance was recorded for AE, PM, OPF and DFO indicating that the preponderance of additive gene action and selection may simply be effectively done phenotypically. Non-additive gene action and high environmental influence were associated with AL similar to what has been reported by Akinwale et al. (2011). Because each of the male and female traits are equally important for redesigning wheat floral biology, positive associations between them may simplify the parental selection.

In the current study, AE, key trait for improving pollen dispersal, shown to be highly correlated with PM and PSH with values of 0.90 and 0.75, respectively. Anther extrusion was also correlated with degree and duration of floret opening with $r = 0.86$ and $r = 0.85$, respectively. These associations showed that only extruded anthers will contribute to the amount to released pollen when the florets are widely opened for an extended period of time (Joppa et al. 1968).

PSH showed also correlations with PFO and DFO, with coefficients of r = 0.68 and r = 0.65, respectively. Given these high associations, PSH and AE seemed to be well suited be taken as proxies to indirectly select the associated traits for large scale breeding programs. However, the large-scale use of anther extrusion based on the counting method seems work-intense and time consuming. Therefore, VAE based on a scale from 1 to 9 is the high-throughput version of the counted AE and thus can be certified as an appropriate trait for practical use especially because both traits showed a coefficient of correlation of 0.75. In addition, many other studies have reported an association between anther extrusion and resistance to Fusarium head blight (Buerstmayr and Buerstmayr 2015; Xu et al. 2019) in a way that genotypes with high AX tend to be resistant. This finding warrants more attention and correlation between the other floral traits seems interesting to check.

Furthermore, AL and their filament beside fully extruded and receptive stigmas are very promising (Beri and Anand 1971; De Vries, 1974, Komaki and Tsunewaki 1981; Fábián et al. 2019). However, influence of AL and SL on the other floral traits remains unclear. Langer et al. (2014) reported a positive correlation between AL with both AE and PM. However, our findings did not show any correlation with other floral traits which mean that the anther size *per se* has no impact on the shed pollen outside the florets.

Moreover, flowering characteristics are highly variable and strongly dependent to environmental conditions. We investigated the floral traits in relation with the flowering characteristics and we did not reveal any effect among each other, except a weak negative correlation between flowering duration and both AE and PSH. In contrast, Langer et al. (2014) reported a moderate correlation between anther extrusion and plant height suggesting further investigation in this regard. Thing that Boeven et al. (2016) made and shown that reduced height genes had a negative effect on male floral traits. Therefore, phenotyping the flowering time, plant height and spike traits requires special attention because non-uniformity of the flowering and inadequate plant height of the parental lines may cause low seed set. High-throughput and genomic tools might be applied to assist the selection of the appropriate traits of the pair lines.

Molecular relatedness identified genotypes that combined high PM, AE as favourable traits for male parents and OPF and DFO for female parents based on the molecular pedigree and trait values. This suggests that an association mapping approach is a promising approach to dissect the genetic basis of the traits that enforce the outcrossing ability. Effective markers could be

established to molecularly screen hybrid potential traits using MAS which can extensively accelerate the breeding cycle and save time, resources, and effort. All floral and flowering traits are laborious to assess, AE, as an example, takes around 10 minutes for each genotype, and given that mostly all genotypes flower at the same time, we had to deep-freeze spikes and count them later in order to level out peak work periods. Nevertheless, genome-wide scan remains a wise strategy to dissect the genetic architecture of complex traits (Reif et al. 2011). However, little is known about the molecular genetic mechanisms controlling male and female flower development of wheat. Therefore, we evaluated them by applying genome wide association mapping technique as defined in Chapter 3. We could identify 70 significant markers associated to the various floral traits at false discovery rate (FDR) < 0.05. Of which, 14 were commonly linked to both AE and VAE highlighting the tight associations between these traits. *Rht-D1b* which is a semi dwarf allele showed to have a negative effect on AE and PM. We have demonstrated that the genotypes carrying the major gene *Rht-D1b* have reduced AE and VAE by 1.45% and by almost two scoring points, respectively. The extent of decrease might be attributed to not only to the proportion of allelic variation of the gene itself but also to significant epistatic interaction with other closely linked genes in the locus (Miedaner et al. 2011; Li et al. 2012). Moreover, further research would be of highest interest to check whether this allele *Rht-D1b*, or any other semi dwarfing genes (*Rht-D1a*, *Rht-B1a*, or *Rht-B1b*), truly influences AE and PM or even PSH since all were shown highly correlated and sharing multi-marker-based associations. This might subsequently open up opportunities to full exploitation of the existing dwarf genetic resources in redesigning the floral potential traits for efficient outcrossing capacity.

Anther and stigma length, as favorable pollinator traits in grain setting, are fairly dissected for Rice and was shown to improve hybrid seed production. However, the genetic control of these two traits is still limited for wheat. We could identify few genes associated and was hard to align them due to the lack of available data. Therefore, further studies are required to dissect the novel genes related to AL and SL. In addition, deep analysis of the genomic regions of wheat-rye chromosome (4R) that increases anther length is noteworthy in order to increase our understanding of the genetic mechanisms of the trait AL (Nguyen et al. 2015).

Degree and the duration of floret opening are two important female traits shown to be challenging due to their intensive work and time requirements. GWAS revealed three potential markers (*Kukri_rep_c103359_233* and *wsnp_Ex_rep_c107911_91350930*) that are commonly associated with OPF, DFO, PM, AE and VAE, suggesting a potential genetic association that

controls these complex traits. These markers merit further validation that could be a MAS target for floral traits. Both the genetic associations and the positive correlations among the important floral traits confirm that AE and especially VAE and PSH as well offer promising proxies for selecting superior parents for hybrid breeding programs based on trait performance *per se*.

The importance of shed viable pollen comprises different aspects of pollen performance such as fertilization ability, and germinability in hybrid wheat breeding (Fritz and Lukaszewski, 1989). Certainly, the proportion of the total viable pollen produced shed outside the florets determines the pollination capacity during the anthesis. In other words, wheat pollen longevity needs to be sufficient to survive the travel through the air from an extruded anther of one plant to the stigma of another plant. However, the genetic basis of these two important traits has not been fairly investigated and the small number of the associated markers with no major gene might be attributed to in part to gaps in the genome with poor marker coverage or to quantitative inheritance and thus the complex genetic control underlying those two traits.

GWAS has enabled us to define the complexity of the traits governing the floral biology of wheat and to detect the minor genes that could be validated and subsequently used in breeding programs *via* MAS. However, MAS has its limitations as only markers/genes with major contributions to phenotypic variation are of interest (Zhao et al. 2014). Therefore, in second step, we have applied genomic prediction for the highly quantitative traits that are controlled by small and even large-effect markers. The results were very positive demonstrating that GS is a feasible quick strategy to identify potential hybrid parents. Prediction accuracy shown to be high for AX (0.85), VAX (0.92), PM (0.83), PSH (0.86), OPF (0.85), DFO (0.76) and AL (0.73) indicating that these traits can be predicted with high reliability. SL was the only trait showing moderate prediction accuracy of 0.52 suggesting that GS can be used to preselect the promising female having hairy long stigmas with acceptable accuracy but subsequent verification in the field might be needed.

