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Fadoua Abdallah

Exploring the Genetic Diversity in the Lathyrus Genus and Identification of Potential Resistance Sources to broomrapes Orobanche spp. and Low Neurotoxin (β-ODAP) Content

JURY

Leila SBABOU	PES, Université Mohammed V, Faculté des Sciences, Rabat.	Président
Leila SBABOU	PES, Université Mohammed V, Faculté des Sciences, Rabat.	Rapporteur/Examinateur
Fatima HENKRAR	PH, Université Mohammed V, Faculté des Sciences, Rabat.	Rapporteur/Examinateur
Mohammed IBRIZ	PES, Université Ibn-Tofaïl, Faculté des Sciences de Kenitra.	Rapporteur/Examinateur
Zakaria KEHEL	EXP, Centre international de Recherche Agricole dans les zones arides, Rabat.	Examinateur
Ahmed AMRI	EXP, Centre international de Recherche Agricole dans les zones arides, Rabat.	Examinateur
Moez AMRI	PES, Université Mohammed VI Polytechnique, Ben Guerir, Marrakesh.	Co-Directeur de thèse
Zine el abidine TRIQUI	PES, Université Mohammed V, Faculté des Sciences, Rabat.	Directeur de Thèse

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Dedication

I humbly dedicate this thesis to:

- *My lovely dad **Ali Abdallah**, without whom I would never have arrived here, this man has always known how to be present for me, at all times and in all circumstances, thus making all the sacrifices, always mindful of me to propel towards the best, nothing in the world is worth the efforts made day and night for my education and my well-being, a very big thank you DAD. This work is the fruit of your pains and all efforts. On this day, I hope to realize one of your dreams.*

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Abstract

Grass pea (*Lathyrus sativus* L.) is considered as a model crop for sustainable agriculture including its nutritional value, resilience to environmental stresses, soil-improving properties, and versatility in food and feed systems. Parasitic weeds and ODAP toxicity threaten the development and the cultivation of grass pea. In this study, several aspects related to the resistance against *Orobanche*, the genetic variability, mineral concentration and ODAP content of grass pea were approached. Two years of field and controlled conditions evaluation showed significant genetic variability and high heritability among crop wild relatives of grass pea for agro-physiological traits related with resistance to *O. crenata* Forsk. in Morocco and *O. foetida* Poir. in Tunisia. Two *L. sativus* accessions such as IG64782 and IG65197 revealed complete resistance to *Orobanche* species. Moreover, accessions from wild species like *L. ochrus*, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius*, and *L. pseudocicera*, originating from different regions; Asia, Cyprus, and Ethiopia, have shown high resistance against broomrape. Some accessions of *Lathyrus* species sucha as *L. sativus*, *L. cicera*, *L. annuus*, *L. hierosolymitanus* and *L. tingitanus*, primarily sourced from Ethiopia and North America, exhibited high levels of parasitism index (PI). Although, a moderately resistant accession, IG116989, which was selected from field, showed high susceptibility in pot experiment. Underground number and weight of emerged *Orobanche* are identified as a criterion for identification and classification of germplasm collection. Through multiple linear regression and correlation analyses, the parasitism index (PI), *Orobanche* incidence (OIN), biological yield (BY), days to flowering (D2F), days to *Orobanche* emergence (D2OE), and *Orobanche* severity (OSV) are classified as the strongest predictors of seed yield (SY). Results reported 25 best-performing accessions of crop wild relatives (CWR) have low *β*- ODAP content and high mineral concentration including *L. ochrus*, *L. cicera* and *L. cassius*, along with one *L. cassius* accession (IG65277), and two *L. sativus* lines (IG117034 and ACC1335) having very low ODAP content. Significant genetic variability and positive correlations was recorded between different studied traits associated to ODAP and mineral level among *Lathyrus* species per, K and P ($r = 0.193^{***}$), K and Fe ($r = 0.177^{***}$), Mn and Fe ($r = 0.210^{***}$), Mn and Se ($r = 0.137^{***}$), ODAP and Mg ($r = 0.158^{**}$), and ODAP and Ca ($r = 0.140^{**}$).

Keywords: Grass pea, *Lathyrus sativus*, Crop Wild Relatives, resistance, *Orobanche*, *β*-ODAP mineral concentration, genetic variability.

Résumé

La gesse (*Lathyrus sativus* L.) est considérée comme une culture modèle pour l'agriculture durable, notamment en raison de sa valeur nutritionnelle, de sa résilience aux stress environnementaux, de ses propriétés d'amélioration des sols et de sa polyvalence dans les systèmes d'alimentation humaine et animale. Dans cette étude, plusieurs aspects liés à la résistance à l'*Orobanche*, à la variabilité génétique, à la concentration en minéraux et à la teneur en ODAP de la gesse ont été abordés. Deux années d'évaluation sur le terrain et dans des conditions contrôlées ont montré une variabilité génétique significative et une héritabilité élevée parmi les espèces sauvages apparentées à la gesse pour les caractères agro-physiologiques liés à la résistance à l'*O. crenata* Forsk. au Maroc et l'*O. foetida* Poir. en Tunisie. Deux accessions de *L. sativus* telles que IG64782 et IG65197 ont révélé une résistance complète aux espèces d'*Orobanche*. De plus, des accessions de cultures sauvages relatives comme *L. ochrus*, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius* et *L. pseudocicera*, originaires de différentes régions ; L'Asie, Chypre et l'Éthiopie ont fait preuve d'une grande résistance à l'*Orobanche*. Certaines accessions d'espèces de *Lathyrus* telles que *L. sativus*, *L. cicera*, *L. annuus*, *L. hierosolymitanus* et *L. tingitanus*, provenant principalement d'Éthiopie et d'Amérique du Nord, présentaient des niveaux élevés d'indice de parasitisme (PI). Cependant, une accession modérément résistante, IG116989, qui a été sélectionnée sur le terrain, a montré une sensibilité élevée lors des expériences en pot. Le nombre et le poids souterrains des *Orobanche* émergés sont identifiés comme critères d'identification et de classification de la collection de matériel génétique. Grâce à de multiples analyses de régression linéaire et de corrélation, l'indice de parasitisme (PI), l'incidence des *Orobanches* (OIN), le rendement biologique (BY), les jours jusqu'à la floraison (D2F), les jours jusqu'à l'émergence des *Orobanches* (D2OE) et la gravité des *Orobanches* (OSV) sont classés comme les meilleurs prédicteurs du rendement en graines (SY). Les résultats ont révélé 25 accessions de plantes sauvages apparentées aux cultures (CWR) les plus performantes ont une faible teneur en *β*-ODAP et une concentration élevée en minéraux, notamment *L. ochrus*, *L. cicera* et *L. cassius*, ainsi qu'une accession de *L. cassius* (IG65277) et deux Lignées de *L. sativus* (IG117034 et ACC1335) ayant une très faible teneur en ODAP. Une variabilité génétique significative et des corrélations positives ont été enregistrées entre les différents caractères étudiés associés à la teneur d'ODAP et la concentration des minéraux parmi les espèces de *Lathyrus* comme, K et P (r =0,193***), K et Fe (r =0,177***), Mn et Fe (r = 0,210***), Mn et Se (r = 0,137***), ODAP et Mg (r = 0,158**), et ODAP et Ca (r = 0,140**).

Mots clés: Gesse, *Lathyrus sativus*, Cultures Sauvages Relatives, résistance, *Orobanche*, *β*-ODAP, concentration minérale, variabilité génétique.

List of abbreviations

AAN: Nitriles aminoacetonitrile.

Ab: *Azospirillum brasiliense*.

AFLP: Amplified Fragment Length Polymorphism.

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

ANFs: Anti-Nutritional Factors.

ANOVA: Analysis of variance.

APG III: Angiosperm Phylogeny Group.

BAPN: b-aminopropionitrile.