So, to bread for improved hybrid seed production, direct trait selection might be done phenotypically or through genomic tools for specific and appropriate characteristics for both the male and female plants which was the major aspects of this thesis work described in Chapter 2 and 3. The assessment of wheat inflorescence for maximum seed setting and high heterosis level, determines the success of a hybrid wheat breeding program regardless of the hybridization mechanism applied. To investigate the relationship between seed set and the floral traits, we placed seven male lines and eleven female testers in crossing block trial, and

we applied a chemical hybridization agent (CHA-Croisor) as a sterility control system at different doses to verify, at the same time, its efficiency under Moroccan environment. Male sterility was induced on almost 95% of the female plants using the 13.5 l ha^{-1} rate and seed set showed a wide variability with high heritability (0.63) as previously found in Boeven et al. (2018). High correlations were observed with VAE (0.94), PM (0.97), PSH (0.91) and AL (0.46). Consequently, VAE, which is easier, faster and more reliable to screen, can be taken as proxy to select for seed set.

The resulting 23 hybrids seed from the best parent combinations were planted in alpha lattice design in two locations (Merchouch and Sidi El Aydi) over two years in Morocco. The germination rate of the hybrid seed was good enough proving the functionality of the CHA-based sterility system applied. The performance of the hybrid combinations over their parental inbred lines was assessed through calculation of the heterosis level and estimation of the combining ability. As shown in Chapter 4, several of the hybrids produced exhibited significant heterosis and heterobeltiosis for yield and its components. Maximum mid-parent heterosis (MPH) for biomass of 13.35% was recorded for the combine data expressed by the hybrid P9/P2. While 7.21% was the value of the best-parent heterosis (BPH) recorded for the cross P13/P11 using the same overall data. For thousand kernel weight MPH and BPH have a maximum gain of 12.5% and 11.26% for the cross combinations P10/P15 and P4/P11, respectively.

Grain yield heterosis in wheat has been a trait of widespread interest of many breeders and several reports have demonstrated yield stability and predictable advantages (Borghi and Perenzin 1990, 1994; Dreisigacker et al. 2005; Kant et al. 2011; Gowda et al. 2012; Mühleisen et al. 2014; Longin et al. 2015; Nie et al. 2019; Adhikari et al. 2020b; Easterly et al. 2020). In the current study, Mid-parent (MPH) and best-parent heterosis (BPH) showed a maximum value of 26.65 % and 24.04 % of yield advantage by more than 1t ha^{-1} each, respectively, for the cross combination P9/P6 using the combine data collected from Merchouch and Sidi El Aydi over two cropping seasons. Stable positive heterosis over MP and BP was recorded for some hybrids in each and across years and environment. This might be attributed to the use of best pair parents with potential floral traits combined with good agronomic performance. In contrast, some of the crosses did not perform well even with two high-yielding parents involved indicating that heterosis depends not only on performance *per se* but also on combining ability.

For this regard, combining ability analysis would be useful to estimate the GCA of the parents and the SCA of crosses in order to select the best performing parents and hybrids (Griffing, 1956; Sprague & Tatum, 1942). In the current study, five (P5, P9, P10, P12 and P14) were identified as good combiners having significant GCA effects for all BM, TKW and YLD. These lines can be used in future crossing strategies as improved parents for F1 hybrid production. Positive significant SCA effects indicated that the hybrids produced were good specific combiners for developing high-yielding hybrids. Eight hybrids (P4/P7, P9/P2, P9/P6, P9/P8, P10/P5, P10/P12, P10/P14 and P10/P15) exhibited positive SCA effects for TKW while only P9/P2 and P10/P5 were significant for BM. We have shown that significant SCA effects may not only obtained from the combination of high × high general combiners but also can be from the combination of high × low general combiners. Thus, high GCA effects of the parents are not necessarily reliable criterion to predict high SCA effects.

High performance of these crosses may be attributed to additive × additive for a cross of high × high or additive × dominance for a cross of high × low (Rewale et al. 2003). Superiority of a hybrid involving high × low general combiners as parents may be attributed to the genetic diversity in the form of number of heterozygous loci of the parents involved in the cross combinations (Kumar et al. 2006). Some parents with high GCA effects may also produce hybrids with low SCA effects (e.g., P4/P17 for yield; Table IV- 6). This may be due to the lack of complementation of the parental genes.

Conclusion and recommendations

Hybrid wheat offers a promising technology that warrants yield advantages and stability under different environments. However, the main limitations of establishing a successful hybrid wheat program are the stringent autogamous nature of wheat, inefficient hybrid seed production and inadequate exploitation of heterosis. Thus, redesigning the floral and flowering traits, together with increasing the power of hybrid via genome-wide association and prediction approaches can greatly contribute to select the best parental combination for better heterosis. To address all these key points, we could:

- Show adequate phenotypic variation for the floral and flowering traits and high heritability coupled with high genetic advance for some of the traits. These results clearly indicate the preponderance of additive gene action and thus effective selection might be done phenotypically.
- Confirm that visual anther extrusion based on a scale from 1 to 9 used as a high-throughput version of the counted anther extrusion could be an appropriate trait for practical use for selection of direct or indirect traits given that most of the floral traits are tightly correlated.
- Detect multi-marker-based associations for hybrid floral traits through GWAS.
- Indicate the feasibility of using genomic selection to predict the performance of hybrid floral traits with high reliability.
- Select promising male and female genotypes based on their potential floral and agronomic performance which were incorporated in experimental hybrid trials.
- Define the correct dose of the CHA-Croisor (13.5 l ha^{-1}) used as sterility control system.
- Confirm the beneficial utilization of wheat phenology in female seed setting.
- Conclude that the heterosis of more than 1t ha^{-1} seems feasible to make hybrid wheat production commercially successful.
- Suggest the use of general combining ability at early generations in order to reduce time and cost in hybrid breeding program.
- Verify that high specific combining ability estimates alone is not always reliable to select future potential hybrids.

So, to make hybrid wheat production even better, we recommend:

- To use visual anther extrusion as proxy for its great contribution in female seed setting and consequently enhancing yield gain.

- To validate the markers linked to the floral traits and subsequently use them in breeding programs *via* marker assisted selection.
- To check whether the GCA classification of the parental lines based on the phenotypic values is consistent with the one based on markers (SNP) data in case of application of genomic selection select superior hybrids. Hence, GS could be more effective than phenotypic method to predict the GCA of wheat lines and their hybrids performance if only the prediction accuracy is high.
- To establish male and female groups with considerable diversity in order to increase the allelic diversity and divergence between the groups for better heterosis increase. Therefore, the use of molecular markers through GWAS can be used as potential tool for grouping the parents. In addition, genomic selection is also useful for predicting quantitative traits controlled by many small-effect QTL.
- To dissect the genetic mechanisms of heterosis using quantitative trait loci mapping which may provide not only the information about the of chromosomal segments involved in heterosis but also can determine the gene effect involved (dominant, over-dominant gene action or epistatic interactions).

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Annexes

Annex 1: List of the 196 elite spring bread wheat used in this study.