BMP: *Bacillus megatherium var Phosphaticum*.

BOAA: β-oxalylaminoalanine (synonym of β-L-ODAP).

BY: Biological yield.

Ca: Calcium.

c**AMP:** Cyclic Adenosine Mono-phosphate.

CBD: The United Nation's Convention on Biological Diversity.

CGIAR: Consortium of International Agricultural Research Centres.

CV: Coefficient of variation.

CWANA: Central and West Asia and North Africa.

D2F: Days to flowering.

D2M: Days to maturity.

D2OE: Days to *Orobanche* emergence.

DABA: L-2,4-diaminobutyric acid.

DAP: α, β-diaminopropionic acid.

DNA: Deoxyribonucleic acid.

ECPGR: European Cooperative Programme for Plant Genetic Resources.

EIAR: Ethiopian Institute of Agricultural Research.

EODW: Emerged *Orobanche* dry weight.

EON: Emerged *Orobanche* number.

EST-SSR: Expressed Sequence Tag-Simple Sequence Repeats.

EURISCO: European Search Catalogue for Plant Genetic Resources.

F: Genotypic effect.

FAO: Food and Agricultural Organization of the United Nations.

FBNYV: Faba bean necrotic yellows virus.

Fe: Iron.

GCDT: Global Crop Diversity Trust.

GLA: ɤ-Linolenic Acid.

GR7: The synthetic strigolactones analogues.

H^2: Heritability.

H_2O_2: Hydrogen Peroxide.

HCA: Hierarchical Cluster Analysis.

HCL: Hydrochloric Acid.

HNO_3: Nitric Acid.

ICARDA: International Center for Agricultural Research in the Dry Areas.

ICP-OES: Inductively Coupled Plasma-Optical Emission Spectroscopy.

INA: National Agronomic Institute of Paris-Grignon.

INRA : Institut National de la Recherche Agronomique.

IPGRI: International Plant Genetic Resources Institute.

ISSR: Inter Simple Sequence Repeat.

ITGC: Technical Institute of Great Crops.

K: Potassium.

KOH: Potassium hydroxide.

LSD: Least significant difference.

MAAN: Methylene aminoacetonitrile.

MANOVA: Multivarite analysis of variance.

MAS: Marker-Assisted Selection.

Mg: Magnesium.

MLR: Multiple linear regression.

Mn: Manganese.

MR: Moderately resistant.

NBS-LRR: Nucleotide-binding site–leucine-rich repeat.

NIST: National Institute of Standards and Technology.

ODAP: *L*-*N*-oxalyl-2,3-diaminopropionic acid, present as α- and, β-isomers.

OIN: *Orobanche* incidence.

OPA: O-phthalaldehyde.

OSV: *Orobanche* severity.

P: Phosphorus.

PCA: Principal component analysis.

PDN: Number of pods.

PGR: Plant Growth Regulators.

ph: Potential hygrogen.

PI: Parasitism index.

PME: Pectin methyl esterase.

PUFA: n-3 Poly-Unsaturated Fatty Acids.

QTL: Quantitative trait locus.

R2: R-squared for a regression.

RAPD: Random Amplified Polymorphic DNA.

RDA: Recommended Dietary Allowance.

RDW: Root dry weight.

RFLP: Restriction Fragment Length Polymorphism.

SDW: Shoot dry weight.

Se: Selenium.

SINGER: System-wide Information Network for Genetic Resources.

SNP: Single Nucleotide Polymorphism.

SRAP: Sequence-Related Amplification Polymorphism.

SSRs: Simple Sequence Repeats.

STS: Sequence Tagged Site.

SXS: Sodium Alkyl Sulfates.

SY: Seed yield.

TAN: Total attachment number.

TI: Trypsin inhibitors.

TIA: Trypsin inhibitor activity.

UK: United Kingdom.

UNCED: UN-Summit on Environment and Development.

UON: Underground *Orobanche* number.

USA: United States of America.

WANA: West Asia and North Africa.

WIEWS: World Information and Early Warning System on Plant Genetic Resources.

Zn: Zinc.

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List of publications

Scientific papers related to this thesis:

- **Abdallah F.**, Kumar S., Amri A., Mentag R., Kehel Z., Mejri R.K., Triqui Z.E.A., Hejjaoui K., Baum M., Amri M., **2020**. Wild *Lathyrus* species as a great source of resistance for introgression into cultivated grass pea (*Lathyrus sativus* L.) against broomrape weeds (*Orobanche crenata* Forsk. and *Orobanche foetida* Poir.). **Crop Science** 61, 263-276.

- **Abdallah F.**, Kehel Z., El Kalchi M.A., Amri A., el Baouchi A., Triqui Z.E.A., Amri M., Kumar S., **2024**. Investigating Genetic Diversity and Correlations Between Mineral Concentration and Neurotoxin (*β*-ODAP) Content in the *Lathyrus* Genus. **Plants** 13, 3202.

- **Abdallah F**., Kehel Z., El Kalchi M.A., Amri A., Triqui Z. E.A., Kumar S., Amri M., **2024**. Genetic variability for resistance to broomrape weeds (*Orobanche crenata* Forsk. and *Orobanche foetida* Poir.) in grass pea (*Lathyrus sativus L*.). (in press).

Other publication:

- **Abdallah F**., Rachidi F., Filali M., Medraoui L., **2024**. Molecular analysis of argan trees "*Argania spinosa* L." by the REMAP markers. (in press).

List of scientific contribution

- **Abdallah F**., El-Haddad N., Z.EA. Triqui., Amri M., Shiv K. Oral communication: « Variation content of ODAP and mineral concentration in *Lathyrus sativus* L. ». 1ere édition des doctoriales du centre Biotechnologies végétale et microbienne, Biodiversité et Environnement. Faculté des Sciences Rabat, 25-26 decembre 2018, Morocco.

- **Abdallah F**., Z.EA. Triqui., Amri A., Amri M., Shiv K. Oral communication: « Micronutrients and neurotoxins analyses of grass pea (*Lathyrus sativus* L.) genotypes ». 1ère édition des journées doctoriales organisées par le Centre de recherche Eau, Resources Naturelles, Environnement et Développement Durable de l'Université Mohammed V de Rabat sous le thème : « Water, Energy and Enivornnement Nexus». Ecole Normale Supérieure de l'Enseignement Technique, 12-14 Juin 2019, Rabat, Morocco.

- **Abdallah F**., Z.EA. Triqui., Amri A., Mentag R., Amri M., Shiv K. Poster: «Best performing accessions in *Lathyrus sativus* resistant to *Orobanche* ». 7ème édition du congrès international (EDE7) sous le thème : « Quel développement Durable pour l'Afrique de Demain ?». Ecole Supérieure de Technologie de Salé, 20-22 November 2019, Morocco.

- **Abdallah F**., El-Haddad N., Z.EA. Triqui., Amri M., Shiv K. Poster: « Nutritional quality and neurotoxins content variations in grass pea genotypes». International Congress-VARENA 2019- Valorization of Natural Resources from Scientific Research to Socio-Economic Feasibility, Faculty of Sciences Rabat, 26th-28th June 2019, Morocco.

- **Abdallah F**., Hejjaoui K., Z.EA. Triqui., Amri A., Amri M., Shiv K. Poster: « Resistance to broomrapes in *Lathyrus sativus* L. accessions». 7th International Food Legumes Research Conference held in Marrakesh, 6-8 May 2018, Morocco.

- **Abdallah F**., EL-Kalchi A., Amri A., Zehel Z., Z.EA. Triqui, Shi K., Amri M. Poster: « Correlation, Heritability and genetic variability among different *Lathyrus* accessions». 3rd Africa Congress on Conservation Agriculture with the theme: « Building a Resilient Future in Africa through Conservation Agriculture and Sustainable Mechanization». National Research Institute for Agriculture, Food and Environment (INRA), Rabat, 5-8 June 2023, Morocco.

- **Abdallah F**., Kehel Z., Z.EA. Triqui., Amri A., Amri M., Shiv K. Poster: « Neurotoxins (*β*-ODAP) and Macro-Micronutrients Variation in Grass Pea (*Lathyrus sativus* L.) genotypes». African Plant Breeders Association (APBA) conference. University Mohammed VI Polytechnic, Ben Guerir, Marrakesh, 23-26 October 2023, Morocco.

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General introduction

General introduction

Exploited by local populations, but benefiting from little attention from decision-makers and researchers, neglected or underutilized crops present considerable potential to increase their production and ensure the food security of populations.

According to the FAO (2011), neglected and underutilized species have been abandoned by those who need them most. Among the 30,000 species of edible plants, only 30 are used to feed people around the world.

In the human diet, legumes and cereals constitute two complementary sources of protein. Graham and Vance (2003) estimate that legumes provide about a third of human dietary protein. For livestock, fodder legumes represent food source rich in protein, fiber and energy.

The cultivation of food legumes dates back 6000 years. These latter appear in the crop rotation systems of the early Egyptian dynasties and later in the Roman period (FAO, 1955).

In the world, these species are consumed mainly in developing countries, which absorb around 90% of world production intended for human food. In many poor countries, legumes provide about 10% of the proteins and 5% of the energy that the population needs (FAO, 2006).

Annual grain and forage legumes such as faba bean (*Vicia faba* L.), vetches (*Vicia* spp.), lentil (*Lens culinaris* Medik.), pea (*Pisum sativum* L.), grass pea (*Lathyrus sativus* L.) and chickling vetch (*Lathyrus cicera* L.) are important crops grown worldwide as a source of protein both for human food and animal feed.

Legume cultivation is hampered in Mediterranean regions by the occurrence of the root parasitic weeds *Orobanche crenata* Forsk. (crenate broomrape) and *Orobanche foetida* Poir. (foetida broomrape) causing important yield losses (Rubiales *et al*., 2006; Joel *et al*., 2007; Parker, 2009; Rubiales, 2014; Cartry *et al*., 2021).

O. crenata has threatened the legume cultivation in the Mediterranean Basin and Middle East crops since antiquity. In Morocco, *O. crenata* infestation was officially reported on faba bean in 1943 in the Fez region. Since then, the parasite has spread to other areas (Zair, Pre-Rif, Chaouia, Sais, Fez, Meknes, Taounate, Sidi Kacem, Chefchaouen, Doukala, and Abda) (Labrada, 2007; FAO, 2014). The estimated average yield losses due to parasitism range from seven to 90%, depending on host

plants, environmental factors, and soil infestation level (Sauerborn, 1991; Torres *et al*., 2006; Ennami *et al*., 2017).

On the contrary, *O. foetida* has been reported to damage faba bean in Tunisia only (Kharrat *et al.*, 1992, Rubiales *et al*., 2005). Kharrat and Halila (1994) had reported that *O. foetida* Poir. is located in the Beja region in the North West. The total areas infested in Tunisia have been estimated to vary in extent from 5000 to 10000 ha and it was indicated that in highly infested areas, farmers generally avoid growing faba bean or other susceptible crops, resulting in substantial reductions to both the extent of cultivated areas and to food legume production (Kharrat and Souissi, 2004). However, it is common in native habitats in the western Mediterranean (Vaz Patto *et al.,* 2008) and has recently been found also in Morocco infecting common vetch (*Vicia sativa* L.) (Rubiales *et al.,* 2005).

Strategies of control have been developed but only marginal successes have been achieved. Most control methods are unfeasible, uneconomical, andhard to achieve or result in incomplete protection. Breeding for resistance is possible, but is hampered by thelack of sufficient levels of resistance, the complexity of its inheritance and the unreliability of available screen-ing methods. Recent achievements in the identification of resistance levels and their deployment in breedingprogrammes will be presented and critically discussed (Rubiales, 2014).

Currently, grass pea (*Lathyrus sativus* L.) is considered a model crop for sustainable agriculture (Vaz Patto *et al*., 2006; Arslan, 2016). *Lathyrus* seeds are nutritious, containing up to 31% protein and 65% carbohydrates and are a good source of minerals (Girma *et al*., 2004), they are cooked like chickpeas. Notwithstanding such virtues, its cultivation has been banned due to the association of its consumption with neurolathyrism, a non-reversible neurological disorder in humans and animals induced by *β*-ODAP [also known as b-*N*-oxalyl-amino-*L*-alanine (BOAA)] (Lambein and Kuo, 2009).

One of the main objectives of genetic improvement of pea is therefore the reduction of ODAP levels present in the seeds. In fact, from 1999, ICARDA was able to develop low-toxic *L. sativus* lines, which contained only a content ranging from 0.07 to 0.02% of ODAP. These lines were obtained by crossing them with low toxicity varieties (Abd El-Moneim, 1999). Several varieties and lines have been developed by combining low *β*-ODAP (<0.1%) with high-yield potential (up

to 1.5 tons/ha) and resistance to an array of biotic and abiotic stresses (Kumar *et al*., 2013; Dixit *et al*., 2016; Sen Gupta *et al*., 2021).

Despite these challenges, abandoning grasspea as a human food source is unlikely given its importance as a safety net for poor subsistence farmers in some of the world's poorest areas. Instead, efforts are being made to breed cultivars with low neurotoxin levels through international collaborations between organizations like ICARDA and Ethiopian breeders.

Grass pea, together with other 'minor' legumes (chickpea, faba bean) has been included in the list of crops selected by the FAO International Treaty to be exchanged and studied among the member countries in order to increase the understanding of plant genetic resources for food and agriculture (List of Crops Covered Under the Multilateral System, 2009).

In fact, given their adaptation, species of the *Lathyrus* genus could play an important role in the resorption of fallow but also in the improvement of fodder production. To promote this culture, a strategic action plan is therefore essential to promote it because it contains numerous nutritional, economic and environmental benefits.

L. sativus is therefore a potential crop, which seems relevant in our current context of global warming, and which deserves attention. For more rational valorization of these rustic plants well adapted to environmental conditions, this study was carried out with the following objectives:

- To identify the source of resistance to *O. crenata* in Morocco and *O. foetida* in Tunisia under field and controlled conditions during two cropping seasons in *Lathyrus* spp. species.
- To explore the genetic variability and the relationships between differents field and pot studied traits.
- To select the best performing accessions with low ODAP content and high mineral concentration among 183 accessions of cultivated and crop wild relatives of *Lathyrus* species.
- To assess the genotypic variance, heritability and correlations between nutritional traits and *β*-ODAP content in *Lathyrus sativus* germplasm collection.

Our thesis is organized in chapters:

- ✓ The first chapter is a bibliographical summary relating to the genus *Lathyrus and Orobanche*, their origin, their situation and their importance.

- ✓ The second chapter contained the evaluation of 285 accessions of *Lathyrus* spp. originating from different regions under open field and controlled conditions during 2017-2018 cropping season to determine the resistance against *Orobanche crenata* Forsk. in Morocco at Marchouch station and *Orobanche foetida* Poir. In Tunisia at Oued-Beja station.
- ✓ The third chapter consisted in analyzing the genetic variability, heritability and correlations between different traits among 285 accessions of cultivated and crop wild relatives (CWR) species under field and pot experiment for resistance to both *O. crenata* and *O. foetida*.
- ✓ The fourth chapter included the analyse for *β*-ODAP content and mineral concentration in 183 germplasm accessions representing 13 different *Lathyrus* species and 11 *L. sativus* breeding lines and to assess the genetic diversity and the correlations among nutritional caracters and *β*-ODAP content.
- ✓ The general conclusion and perspectives will be developed at the end of this study.