Genotype	GID	Panel	Name/Pedigree
Elt-001	1401001	R-H&D	ATTILA*2/PBW65//PFAU/MILAN
Elt-002	1401002	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN
Elt-003	1401003	R-H&D	QAFZAH-21/ICARDA-SRRL-9
Elt-004	1401004	R-H&D	KAUZ'S'/SERI/3/KAUZ//KAUZ/STAR
Elt-005	1401005	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN
Elt-006	1401006	R-H&D	ATTILA*2/PBW65//PFAU/MILAN
Elt-007	1401007	R-H&D	CROC1/AE.SQUARROSSA(205)//KAUZ/3/ATTILA/4/FLAG-1
Elt-008	1401008	R-H&D	BACANORA T 88/SHIHAB-8
Elt-009	1401009	R-H&D	KAUZ'S'/BOCRO-3//ANGI-2
Elt-010	1401010	R-H&D	GOUBARA-1/ANGI-1
Elt-011	1401011	R-H&D	ATTILA 50Y//ATTILA/BCN/3/PFAU/MILAN
Elt-012	1401012	R-H&D	HUBARA-5/ANGI-1
Elt-013	1401013	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/KAUZ/FLORKWA-1
Elt-014	1401014	R-H&D	KAUZ'S'/SERI/3/KAUZ//KAUZ/STAR
Elt-015	1401015	R-H&D	GIRWILL-13/2*PASTOR-2
Elt-017	1401017	R-H&D	HIDDAB/CHAM-8
Elt-018	1401018	R-H&D	CROC1/AE.SQUARROSSA(205)//KAUZ/3/ATTILA/4/FLAG-1
Elt-019	1401019	R-H&D	SERI 82/SHUHA'S'//GRU90-204782/4/PASTOR/3/KAUZ*2/OPATA//KAUZ
Elt-020	1401020	R-H&D	ZEMAMRA-1/2*SOMAMA-3
Elt-021	1401021	R-H&D	ZOLOTARA//SHA3/SERI/3/SKAUZ/2*STAR
Elt-022	1401022	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9
Elt-023	1401023	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9
Elt-024	1401024	R-H&D	ESDA/SHWA//BCN/3/MILAN/PASTOR
Elt-025	1401025	R-H&D	PASTOR-2
Elt-026	1401026	R-H&D	HUBARA-13/4/TRAP#1/BOW//PFAU/3/MILAN
Elt-027	1401027	R-H&D	HUBARA-5/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR
Elt-028	1401028	R-H&D	IZAZ-1/KATILA-11//GOUMRIA-3
Elt-029	1401029	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN
Elt-030	1401030	R-H&D	BOUSHODA-1/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR
Elt-031	1401031	R-H&D	ANGI-2/HUBARA-3
Elt-032	1401032	R-H&D	QIMMA-12/PASTOR-6//QIMMA-12
Elt-033	1401033	R-H&D	SOMAMA-9/ICARDA-SRRL-2
Elt-034	1401034	R-H&D	VEE/PJN//2*KAUZ/3/SHUHA-4/FOW-2
Elt-035	1401035	R-H&D	QAFZAH-2/FERROUG-2//ZEMAMRA-8
Elt-036	1401036	R-H&D	SOMAMA-9/NEJMAH-18
Elt-037	1401037	R-H&D	KAUZ'S'/SERI/3/KAUZ//KAUZ/STAR
Elt-038	1401038	R-H&D	HUBARA-5/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR
Elt-039	1401039	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN
Elt-040	1401040	R-H&D	QAFZAH-35/AMIR-2

Elt-042	1401042	R-H&D	QAFZAH-16/ICARDA-SRRL-5
Elt-043	1401043	R-H&D	ESDA/SHWA//BCN/3/MILAN/PASTOR
Elt-044	1401044	R-H&D	KAUZ//ALTAR 84/AOS/3/MILAN/DUCULA
Elt-045	1401045	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-2
Elt-046	1401046	R-H&D	HUBARA-5/PASTOR-2
Elt-047	1401047	R-H&D	GIRWILL-13/2*PASTOR-2
Elt-048	1401048	R-H&D	HUBARA-7/4/PASTOR/3/KAUZ*2/OPATA//KAUZ
Elt-049	1401049	R-H&D	HIDDAB/CHAM-8
Elt-050	1401050	R-H&D	QIMMA-12
Elt-051	1401051	R-H&D	ANGI-2/HUBARA-3
Elt-052	1401052	R-H&D	ESDA/SHWA//BCN/3/MILAN/PASTOR
Elt-053	1401053	R-H&D	SOMAMA-9/ICARDA-SRRL-2
Elt-054	1401054	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN
Elt-055	1401055	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9
Elt-056	1401056	R-H&D	HIDDAB/CHAM-8
Elt-057	1401057	R-H&D	DEBEIRA/ANGI-2
Elt-058	1401058	R-H&D	QAFZAH-2/FERROUG-2//ZEMAMRA-8
Elt-059	1401059	R-H&D	KAUZ//MON/CROW?S?/3/VEE/PJN//2*KAUZ
Elt-060	1401060	R-H&D	KAUZ'S'/SERI/3/KAUZ//KAUZ/STAR
Elt-061	1401061	R-H&D	ANGI-5/ZEMAMRA-8
Elt-062	1401062	R-H&D	KAUZ'S'/FLORKWA-1//GOUMRIA-3
Elt-063	1401063	R-H&D	YMI #6/GEN//TIA.1/3/VEE#5//DOVE/BUC/4/MILAN/PASTOR
Elt-064	1401064	R-H&D	SERI 82/SHUHA'S'//GRU90-204782/3/MUNIA/CHTO//MILAN
Elt-065	1401065	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-1
Elt-066	1401066	R-H&D	HUBARA-5/PASTOR-2
Elt-067	1401067	R-H&D	HUBARA-15/CATBIRD//PASTOR-2
Elt-068	1401068	R-H&D	QAFZAH-23/SOMAMA-3//GOUMRIA-3
Elt-069	1401069	R-H&D	ATTILA*2/PBW65//PFAU/MILAN
Elt-070	1401070	R-H&D	PASTOR-2/HUBARA-5
Elt-071	1401071	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/KAUZ/FLORKWA-1
Elt-072	1401072	R-H&D	KAUZ'S'/FLORKWA-1//GOUMRIA-3
Elt-073	1401073	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN
Elt-074	1401074	R-H&D	HAAMA-17/ANGI-2
Elt-075	1401075	R-H&D	ATTILA-7
Elt-076	1401076	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-2
Elt-077	1401077	R-H&D	ATTILA*2/PBW65//PFAU/MILAN
Elt-078	1401078	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9
Elt-079	1401079	R-H&D	KAUZ//MON/CROW'S'/3/KAUZ//KAUZ/STAR/5/SHAMIEKH-7
Elt-080	1401080	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-1
Elt-081	1401081	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/PFAU/MILAN
Elt-082	1401082	R-H&D	GOUMRIA-15/ANGI-2
Elt-083	1401083	R-H&D	HUBARA-16/4/PASTOR/3/KAUZ*2/OPATA//KAUZ
Elt-084	1401084	R-H&D	SERI 82/SHUHA'S'//GRU90-204782/3/MUNIA/CHTO//MILAN
Elt-085	1401085	R-H&D	HIDDAB/CHAM-8
Elt-086	1401086	R-H&D	SOMAMA-9/ICARDA-SRRL-2
Elt-087	1401087	R-H&D	KAUZ'S'/SERI/3/KAUZ//KAUZ/STAR

Elt-088	1401088	R-H&D	VEE/PJN//2*KAUZ/3/SHUHA-4/FOW-2
Elt-089	1401089	R-H&D	VEE/PJN//2*KAUZ/3/SHUHA-4/FOW-2
Elt-090	1401090	R-H&D	ATTILA*2/PBW65//PFAU/MILAN
Elt-091	1401091	R-H&D	SERI 82/SHUHA'S'//GRU90-204782/4/PASTOR/3/KAUZ*2/OPATA//KAUZ
Elt-092	1401092	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9
Elt-093	1401093	R-H&D	QAFZAH-33*2/SALSAL-2
Elt-094	1401094	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9
Elt-095	1401095	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-1
Elt-096	1401096	R-H&D	BOUSHODA-1/5/CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/VEE#7/BOW/4/PASTOR
Elt-097	1401097	R-H&D	PASTOR-2/HUBARA-5
Elt-098	1401098	R-H&D	SERI 82/SHUHA'S'//GRU90-204782/3/MUNIA/CHTO//MILAN
Elt-099	1401099	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-2
Elt-100	1401100	R-H&D	KABOWSH-1
Elt-101	1401101	R-H&D	QAFZAH-35/AMIR-2
Elt-102	1401102	R-H&D	LAKTA-1/QAFZAH-21
Elt-103	1401103	R-H&D	SOMAMA-9
Elt-104	1401104	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15
Elt-105	1401105	R-H&D	VEE7/KAUZ//PFAU/MILAN/3/MILAN/PASTOR
Elt-106	1401106	R-H&D	MUNIA/ALTAR 84//AMSEL
Elt-107	1401107	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/PYN/BAU//MILAN/4/ZEMAMRA-8
Elt-108	1401108	R-H&D	ATTILA-7//MILAN/PASTOR/3/HXL8088/DUCULA
Elt-109	1401109	R-H&D	VEE7/KAUZ//PFAU/MILAN/3/MILAN/PASTOR
Elt-110	1401110	R-H&D	URES/BOW//OPATA/3/HD2206/HORK'S'
Elt-111	1401111	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15
Elt-112	1401112	R-H&D	SHIHAB-16
Elt-113	1401113	R-H&D	VEE7/KAUZ//PFAU/MILAN/3/MILAN/PASTOR
Elt-114	1401114	R-H&D	KAUZ'S'/SERI/3/KAUZ//KAUZ/STAR
Elt-115	1401115	R-H&D	MILAN/DUCULA
Elt-116	1401116	R-H&D	MILAN/DUCULA//AL-ZEHRAA-1
Elt-117	1401117	R-H&D	QADANFER-11/REBWAH-11
Elt-118	1401118	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15
Elt-119	1401119	R-H&D	P1.861/RDWG//PBW343/3/MUNIA/ALTAR 84//AMSEL
Elt-120	1401120	R-H&D	KAUZ//ALTAR 84/AOS 3/KAUZ/3/SHUHA-4//NS732/HER/4/QAFZAH-33
Elt-121	1401121	R-H&D	P1.861/RDWG//PBW343/3/MUNIA/ALTAR 84//AMSEL
Elt-122	1401122	R-H&D	OPATA/RAYON//KAUZ/3/2*MILAN/DUCULA
Elt-123	1401123	R-H&D	KAUZ'S'/SHUHA-15
Elt-124	1401124	R-H&D	VEE/PJN//2*TUI/3/WH576/4/AL-ZEHRAA-5
Elt-125	1401125	R-H&D	SIDS-1
Elt-126	1401126	R-H&D	ANGI-2
Elt-127	1401127	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-1/5/KABOWSH-1
Elt-128	1401128	R-H&D	MILAN/DUCULA//AL-ZEHRAA-1
Elt-129	1401129	R-H&D	BOW/PRL//BUC/3/WH576
Elt-130	1401130	R-H&D	CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/2*WEAVER/4/IPA-95

Elt-131	1401131	R-H&D	QADANFER-11
Elt-132	1401132	R-H&D	QADANFER-11/REBWAH-11
Elt-133	1401133	R-H&D	PASTOR/3/KAUZ*2/OPATA//KAUZ
Elt-134	1401134	R-H&D	P1.861/RDWG/3/VEE/PJN//2*KAUZ/4/CMH82A.1294/2*KAUZ//MUNIA /CHTO/3/MILAN
Elt-135	1401135	R-H&D	VEE7/KAUZ/3/KAUZ//MON/CROW'S'/4/QAFZAH-33
Elt-136	1401136	R-H&D	MILAN//PSN/BOW
Elt-137	1401137	R-H&D	KAUZ//MON/CROW'S'/3/SOMAMA-3/4/MILAN/DUCULA
Elt-138	1401138	R-H&D	GOUBARA-1/ANGI-1//QAFZAH-21
Elt-139	1401139	R-H&D	ATTILA 50Y//ATTILA/BCN/3/KAUZ//MON/CROW'S'/4/MILAN/PASTOR
Elt-140	1401140	R-H&D	VEE7/KAUZ//PFAU/MILAN/3/MILAN/PASTOR
Elt-141	1401141	R-H&D	VEE7/KAUZ//PFAU/MILAN/3/MILAN/PASTOR
Elt-142	1401142	R-H&D	QADANFER-11/REBWAH-11
Elt-143	1401143	R-H&D	P1.861/RDWG/3/VEE/PJN//2*KAUZ/4/CMH82A.1294/2*KAUZ//MUNIA /CHTO/3/MILAN
Elt-144	1401144	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/ANGI-1/5/KABOWSH-1
Elt-145	1401145	R-H&D	ZEMAMRA-1
Elt-146	1401146	R-H&D	VEE/PJN//2*TUI/3/WH576
Elt-147	1401147	R-H&D	CHEN/AEGILOPS SQUARROSA (TAUS)//FCT/3/2*WEAVER
Elt-148	1401148	R-H&D	P1.861/RDWG//DAJAJ-10/3/MILAN/PASTOR
Elt-149	1401149	R-H&D	PASTOR-6
Elt-150	1401150	R-H&D	DEBIRA
Elt-151	1401151	R-H&D	QAMAR-6
Elt-152	1401152	R-H&D	KAUZ//ALTAR 84/AOS/3/TNMU/MILAN/4/MILAN//PSN/BOW
Elt-153	1401153	R-H&D	PFAU/MILAN
Elt-154	1401154	R-H&D	KAUZ//ALTAR 84/AOS/3/TNMU/MILAN/4/MILAN//PSN/BOW
Elt-155	1401155	R-H&D	KASYON/GENARO 81//TEVEE-1/../3/2*QADANFER-11
Elt-156	1401156	R-H&D	VEE/PJN//2*KAUZ
Elt-157	1401157	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KA UZ'S'/SHUHA-15
Elt-158	1401158	R-H&D	OPATA/RAYON//KAUZ/3/CHAM-6/FLORKWA-2/5/SHAMIEKH-7
Elt-159	1401159	R-H&D	P1.861/RDWG//PBW343/3/MUNIA/ALTAR 84//AMSEL
Elt-160	1401160	R-H&D	WEAVER/WL 3928//SW 89.3064/3/SOMAMA-3/4/BOW/PRL//BUC/3/WH576
Elt-161	1401161	R-H&D	FLAG-1
Elt-162	1401162	R-H&D	KAUZ'S'/FLORKWA-1
Elt-163	1401163	R-H&D	QAFZAH-35
Elt-164	1401164	R-H&D	ZEMAMRA-8
Elt-165	1401165	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/PYN/BAU//MILAN/4/ZEMAMRA-8
Elt-166	1401166	R-H&D	QAFZAH-33
Elt-167	1401167	R-H&D	KAUZ//ALTAR 84/AOS 3/KAUZ/3/ATTILA 50Y//ATTILA/BCN/4/PASTOR-6
Elt-168	1401168	R-H&D	VEE/PJN//2*TUI/3/WH576/4/AL-ZEHRAA-5
Elt-169	1401169	R-H&D	P1.861/RDWG//DAJAJ-10/3/MILAN/PASTOR
Elt-170	1401170	R-H&D	ATTILA 50Y//ATTILA/BCN/3/KAUZ//MON/CROW'S'/4/MILAN/PASTOR
Elt-171	1401171	R-H&D	OPATA/RAYON//KAUZ/3/2*MILAN/DUCULA
Elt-172	1401172	R-H&D	QADANFER-11/REBWAH-11