Chapter I: Literature review

1. The genus *Lathyrus* L.

The scientific name *Lathyrus* comes from the Greek work "pea". Common names of *Lathyrus sativus* are khesari in Bengladesh and India, Sabberi in Ethiopia, Grass pea in United states, gesse blanche or commune in France, Cicerchia coltivata in Italy (Campbell, 1997).

Lathyrus sativus is an herbaceous, annual plant, straggling or climbing, with a dense branching and taproot system. The stems are angular or winged. They are 0.6-9.0 m tall and the leaves are pinnately compound with usually two leaflets (linear-lanceolate 25-150 mm long, 3-9 mm broad) (Kay, 1979; Mannetje, 1980; Duke, 1981).

Plant height in *L. sativus* varies greatly and in India it ranges from 15 up to 68 cm, while in Canada from 24.5 to 172 cm. This plant trait is conditioned, to a high degree, by rainfall (Campbell, 1997; Grela *et al*., 2010).

The genus *Lathyrus* is characterized by paripinnate leaves, i.e. composed of leaflets arranged in two opposite rows in an even number (i.e. without terminal leaflet) and provided with halberd-shaped stipules. The leaves are reduced to a branching tendril in *Lathyrus aphaca*, replaced by 2 large oval and acute stipules imitating 2 opposite leaves. The terminal tendril is sometimes absent in *Lathyrus linifolius* and *Lathyrus palustris* with almost no stipules. Parallel veined phyllodes are shown in *Lathyrus nissolia*, with almost no stipules.

Lathyrus exhibits a typical bee-pollinated papilionoid flower. Flowers are solitary, axillary and are borne on peduncles 30-60 mm long; corolla 12-24 mm long and are reddish-purple, pink, red, yellow, orange, blue or white (Kay, 1979; Asmussen and Liston, 1998).

The fruit of leguminous species develops into a pod. They are oblong, 2.5-4.0 cm long, flat and slightly curved and each pod has 3-5 seeds that are 4-7 mm in diameter, angled and wedge-shaped. The color is white, grayish-brown or yellowish and usually spotted or mottled (Campbell, 1997; Duke, 1981). By Desphande and Campbell 1992, a highly correlation have been revealed in *Lathyrus sativus* between the flower and seed color; the colorful flowers produce a colorful seed while the white flowers produce a creamy yellow seeds.

Figure 1: Grass pea cultivation in Morocco, in the field at Marchouch station (a, b, c), in greenhouse (d) and different grass pea flowers species (e, f, i, j, k) (Abdallah F., 2018).

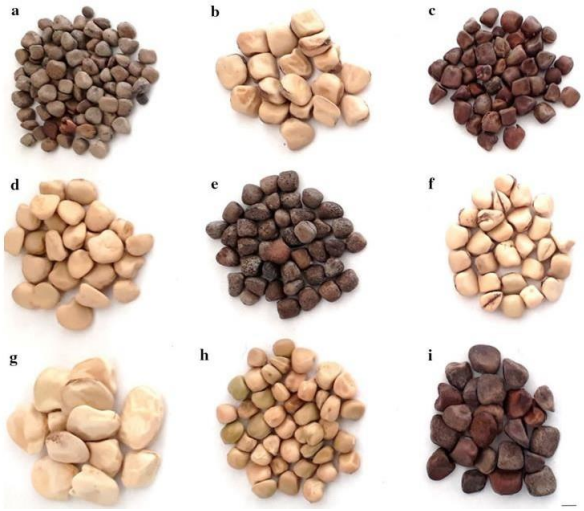


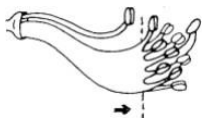
Figure 2 : Diversity of *Lathyrus sativus* seed varieties. Broadly, grass pea germplasms can be divided into two groups: The Asian and Mediterranean groups with small and large seeds and on average a high and low β-ODAP content, respectively, Bangladesh (a), China (b), Ethiopia (c), Canada (d), India (e), Nepal (f), Portugal (g), Poland (h), China (i) (scale: 5 mm) (Lambein *et al*., 2019).

In the Mediterranean basin, grass pea is used as food (*Lathyrus clymenum*, *Lathyrus cicera*). Another species like *Lathyrus tuberosus* provide edible tubers. *Lathyrus tingitanus* is planted in green fodder.

Grass pea is also ornamental plants. *Lathyrus odoratus* (pois de senteur) is the best known species. *Lathyrus latifolius* 'Pink Pearl' or 'White Pearl' and *Lathyrus maritimus* will fit perfectly into a romantic or seaside garden.

The genus *Lathyrus* slightly larger than the sister genus *Vicia*, is easier to be identified, it has more clear vegetative characters than *Vicia* (Kupicha, 1983). It has an interesting floral variation similar to that of *Vicia*. The extensive use of characters has led to a great improvement in the infra-generic classification of *Lathyrus*. But, there is still a need to study this genus in more details to be well known.

Table 1: Difference between some *Lathyrus* and *Vicia* characters (Leurquin J, 2004).

Discriminating characters	G.*Vicia*	G. *Lathyrus*
Stems	rounded or angular, never winged	angular or winged, never rounded
Leaves (paripinnate compound)	+ more than 3 pairs of leaflets (except in *V. faba*, *V. bithynica*, *V. narbonensis*: cultivated or weedy species)  + veins pinnate	+ 0 to 1 pair of leaflets (except in *L. linifolius* and very rare or weedy species: *L. vernus*, *L. niger*, *L. pannonicus*, *L. palustris*, *L. ochrus*, *L. clymenum*)  + veins parallel
Stipules	smaller than leaflets, nectariferous glands in some species	variable, without nectariferous glands
Flowers	+ corolla with wings united to the keel + staminal tube truncates obliquely at the top  + filiform style pubescent in its upper part, not twisted	+ corolla with wings not united to the keel + truncate staminal tube perpendicular to base  + flattened style, provided with a line hair on the inner side, often twisted

2. Origin and classification of *Lathyrus*:

The genus *Lathyrus* belongs to the family Leguminosae (=Fabaceae), subfamily Papilionoideae, tribe Vicieae (Campbell, 1997). The origin of *L. sativus* was unknown it was thought that the natural distribution has been obscured by plants appears as a weed among the other crops and growing in the wild as escapees from cultivation (Jackson and Yunus, 1984; Smartt, 1984).

However, it would seem that its presumed center of origin is Central Asia (Smartt, 1990). Vavilov (1951) described two separate centers, one was the Central Asiatic and the second was the Abissiyian Center. Moreover, this author, Jackson and Yunus (1984), have noted a certain diversity in seeds forms. The small seeds forms were found in southern and southwest Asia, whereas around the Mediterranean region, almost all were highly cultivated forms with large white seeds and flowers.

Based on archeobotanical and phytogeographical evidence, the origin of cultivation of *L. sativus* has been suggested to be the Balkan peninsula (Kislev, 1989). Grass pea is extensively cultivated in Central, South and Eastern Europe (Germany, Portugal, Spain, Russia), in Crete, Rhodes, Cyprus, in West Asia, North Africa (Palestine, Syria, Lebanon, Iraq, Afghanistan, Algeria and Morocco) (Campbell, 1997) and Ethiopia (Butler *et al*., 1999). Also, it is found in Eurasia, North America, temperate South America and East Africa (Smartt, 1990).

The species *L. sativus* is likely derived from its closed wild relative *L. cicera* (red pea) (Belaid *et al.*, 2006). Fossilized seeds of *L. sativus* and allied species (*L. cicera* and *L. ochrus*) have been found in several Neolithic sites in the fertile crescent, Eastern Europe and the Mediterranean, dated to earlier than 6000 BCE (Kislev, 1989; Marinova, 2007; Coward *et al*., 2008; Peña-Chacarro *et al*., 2013). It has been reported by several authors that *L. sativus* and *L. cicera* represented similar characters (Jackson and Yunus, 1984; Croft *et al.*, 1999; Chtourou-Ghorbel *et al.*, 2001). According to these authors, this adaptability facilitated the dispersal of plants outside the American continent, Madagascar, South Est Asia and in many Pacific islands.