Elt-173	1401173	R-H&D	SHAMISS-3
Elt-174	1401174	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15
Elt-175	1401175	R-H&D	GOUMRIA-3
Elt-176	1401176	R-H&D	OPATA/RAYON//KAUZ/3/2*MILAN/DUCULA
Elt-177	1401177	R-H&D	QADANFER-11/REBWAH-11
Elt-178	1401178	R-H&D	KATILA-8/4/SKAUZ/BAV92/3/CROC-1/AE.SQUARROSA (224)//OPATA/5/MUNIA/ALTAR 84//MILAN
Elt-179	1401179	R-H&D	FAYEQ-1
Elt-181	1401181	R-H&D	ATTILA-7//MILAN/PASTOR/3/ICARDA-SRRL-2
Elt-182	1401182	R-H&D	QADANFER-11/REBWAH-11
Elt-183	1401183	R-H&D	QADANFER-9
Elt-184	1401184	R-H&D	KASYON/GENARO 81//TEVEE-1/../3/2*QADANFER-11
Elt-185	1401185	R-H&D	KAUZ//ALTAR 84/AOS 3/KAUZ/3/SHUHA-4//NS732/HER/4/QAFZAH-33
Elt-186	1401186	R-H&D	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/KAUZ/GYS//KAUZ/5/MUNIA/ALTAR 84//MILAN
Elt-187	1401187	R-H&D	VEE/PJN//2*TUI/3/WH576/4/AL-ZEHRAA-5
Elt-188	1401188	R-H&D	HAAMA-17/QIMMA-12
Elt-189	1401189	R-H&D	KAUZ//MON/CROW'S'/3/SHUHA-4//NS732/HER/4/MILAN/PASTOR
Elt-190	1401190	R-H&D	QADANFER-11/REBWAH-11
Elt-191	1401191	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15
Elt-192	1401192	R-H&D	KAUZ'S'/SERI//STAR'S'/FLORKWA-2/3/FLAG-1
Elt-193	1401193	R-H&D	MILAN/PASTOR
Elt-194	1401194	R-H&D	ANGI-1
Elt-195	1401195	R-H&D	SAMIRA-9
Elt-197	1401197	R-H&D	KAUZ//MON/CROW'S'/3/KAUZ//KAUZ/STAR/5/SHAMIEKH-7
Elt-198	1401198	R-H&D	SHIHAB-19
Elt-199	1401199	R-H&D	VEE7/KAUZ/3/KAUZ//MON/CROW'S'/4/QAFZAH-33
Elt-200	1401200	R-H&D	HIDDAB

Annex 2: Preliminary work (publication of the study: Genome wide association study for adult plant resistance to yellow rust in spring bread wheat)

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Genome-wide association study for adult plant resistance to yellow rust in spring bread wheat (*Triticum aestivum* L.)

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Abstract Yellow rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a common and serious fungal disease of wheat (*Triticum aestivum* L.) all over the world and particularly in the Central and West Asia and North Africa region. To identify effective yellow rust resistance loci, genome-wide association study (GWAS) was performed using 196 bread wheat genotypes based on 10,477 single nucleotide polymorphisms markers. Adult plants were evaluated under field conditions for resistance to yellow rust for 2 years (2014–2015) at ICARDA station, Marchouch, Morocco. Out of the 196 genotypes, 85 genotypes (43.37%) were resistant, 22 genotypes (11.22%) were moderately resistant, 13 genotypes (6.63%) were moderately susceptible, 48 genotypes (24.49%) were moderately susceptible to susceptible and 28 genotypes (14.29%) were susceptible to *Puccinia striiformis* f. sp. *tritici*. GWAS using mixed linear model identified 23 markers on chromosomes 2A, 2B, 2D and 7B significantly associated with adult plant resistance at false discovery rate (FDR-adjusted $P \leq 0.05$). Of which, three markers were within 10 wheat functional genes involved in several plant disease resistance and defense mechanism. Five of the reported functional genes are annotated as disease resistance proteins with nucleotide-binding site leucine repeat domains. BLAST analysis confirmed that *YrR61* and *Yr17* were mostly the candidate genes linked to the marker *Tdurum_contig29983_490* on chromosome 2A. Moreover, markers identified on chromosome 7B and *Kukri_c12648_434* on 2D were not mapped within any of previously reported gene/QTL region hence, representing novel resistance loci for *Pst* and needs to be confirmed using an allelism test.

Keywords Adult plant resistance · GWAS · *Puccinia striiformis* f. sp. *Tritici* · SNP · Stripe rust · Wheat

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Annex 3: Number of favorable and unfavorable alleles per genotypes versus AE-BLUEs values.

	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	M-9	M-10	M-11	M-12	M-13	M-14	M-15	M-16	M-17	UNF-A'	F-A"	AE score
Elt-001	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	0	0	1	3	35.83
Elt-002	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	41.56
Elt-003	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	4	11	73.37
Elt-004	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	4	11	54.04
Elt-005	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	32.65
Elt-006	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	37.65
Elt-007	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	85.87
Elt-008	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	4	11	39.86
Elt-009	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	11	29.68
Elt-010	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	11	22.11
Elt-011	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	38.61
Elt-012	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	60.41
Elt-013	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	4	11	65.04
Elt-014	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	11	25.97
Elt-015	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	4	10	83.23
Elt-017	0	0	0	1	1	0	1	1	0	0	0	0	0	0	0	0	0	2	2	40.13
Elt-018	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	70.31
Elt-019	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	5	11	45.69
Elt-020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69.04
Elt-021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32.79
Elt-022	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	4	10	59.44
Elt-023	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	48.75

M-1:wsnp_Ex_c13109_20725640; M-2:wsnp_Ku_rep_c89089_82009060; M-3:IAAV3802; M-4:Excalibur_c10496_651; Mm-5:snp_Ex_rep_c103167_88182254; M-6:Ex_c11930_509; M-7:Rht-D1b; M-8:wsnp_Ku_c13043_20902807; M-9:wsnp_Ex_c23230_32466568; M-10:Tdurum_contig30517_578; M-11:Tdurum_contig30517_310; M-12:Kukri_rep_c103359_233; M-13:wsnp_Ex_rep_c107911_91350930; M-14:wsnp_Ex_rep_c107911_91350866; M-15:wsnp_Ex_c21198_30327016; M-16:BS00062724_51; M-17:wsnp_Ex_c23235_32471767; UNF-A': sum of present unfavorable allele; F-A":sum of present favorable allele.

Annex 4: Number of favorable and unfavorable alleles per genotypes versus VAE-BLUEs values

	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	M-9	M-10	M-11	M-12	M-13	M-14	M-15	M-16	M-17	M-18	UNF-A’	F-A”	VAE score
Elt-001	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	1	1	0	5	4.83
Elt-002	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	4.56
Elt-003	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	7.37
Elt-004	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	5.4
Elt-005	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	3	3.65
Elt-006	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	3	3.65
Elt-007	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	3	7.87
Elt-008	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	3.86
Elt-009	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	2.68
Elt-010	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	2.11
Elt-011	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	3.61
Elt-012	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	6.41
Elt-013	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	6.4
Elt-014	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	2.97
Elt-015	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	4	13	8.23
Elt-017	0	0	0	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	4	3.13
Elt-018	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.31
Elt-019	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	4.69
Elt-020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.4
Elt-021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	4.79
Elt-022	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	4	13	5.44
Elt-023	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.75
Elt-024	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4	14	3.28

M-1:wsnp_Ex_c13109_20725640; M-2:Ex_c11930_509; M-3:wsnp_Ku_rep_c89089_82009060; M-4:Excalibur_c10496_651; M-5:IAAV3802; M-6:Rht-D1b; M-7:Tdurum_contig30517_578; M-8:wsnp_Ex_c23230_32466568; M-9:Tdurum_contig30517_310; M-10:wsnp_Ku_c13043_20902807; M-11:Kukri_rep_c103359_233; M-12:wsnp_Ex_rep_c107911_91350930; M-13:wsnp_Ex_rep_c107911_91350866; M-14:wsnp_Ex_c21198_30327016; M-15:BS00062724_51; M-16:wsnp_Ex_c23235_32471767; M-17:Excalibur_c20309_539; M-18:Kukri_rep_c110544_248; UNF-A’: sum of present unfavorable allele; F-A”:sum of present favorable allele.

Annex 5: Number of favorable and unfavorable alleles per genotypes versus PM-BLUEs values

	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	M-9	M-10	M-11	M-12	M-13	M-14	M-15	UNF-A’	F-A”	PM score
Elt-001	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	1	17.72
Elt-002	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	17.41
Elt-003	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	5	9	39.74
Elt-004	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	5	9	22.07
Elt-005	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	15.94
Elt-006	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	20.79
Elt-007	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	41.11
Elt-008	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	5	9	19.48
Elt-009	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	6	9	13.68
Elt-010	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	6	9	16.19
Elt-011	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	18.52
Elt-012	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	25.03
Elt-013	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	5	9	26.18
Elt-014	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	6	9	17.44
Elt-015	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	5	9	39.33
Elt-017	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	2	1	22.16
Elt-018	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	34.82
Elt-019	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	6	9	21.45
Elt-020	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29.65
Elt-021	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15.82
Elt-022	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	5	9	25.92
Elt-023	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	25.2
Elt-024	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	5	9	16.87

M-1:wsnp_Ex_c13109_20725640;,M-2:wsnp_Ku_rep_c89089_82009060; M-3:Ex_c11930_509; M-4:IAAV3802; M-5:wsnp_Ex_rep_c103167_88182254; M-6:Excalibur_c10496_651; M-7:wsnp_Ku_c13043_20902807; M-8:wsnp_Ex_c23230_32466568; M-9:Tdurum_contig30517_578; M-10:wsnp_Ex_c21198_30327016; M-11:wsnp_Ex_rep_c107911_91350866; M-12:Kukri_rep_c103359_233; M-13:wsnp_Ex_rep_c107911_91350930; M-14:BS00062724_51; M-15:wsnp_Ex_c23235_32471767; UNF-A’: sum of present unfavorable allele; F-A”:sum of present favorable allele.

Annex 6: Number of favorable and unfavorable alleles per genotypes versus OPF-BLUEs values

	M-1	M-2	M-3	M-4	M-5	M-6	M-7	M-8	M-9	M-10	M-11	UNF-A’	F-A”	OPF score
Elt-001	0	0	0	0	1	0	0	0	0	0	1	1	1	21.8
Elt-002	0	0	0	0	1	0	0	0	0	0	0	1	0	23.35
Elt-003	1	1	1	1	0	1	1	1	1	1	1	5	5	31.85
Elt-004	1	1	1	1	0	1	1	1	1	1	1	5	5	28.31
Elt-005	0	0	0	0	1	0	0	0	0	0	0	1	0	17.31
Elt-006	0	0	0	0	1	0	0	0	0	0	0	1	0	25.08
Elt-007	0	0	0	0	1	0	0	0	0	0	0	1	0	36.19
Elt-008	1	1	1	1	0	1	1	1	1	1	1	5	5	24.87
Elt-009	1	1	1	1	1	1	1	1	1	1	1	6	5	17.75
Elt-010	1	1	1	1	1	1	1	1	1	1	1	6	5	17.4
Elt-011	0	0	0	0	1	0	0	0	0	0	0	1	0	26.31
Elt-012	0	0	0	0	1	0	0	0	0	0	0	1	0	30.1
Elt-013	1	1	1	1	0	1	1	1	1	1	1	5	5	30.02
Elt-014	1	1	1	1	1	1	1	1	1	1	1	6	5	20.88
Elt-015	1	1	1	1	0	1	1	1	1	1	1	5	5	33.09
Elt-017	0	0	0	0	1	0	0	0	0	0	0	1	0	27.08
Elt-018	0	0	0	0	1	0	0	0	0	0	0	1	0	33.3
Elt-019	1	1	1	1	1	1	1	1	1	1	1	6	5	25.55
Elt-020	0	0	0	0	0	0	0	0	0	0	0	0	0	30.5
Elt-021	0	0	0	0	0	0	0	0	0	0	0	0	0	19.69
Elt-022	1	1	1	1	0	1	1	1	1	1	1	5	5	33.1
Elt-023	0	0	0	0	1	0	0	0	0	0	0	1	0	26.67
Elt-024	1	1	1	1	0	1	1	1	1	1	1	5	5	20.04

M-1:wsnp_Ex_c13109_20725640; M-2:wsnp_Ku_rep_c89089_82009060; M-3:Ex_c11930_509; M-4:IAAV3802; M-5:wsnp_Ex_rep_c103167_88182254; M-6:wsnp_Ex_c21198_30327016; M-7:wsnp_Ex_rep_c107911_91350866; M-8:Kukri_rep_c103359_233; M-9:wsnp_Ex_rep_c107911_91350930; M-10:wsnp_Ex_c23235_32471767; M-11:Tdurum_contig30517_310; UNF-A’: sum of present unfavorable allele; F-A”:sum of present favorable allele.