These regions represent an important centers of secondary diversity. *Lathyrus* species classification is based on seed color and size, flower color and pod spotting. *Lathyrus sativus* showed an enormous variation in morphological characters, such as leaf length. On the other hand,

there are fewer variations for the floral characteristics (Jackson and Yunus, 1984). These authors have also revealed the geographical distribution of certain species; the blue-flowered varieties all came from South-West and South Asia, and Ethiopia, whereas the white and mixed colored varieties had a more western distribution, from the Canary Isles to the western republics of the Soviet Union.

Grass pea lines can be divided into two large groups; the Indian subcontinent group and the Mediterranean group characterized by a higher potential yield (Hanbury *et al.*, 1999).

Generally, annual species are derived from perennial species and the autogamous species from allogamous species (Jackson and Yunus, 1984). A study carried out on two perennial species (*L. nervosus* and *L. pubescents*) and two annual species (*L. crassiers* and *L. paramnesies*) shows that the evolution of *Lathyrus* studied species was followed by a decreasing in the size of chromosomes and that *L. crassiers* is a derived species. This interpretation is based on the chromosomes length, cycle life and mode of reproduction of studied species (Klimt and Schifonno-wittman, 2000).

Cytogenetic studies have shown that the genus *Lathyrus* is diploid dominance (2n = 2x = 14) (Smartt *et al.*, 1994). Two species *L. pratensis* and *L. venosus* are tetraploid with (2n = 28) chromosomes and one specie *L. palustris* is hexaploid with (2n = 42). Another's species have been studied cytologically and have been shown to be autopolyploids (Campbell *et al.*, 1994).
Total chromosome length in perennial *Lathyrus* species is generally greater than for annual species (Rees and Hazarika, 1969; Yamamoto *et al.*, 1984). A similar difference has also been found in *Vicia*, a closely related genus (Rees *et al.*, 1966).

The Taxonomic studies done by Smartt *et al*., (1994), have shown that only these crosses *L. cicera* x *L. sativus* and *L. amphicarpus* x *L. sativus* produce the variables hybrids.

Lathyrus sativus is a self-pollinating culture (Ben Brahim *et al*., 2002), however, Smartt (1984) reports that the cross pollination is higher than the truly self-pollinated species. The frequency of outcrossing has been shown a varying amounts depending on the flower's color as high as 27,8% in Bangladesh (Rahman *et al*., 1995). *L. cicera* and *L. sativus* have similar growth habit and are

very closely related (Kupicha, 1983; Jackson and Yunus, 1984). Hanruby *et al*., (1994) suggested that *L. sativus* is considered to be descended from *L. cicera* and the cross pollination level of *L. cicera* would be similar for *L. sativus* because their similarities in floral biology.

The total number of *Lathyrus* species is 189 species and sub-species according to Allkin *et al*., (1985), approximately 150 species according to Kupicha (1983) and 187 (Lewis *et al*., 2005; Kenicer, 2008) are from different origins. About 52 are from Europe, 30 from North America, 78 from Asia, 24 from Tropical Africa and 24 from temperate regions of South America (Asmussen and Liston, 1998). Of these only a small number are cultivated. Jackson and Yunus (1984) and Campbell (1997) report that about 56 species are distinguished in India alone (45 *L. cyaneus*, 10 *L. roseus*, 1 *L. albus*).

The classification of *Lathyrus* has been modified over the years before the classification of Gordon (1848) when the botanists had accepted the two Linnaean genera *Lathyrus* and *Orobus*, which were separated based on different criteria by different authors. Gordon united the two into *Lathyrus* and recognized six sections within *Lathyrus*: *Eulathyrus*, *Cicerula*, *Clymenum*, *Nissolia*, *Aphaca*, and *Orobus* (Kupicha, 1983). The six important studies that resulted in generic classifications are reported in Table 2.

According Asmussen and Liston (1998), the genus *Lathyrus* has been devised in 13 sections (Figure 3). Phylogenetic molecular studies have also published by Asmussen and Liston (1998), Croft *et al*., (1999), Chtourou-Ghorbel *et al*., (2001), Badr et *al*., (2002) and Ben Brahim *et al*., (2002). All these studies are geographical or taxonomic limited scope, except that Asmussen and Liston; they focused on Mediterranean taxa, especially on the *Lathyrus* section, containing an important economically and ecologically species (Kenicer *et al*., 2005).

The largest molecular study was carried out by Asmussen and Liston (1998), which is based on RFLP markers (Kenicer *et al*., 2005). Forty-two *Lathyrus* species were examined come from Eurasia and another's species from the new world and suggest merge certain groups; monotypic sections *Orobon* and *Orobastrum* with *Lathyrus* section and *Notolathyrus* South-America section with *holarctic* section.

Table 2: Taxonomic studies of genus *Lathyrus* L. (Kenicer *et al.*, 2005).

Gordon (1848)	Boissier (1872)	Bassler (1966)	Davis (1970)	Czefranova (1971)	Kupicha (1983)	Dogan, Kence and Tigin (1992)	Asmussen & Liston (1998)	Kenicer et. al,(2002)
					Notolathyrus		*Orobus*	*Notolathyrus*
Orobus	*Orobus*	*Orobus*	*Orobus*	*Lathyrobus*	*Orobus*	*Orobus*		*Orobus*
	Orobastrum	*Lathyrostylis*	*Lathyrostylis*	*Orobus*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*	*Lathyrostylis*
		Pratensis	*Pratensis*	*Pratensis*	*Pratensis*		*Pratensis*	*Pratensis*
		Eurytrichon		*Eurytrichon*				
Aphaca	*Aphaca*		*Aphaca*	*Aphaca*	*Aphaca*	*Aphaca*	*Aphaca*	*Aphaca*
Orobus	*Orobastrum*	*Neurolobus*	*Neurolobus*	*Neurolobus*	*Neurolobus*		*Neurolobus*	*Neurolobus*
		Orobon	*Orobon*	*Orobon*	*Orobon*	*Orobon*	*Lathyrus*	
Eulathyrus	*Eulathyrus*		*Lathyrus*	*Lathyrus*	*Lathyrus*			*Lathyrus*
						Lathyrus		
Cicerula	*Cicerula*		*Cicerula*	*Cicerula*		*Gorgonia*		
						Cicerula	*Cicerula*	
Orobus	*Orobastrum*		*Orobastrum*	*Orobastrum*	*Orobastrum*	*Clymenum*	*Orobastrum*	
					Linearicarpus		*L. sphaericus*	*L. sphaericus*
							L. angulatus	*L. angulatus*
					Viciopsis			
Nissolia	*Nissolia*		*Nissolia*	*Nissolia*	*Nissolia*	*Nissolia*	*Nissolia*	*Nissolia*
Clymenum	*Clymenum*		*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*	*Clymenum*
							L. gloeospermus	

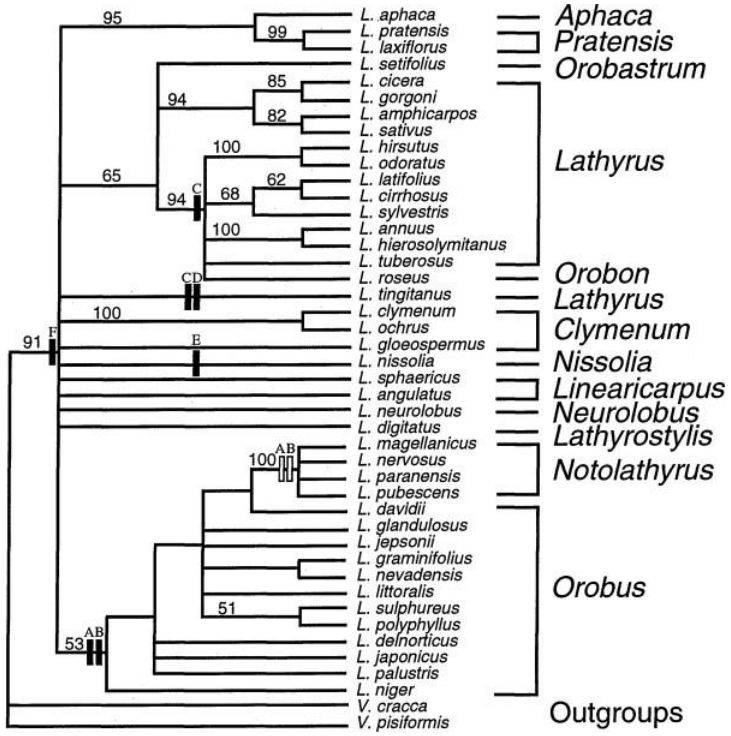


Figure 3 : Sectional classification of the genus *Lathyrus* (Asmussen and Liston, 1998).