Annex 7: Number of favorable and unfavorable alleles per genotypes versus DFO-BLUEs values

	M-1	M-2	M-3	UNF-A'	F-A"	DFO score
Elt-001	1	0	0	1	0	35.12
Elt-002	1	0	0	1	0	45.72
Elt-003	0	1	1	1	1	84.67
Elt-004	0	1	1	1	1	66.58
Elt-005	1	0	0	1	0	53.66
Elt-006	1	0	0	1	0	47.08
Elt-007	1	0	0	1	0	85.43
Elt-008	0	1	1	1	1	43.46
Elt-009	1	1	1	2	1	34.61
Elt-010	1	1	1	2	1	30.89
Elt-011	1	0	0	1	0	61.62
Elt-012	1	0	0	1	0	64.46
Elt-013	0	1	1	1	1	73.33
Elt-014	1	1	1	2	1	36.62
Elt-015	0	1	1	1	1	98.67
Elt-017	1	0	0	1	0	64.29
Elt-018	1	0	0	1	0	91.22
Elt-019	1	1	1	2	1	47.29
Elt-020	0	0	0	0	0	72.83
Elt-021	0	0	0	0	0	38.08
Elt-022	0	1	1	1	1	81.29
Elt-023	1	0	0	1	0	70.29
Elt-024	0	1	1	1	1	44.56
Elt-025	1	1	1	2	1	62.15
Elt-026	0	0	0	0	0	86.16
Elt-027	0	0	0	0	0	49.29
Elt-028	0	1	1	1	1	92.83
Elt-029	0	0	0	0	0	65.95
Elt-030	1	0	0	1	0	70.39
Elt-031	0	1	1	1	1	86.83
Elt-032	0	1	1	1	1	55.24
Elt-033	1	1	1	2	1	66.02
Elt-034	1	0	0	1	0	65.69
Elt-035	0	0	0	0	0	71.5
Elt-036	0	0	0	0	0	75.11
Elt-037	1	1	1	2	1	31.29
Elt-038	0	0	0	0	0	46.85
Elt-039	1	1	1	2	1	54.79
Elt-040	0	1	1	1	1	67

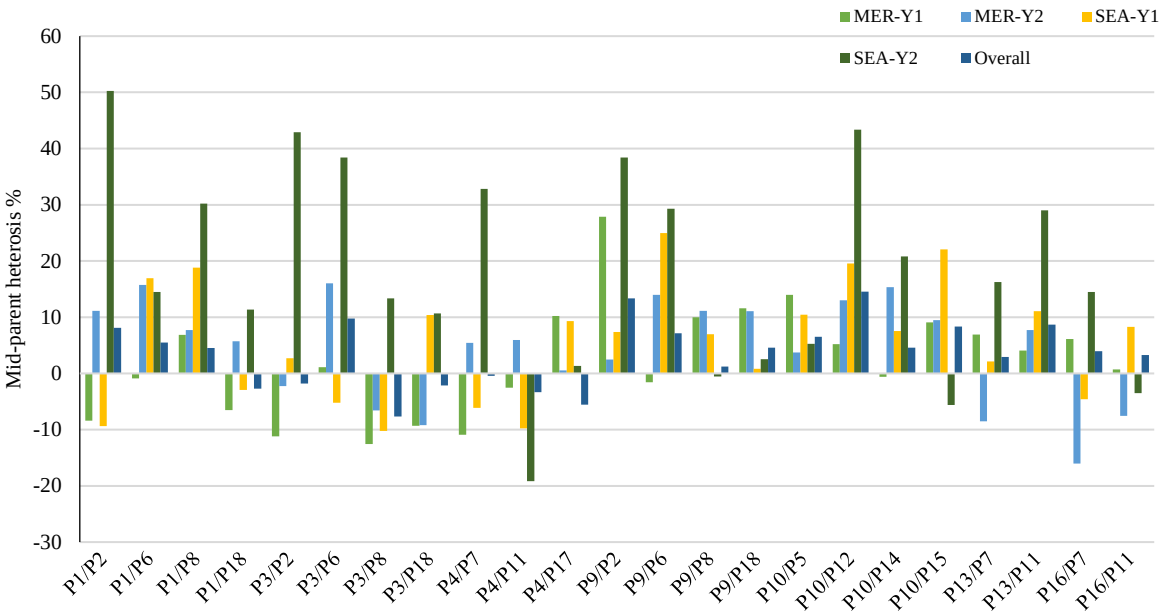
Annex 8: List of the 18 elite spring bread wheat parental lines used for hybridization and seed set activities.

Parent	Parent code	GID	Panel	Name/Pedigree
P1	Elt-151	1401151	R-H&D	QAMAR-6
P2	Elt-152	1401152	R-H&D	KAUZ//ALTAR 84/AOS/3/TNMU/MILAN/4/MILAN//PSN/BOW
P3	Elt-172	1401172	R-H&D	QADANFER-11/REBWAH-11
P4	Elt-141	1401141	R-H&D	VEE7/KAUZ//PFAU/MILAN/3/MILAN/PASTOR
P5	Elt-120	1401120	R-H&D	KAUZ//ALTAR 84/AOS 3/KAUZ/3/SHUHA-4//NS732/HER/4/QAFZAH-33
P6	Elt-157	1401157	R-H&D	SERI.1B//KAUZ/HEVO/3/AMAD/4/ATTILA//PSN/BOW/3/ATTILA/5/KAUZ'S'/SHUHA-15
P7	Elt-119	1401119	R-H&D	P1.861/RDWG//PBW343/3/MUNIA/ALTAR 84//AMSEL
P8	Elt-100	1401100	R-H&D	KABOWSH-1
P9	Elt-051	1401051	R-H&D	ANGI-2/HUBARA-3
P10	Elt-053	1401053	R-H&D	SOMAMA-9/ICARDA-SRRL-2
P11	Elt-066	1401066	R-H&D	HUBARA-5/PASTOR-2
P12	Elt-069	1401069	R-H&D	ATTILA*2/PBW65//PFAU/MILAN
P13	Elt-026	1401026	R-H&D	HUBARA-13/4/TRAP#1/BOW//PFAU/3/MILAN
P14	Elt-068	1401068	R-H&D	QAFZAH-23/SOMAMA-3//GOUMRIA-3
P15	Elt-093	1401093	R-H&D	QAFZAH-33*2/SALSAL-2
P16	Elt-022	1401022	R-H&D	ATTILA//VEE#5/DOBUC'S'/3/QADANFER-9
P17	Elt-075	1401075	R-H&D	ATTILA-7
P18	Elt-089	1401089	R-H&D	VEE/PJN//2*KAUZ/3/SHUHA-4/FOW-2

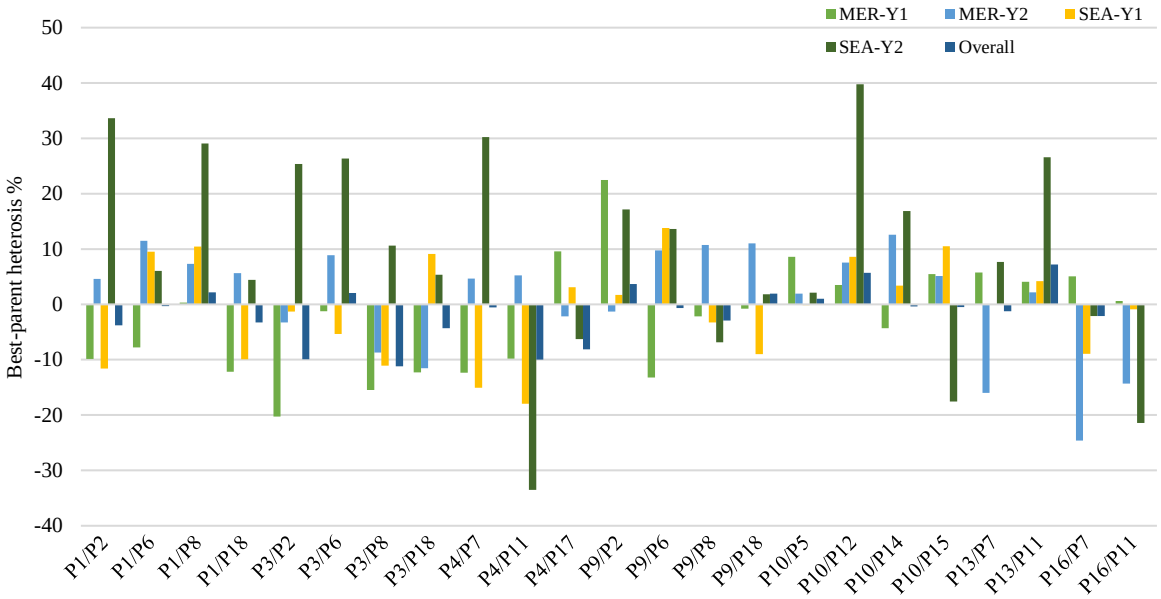
Annex 9: Hybrid seed set using CHA at 13.5 l/ha.