Lathyrus is well represented in the New World by two separate endemic groups in North and South America (Kupicha, 1983). They have been included in an infrageneric classification, but both groups have been revised on a regional basis (Burkart, 1935, 1942; Hitchcock, 1952) and their vegetative and floral characters are described in recent surveys (Simola, 1968; Gunn and Kluve, 1976). Table 3 summarized the classification and geographic distribution of *Lathyrus* (Kupicha, 1983).

Table 3 : Geographic distribution's species of genus *Lathyrus* (Kupicha, 1983).

Section	Species	Geographical distribution
***Aphaca* (J. Mill.) Dumort.**	2 species (incl. *L. aphaca*)	Europe, W. and C. Asia and N. Africa
***Clymenum* (J. Mill.) DC. ex Ser.**	3 species (incl. *L. clymenum*, *L. ochrus*)	Mediterranean
***Lathyrostylis* (Griseb.) Bassler**	20 species	C. and S. Europe, W. Asia and N.W. Africa
***Lathyrus* L.**	33 species (incl. *L. annuus*, *L. blepharicarpus*, *L. cicera*, *L. gorgoni*, *L. hirsutus*, *L. latifolius*, *L. odoratus*, *L. rotundifolius*, *L. sativus*, *L. sylvestris*, *L. tingitanus*, *L. tuberosus*)	Europe, Canaries, W. and C. Asia and N. Africa
***Linearicarpus* Kupicha**	7 species	Europe, W. Asia and N. and E. Africa
***Neurolobus* Bassler**	1 species	W. Crete
***Nissolia* (J. Mill.) Dumort.**	1 species	Europe, W. Asia and N.W. Africa
***Notolathyrus* Kupicha**	23 species	Temperate S. America and S.E. USA
***Orobastrum* Boiss.**	1 species	Mediterranean, Crimea and Caucasia
***Orobon* Tamamsch.**	1 species	Anatolia, Caucasia, Crimea and Iran
***Orobus* (L.) Godr.**	54 species	Europe, W. and E. Asia, N.W. Africa and N and C.
***Pratensis* Bassler**	6 species (incl. *L. pratensis*)	Europe, W. and C. Asia and N.W. and N. E. Africa
***Viciopsis* Kupicha**	1 species	S. Europe, E. Anatolia and N. Africa

3. Crop species of *Lathyrus*:

Three species *L. sativus*, *L. cicera*, and *L. ochrus* are grown and used for human consumption and to a lesser extent *L. clymenum* and *L. tuberosus* but for its edible tubers rather than its seed.

Several *Lathyrus* species have potential commercial importance, especially for their ornamental value or as forages and feed, including:

- *L. aureus* (Golden Pea)
- *L. annuus* (Red Fodder Pea)
- *L. japonicus* (Sea Pea)
- *L. latifolius* (Everlasting Pea)
- *L. linifolius* (Bitter Vetch)
- *L. nervosus* (Lord Anson's Blue Pea)
- *L. nissolia* (Grass Vetchling)
- *L. odoratus* (Sweet Pea)
- *L. pratensis* (Meadow Vetchling)
- *L. sphaericus* (Spring Vetchling)
- *L. sylvestris* (Flat Pea-vine)
- *L. tingitanus* (Tangier Pea) (Shehadeh, 2011).

Another species *L. amphycarpus* is currently observed in the Middle East and has a great potential as a self-seeding fodder plant (Campbell, 1997).

Table 4 provides a list of all cultivars, landraces as well as the wild species of genus *Lathyrus*, known to be historically or currently cultivated for agriculture and horticulture (Rizvi *et al.*, 2016).

The annual grass pea, *L. sativus*, is a dual purpose crop cultivated as a winter pulse, and grown both as stock feed (Hanbury, 2000) and for human consumption (Muehlbauer and Tullu, 1997). It can be consumed uncooked as a green snack, like chickpea, cooked in a stew, milled into flour or by roasting the seed (Peña-Chocarro and Peña, 1999). Present importance of *L. sativus* world cultivation is summarized in Table 5 (Vaz Patto *et al.*, 2006).

Table 4 : Historic or currently cultivated *Lathyrus* species (Rizvi *et al.*, 2016).

Species	Use	Status of uses	Location
L. annuus	Pulse, fodder	Rare	Europe, N. America
L. aphaca	Fodder	Rare	India
L. blepharicarpus	Pulse	Historic	Near East
L. cicera	Pulse, fodder	Rare	S. Europe, N. Africa
L. clymenum	Pulse	Rare	Greece
L. gorgoni	Fodder	Historic	Middle East
L. hirsutus	Forage	Common	USA
L. latifolius	Horticulture	Common	Europe
L. ochrus	Pulse, fodder	Rare	Greece, Middle East
L. odoratus	Horticulture	Common	Widespread
L. pratensis	Forage	Rare	S. Europe, N. Africa
L. rotundifolius	Horticulture	Common	Widespread
L. sativus	Pulse, forage	Common	Widespread
L. sylvestris	Forage	Rare	S. Europe, N. Africa
L. tingitanus	Fodder	Rare	N. Africa
L. tuberosus	Tubers	Rare	W. Asia

Table 5: Current importance of *Lathyrus sativus* world cultivation (Vaz Patto *et al.*, 2006).

Countries where *L. sativus* is currently an important crop	
Bangladesh	Campbell *et al.* (1994) ; Rahman *et al.* (2001)
China	Campbell *et al.* (1994); Zhou and Arora (1995)
Ethiopia	Campbell (1997); Tadesse and Bekele (2003a)
India	Campbell *et al.* (1994) ; Pandey *et al.* (1995), Sarkar *et al.* (2003)
Nepal	Campbell *et al.* (1994) ; Neupane (1995)
Pakistan	Campbell *et al.* (1994) ; Haqqani and Arshad (1995)
Countries where *L. sativus* is grown to a lesser extent	
Australia	Hanbury *et al.* (1999)
Europe (Italy, France, Poland, Portugal...)	Campbell *et al.* (1994); Tavoletti and Capitani (2000); De la Rosa and Martìn (2001); Milczak *et al.* (2001)
North Africa (Egypt, Morocco, Algeria...)	Campbell (1997)
South America (Chile, Brazil...)	Campbell *et al.* (1994); Mera *et al.* (2000)
West Asia (Syria, Lebanon, Iraq, Afghanistan...)	Campbell *et al.* (1994); Campbell (1997)

The most consumption of *L. sativus* is observed in Asia and in the Middle East when the whole seed is used in soups and ground to make unleavened bread (Tsegaye *et al.*, 2007).

In India, the grains are sometimes boiled but they are most often processed to obtain the split dhal (soupe). In Canada, the seed are used for cattle feed (Tsegaye *et al.*, 2007).

In Ethiopia, grass pea is utilized as boiled (nifro) or roasted (kolo) whole seed, shiro (powdered seeds). Shiro is used in the preparation of shiro wott, a traditional Ethiopian sauce. Grass pea flour increasingly is being used to adulterate legume flours such as chickpea or dry peas (Urga *et al.*, 2005). Pancake like unleavened bread-enjera, made from teff, wheat, barley, maize or sorghum are eaten with Wott (Deshpande and Campbell, 1992).

In China, the crop is used as animal feed and a supplement in food processing in Shaanxi and Gansu province. The farmers like this crop because of its high yield potential (20% higher than pea) and wide adaptability (Zhou and Arora, 1996).

In Turkey, among all *Lathyrus* species, *L. sativus* appears to be a highest producer of biomass and grain. Therefore, the species is ideal for feeding the cattle and is preferred by the farmers (Kumar, 1997).

4. Areas and production:

4.1 In the world:

4.1.1 Economic importance:

Globally, the area under grass pea cultivation is estimated at 1.50 million ha, with annual production of 1.20 million tons (Kumar *et al*., 2011).

The area devoted to grass pea cultivation varies in different parts of the world. In Ethiopia, the area sown to grass pea is about 9% of the total area used for grain legume production up to 1999 (Teklehaimanot *et al.*, 1993; Butler *et al.*, 1999) and may have increased more recently (Patto *et al*., 2006). In Bangladesh, Malek *et al*., (1996) estimated that this crop, during the late 1980s and early 1990s, occupied 36% of the total area under grain legumes.

Grass pea grain yields are variable depending on variety, ultimate crop use and cropping system. In Ethiopia, where the crop is grown as a food crop, under favorable conditions, *L. sativus* grain yields for local cultivars were 3.7 t ha^{-1} and for improved cultivars, 4.2-5.2 t ha^{-1} (Tsegaye *et al*., 2005). A similar seed yield was also reported for a new large-seeded cultivar Luanco-INIA in Chile (Mera *et al.*, 2003). This yield was still noted in 2000 (Rahman *et al.*, 2001). Grain yield of 17 lines of *L. sativus* from ICARDA grown in three agro-climatically different sites in the Mediterranean-type environments of southern Australia ranged from 0.72 to 1.56 t ha^{-1} at Northam, 0.24 et 0.68 t ha^{-1} at Merredin, and 0.24 to 0.75 t ha^{-1} at Mullewa (Siddique *et al.*, 1996). Yield of improved grass pea lines observed at Tel Hadaya, Aleppo in Syria, ranged from 0.8 to 1.6 t ha^{-1} (Kumar *et al.*, 2010). These authors also showed a high variation in seed size of improved grass pea varieties ranging from 0.06 g per seed for variety Bari in Bangladesh to 0.19 per seed for variety Krab in Poland. Interestingly, the higher seed size of 0.20 g per seed was found from plants that had experienced sever water deficit (Herwig, 2001).

A high yield of fodder of 7-10 t ha^{-1} was reported for *L. sativus* when intercropped with maize (Campbell, 1997). A six-year study on legumes as green manure in dryland cropping systems found that *L. sativus* yielded 2.2 t ha^{-1} vegetative growth and concluded that this crop was one of the most suited to green manure in semi-arid climates (Biederbeck *et al.*, 1993).

In some countries (Poland, Italy, Spain and Hongaria), grass pea was not completely abandoned and still cultivated on small farms which continued to produce it on a small scale for family consumption and local market (Hammer *et al*., 1999; Tavoletti and Capitani, 2000). Generally, grass pea is overlooked and underused (Campbell, 1997).

In 1996, the grass pea area in Spain was 273 ha, with 174 t grain production and 52 t of hay (De la Rosa and Martin, 2001). In this country, the seeds sown are local cultivars. In Turkey, it's a marginal culture and cultivated as forage (Basaran *et al*., 2013) over an area of approximatively 24300 ha (1270 ha for seed production and 23030 ha for forage production) (Tuik, 2014). Seed yield varies from 1142,4 kg/ha to 2046,4 kg/ha and an average yield of 1521,1 kg/ha in forage (Basaran *et al.*, 2016). A study of landraces from the south of France grown in Serbia showed that the average seed yield per plant for three years varied from 4.37 to 7.20 per plant, thus confirming

that local varieties of grass pea, accompanied by appropriate cultural practices and a plant density of 1 000000 ha^{-1}, can produce high grain yields (Mikic´ *et al*., 2010).

L. cicera and *L. odoratus* species are economically important. *L. cicera* has been cultivated since antiquity and was domesticated in southern France and the Iberian Peninsula soon after the introduction of agriculture in the region (kislev, 1989). It is used for animal feed (White *et al*., 2002). *L. odoratus* originated from South Italy, is valuated as ornamental plants and economically important and cultivated for its flowers and garden decoration.

Species such as; *L. belinensis, L. chloranthus, L. vernus, L. tingitanus, L. grandiflorus, L. latifolius, L. rotundifolius* and *L. sativus* can also be used for ornamental purposes (Parsons, 2009). Other species are important for human consumption only in certain countries, such as *L. clymenum* and *L. ochrus* in regions of Greece, Italy, Chypre and Turkey (Sarpaki and Jones, 1990; Jones, 1992).

4.1.2. Agronomic importance:

More than 100 million people in drought-prone areas of Asia and Africa consider grass pea as a traditional popular crop, because of its easy cultivation, its relative resistance to drought, flood, moderate salinity and insect attack, and its good yield of tasty seeds (Rutter and Percy, 1994; Abd-El Moneim *et al*., 1999).

The incorporation of grass pea in the rotation can make the production system more sustainable by improving soil fertility and breaking disease and pest cycles (Abd-El Moneim *et al*., 2000).

Production systems that include grass pea vary in different parts of the world. In Bangladesh, grass pea is grown as a relay crop with rice. Grass pea seeds are simply broadcast into standing rice one or two weeks before harvesting. *Lathyrus* yields were higher when seeds were sown under minimal tillage after rice harvest. Application of N: P: K at 80: 60: 30 $kg\ ha^{-1}$ to rice and N of 10 $kg\ ha^{-1}$ to *Lathyrus* at planting resulted in the greatest yields for both rice and *Lathyrus*. Leaving 20 cm of rice stubble height resulted in improved growth and highest yields of *Lathyrus* (Dixit *et al*., 2016).

Herwig (2001) reported a similar cultivation method used in India, Nepal and Pakistan. In Spain both *L. sativus* and *L. cicera* are grown in winter, but only *L. sativus* is grown in summer due to its potential for human consumption (Peña-Chocarro and Peña, 1999). *L. sativus* and *L. cicera* are also considered multipurpose grain legumes that adapt well to a range of environments in the grain belt in western Australia (Siddique *et al*., 1996; Hanbury and Siddique, 1998; Leport *et al*., 1998; Hanbury *et al*., 2000; Siddique *et al*., 2001; Hanbury *et al*., 2005). In this region, *L. sativus* has potential for rotation with cereal crops (Hanbury *et al*., 2005). They also reported that *L. sativus* in rotation will act as a break crop for root-and stubble-borne diseases of cereals, weed control and nitrogen fixation, and also produce a good yield.

Lathyrus sativus is a Fabaceae capable of fixing atmospheric nitrogen via rhizobium agrobacteria. Furthermore, it nodules effectively with *Rhizobium leguminosarium* (Yadav and Bejiga, 2006), leaving the soil rich in nitrogen for the next crop (Duke, 1981; Campbell *et al*., 1994) by adding about 67kg/ha of nitrogen to the soil per season (Wang *et al*., 2000).

In many parts of India, grass pea is cultivated up to 1300 m altitude (Duke, 1981), while in some parts of Ethiopia the altitude can reach 2500-3000 m above sea level with rainfall average annuals of 1000 mm. Grass pea grows on many soil types, including marginal areas when soil fertility is poor and soils are waterlogged, and in areas receiving only 380-650 mm of annual rainfall.