Parental combination	cross code	DH	PLH	SPS	GYS	PSH	VAE	PM	AL	Seed set
P1/P18	151/89	100.5	100	21.35	2.2075	9	9	39.58	3.7	47.05
P10/P5	53/120	109	99	20.75	1.873	8.25	8.25	35.78	4	38.75
P1/P6	151/157	99	99.5	20.4	2.208	8.375	8.625	39.02	3.85	45.1
P10/P15	53/93	108	98	20.4	1.944	8.625	8.75	38.17	3.85	39.6
P10/P12	53/69	109	98	20.2	1.924	8.5	8.5	36.83	3.95	38.6
P1/P2	151/152	100.5	99	20.1	2.177	8.625	9	38.87	3.9	43.65
P9/P6	51/157	105	99.5	20	1.584	7.625	7.375	32.39	3.9	31.6
P16/P7	22/119	109	97.5	19.8	1.898	8.125	8.625	35.58	3.85	37.85
P10/P14	53/68	107.5	97	19.7	1.9105	7.875	7.875	34.41	3.95	37.45
P16/P11	22/66	107	97.5	19.6	1.9455	8.125	7.875	35.31	3.9	38.1
P3/P18	172/89	104.5	98	19.6	1.782	7.75	7.875	33.01	4.05	35.05
P3/P2	172/152	104	98.5	19.55	1.754	7.125	7.25	30.67	3.6	33.9
P3/P8	172/100	100.5	98.5	19.45	1.618	6.875	7.125	29.94	3.65	31.35
P3/P6	172/157	102	98.5	19.35	1.6755	7	7.25	31.02	3.6	32.45
P4/P17	141/75	109	97	19.15	1.8705	7.75	8	33.54	4	35.7
P4/P11	141/66	107.5	97	19.1	1.857	7.375	7.375	32.78	3.85	35.4
P1/P8	P1/P8	106.5	98.5	19.05	1.956	7.25	7.625	33.22	3.9	37.15
P4/P7	141/119	107	97	18.95	1.8945	7.625	7.25	34.90	3.95	35.8
P9/P8	51/100	102.5	99	18.95	1.372	6.875	6.25	26.80	3.75	25.95
P13/P11	26/66	105	97	18.85	1.832	7.375	7.75	32.02	3.9	34.65
P13/P7	26/119	104.5	96.5	18.75	1.7875	7.125	7.125	31.43	3.7	33.35
P9/P18	51/89	99.5	98	18.55	1.4935	6.375	6.25	27.74	3.5	27.5
P9/P2	51/152	99.5	97.5	18.3	1.336	6	6	26.55	3.6	24.5

HD: heading date, PLH: plant height, SPS: spikelets per spike, GYS: grain yield per spike, PSH: pollen shedding, VAE: visual anther extrusion, PM: pollen mass, AL: anther length

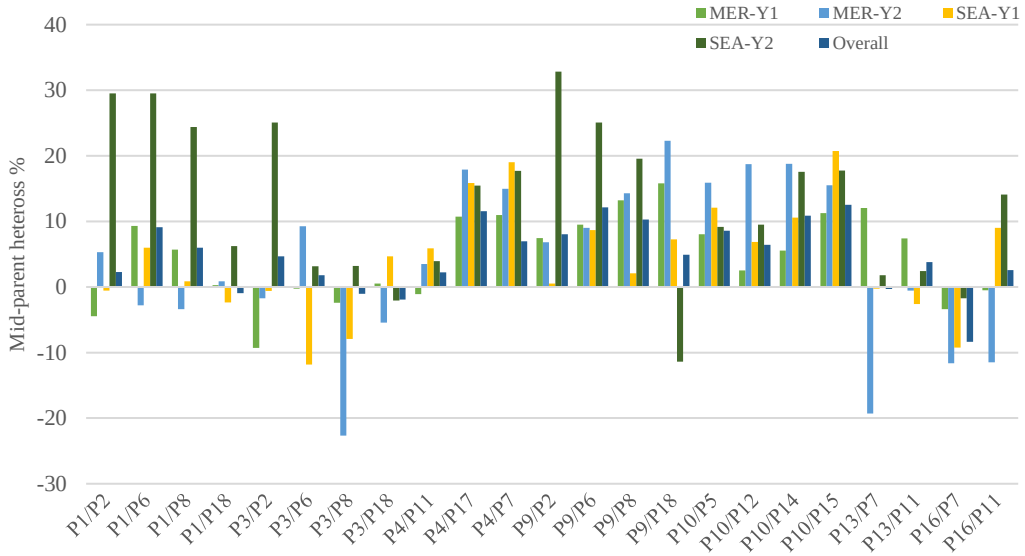


(a)

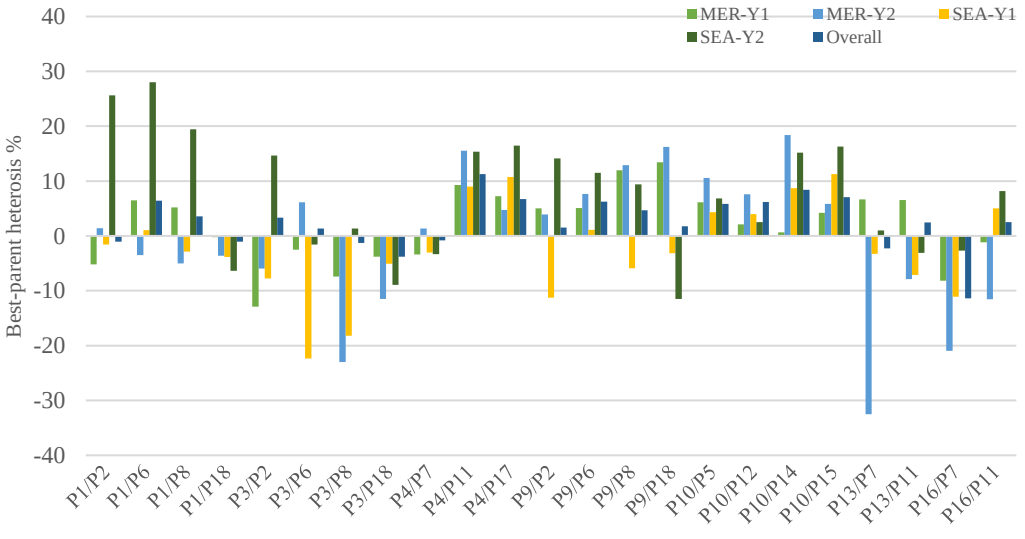


(b)

Annex 10: Mid- (a) and best-parent heterosis (b) for biomass in Merchouch and Sidi El Aydi during 2019 and 2020 seasons



(a)



(b)

Annex 11: Mid- (a) and best-parent heterosis (b) for thousand kernel weight in Merchouch and Sidi El Aydi during 2019 and 2020 seasons