4.1.3. Alimentary importance:

The chemical composition of grass pea may vary according to varieties/ genotype, soil biology, agronomic practices, geographical region of their growing and maturity and environmental factors (soil fertility (nutrition), nitrogen nutrition, temperature, water stress and soil pH) (biotic and abiotic) (Rotter *et al*., 1991).

With regards to nutritional composition, there appears to be few studies on the nutritional aspects of grass pea. In general, the cotyledon and embryo make up most of the nutritionally beneficial part of the seed whilst the seed coat contains many of the antinutritional factors (ANFs). As any legume, grass pea is rich in protein (28-32%) and contains good quantities of essential amino acids except the sulfur containing amino acids (Urga *et al*., 2005). These latter reported the protein

content of grass pea to range from 27.29% to 31.98% in Ethiopian varieties. Protein in the diet is essential in providing the body with amino acids to build new proteins for tissue repair and replacement, and to synthesize enzymes, antibodies and hormones. By Chinnusamy *et al*., (2005), the seeds also have a high level of polyunsaturated fatty acids. Grass pea is very suitable for human consumption as 58% of fatty acids are polyunsaturated (Grela *et al*., 2010).

In addition to being important source of protein and calories, grass pea is rich in minerals. The seeds have a higher concentration of magnesium and phosphorus followed by calcium (Urga *et al*., 2005). Grass pea plants obtain minerals from their soil environment and partition these to their seeds. Roots utilize specific and/or selective transport proteins to obtain all the minerals (usually as ions) that are essential for plant growth and development (Ca, Mg, K, P, S, Cl, B, Fe, Mn, Zn, Cu, Ni and Mo (Urga *et al*., 2005).

The ash and crude fiber concentration of grass pea is also reported to range from 3.56 and 1.2% to 8.62 and 4.14 %. Fats and carbohydrates concentrations also range from 0.92% to1.47% and 55.05% to 85.17% respectively (Rotter *et al*., 1991).

In summary, grass pea is a good source of energy, protein, minerals, vitamins, fiber. It is generally the cheapest food legume for low income families, being a major component of their diet (Aletor *et al*., 1994). Since, grass pea contains a high concentration of free L-homoargmine, a precursor for lysine, it is a valuable animal feed (Qureshi *et al*., 1977).

4.2 In Morocco:

4.2.1 Superficies of *Lathyrus sativus* L.:

Legumes are an essential component of human and animal food, particularly in the Mediterranean area. While some legumes are widely cultivated and consumed, others are neglected and underused. This is the case of an ancient Mediterranean legume, chickling-vetch (*Lathyrus cicera* L.), currently considered as a marginal crop. In Morocco, this crop persists in some traditional mountain agroecosystems in the Tadla-Azilal region (Figure 4) (El Fatehi *et al*., 2021).

L. cicera L., known in Morocco as '*ikiker*', '*kiker*' or '*ichicher*', is marginally cultivated in the region. Landraces of this crop species, which are maintained locally by traditional agricultural practices, correspond to ecotypes adapted to local agro climatic conditions. We have surveyed the traditional cultivation sites of this crop to identify specific associated agro-ecosystems in the Middle and High Atlas Mountains of Morocco (El Fatehi *et al*., 2021).

In Morocco, this species persisting in some refuge areas, with scarce data for its cultivation and its history. Archaeological data (Ballouche and Marinval 2003; Morales *et al*., 2013) confirmed the presence of pea in some Neolithic sites, without confirming whether it is *L. sativus* or *L. cicera*.

On the other hand, although the cultivation, cropping, and uses of this species are relatively well described by Christian and Muslim authors during the medieval period in Spain (Carabaza, 1991; Garcia Sanchez, 1992; FAO, 1997), the citations during the same period are rarer in Morocco (Berque, 1978; Rosenberger, 1980; Ruas *et al*., 2011).

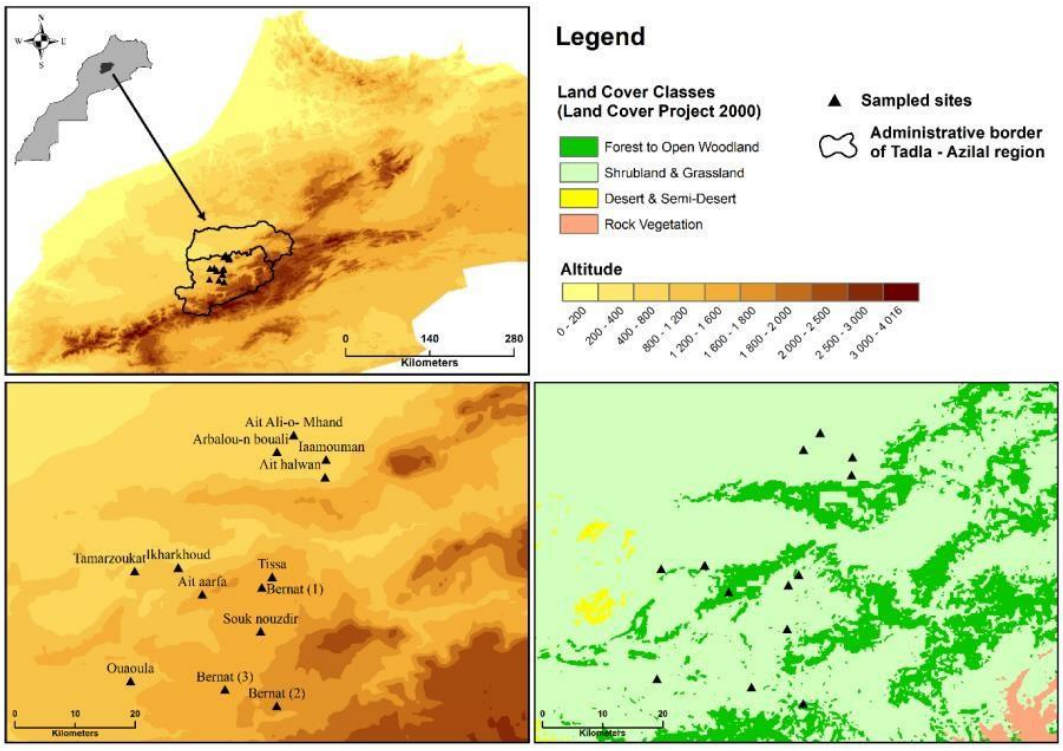


Figure 4 : Maps showing the distribution area of the chickling-vetch crop and location of the sampled sites (El Fatehi *et al*., 2021).

4.2.2 Production of *Lathyrus sativus* L.:

The cultivated *Lathyrus* species are considered as orphan crops. Widely cultivated in poor countries, they constitute a reliable resource in marginal low-input agricultural ecosystems (Vaz Patto *et al*., 2006).

In the Mediterranean area and especially in North Africa, production systems are commonly affected by the scarcity of water resources. This constraint will worsen in the current context of climate change (Hopkins, 2012; Nefzaoui *et al*., 2012; Norton and Volaire, 2012). Therefore, it is important to seek alternative crops in forage legumes capable of ensuring a qualitative and quantitative satisfactory production under an arid environment. Hence the interest in the rehabilitation of an ancient legumes, currently neglected and marginalized (López-i-Gelats and Bartolomé, 2012; Enneking and Miller, 2014).

The example of chickling-vetch local ecotypes, still cultivated in Morocco, represents a good opportunity where the conservation of scarce genetic resources coincides with the search for alternative crops as response to global changes.

El Fatehi *et al*., (2021) have shown that the average size of chickling-vetch plots is 0.7 ha and varies between 0.01 ha and two ha. Field management is not mechanized and agricultural work is carried out according to traditional local practices: plowing by animal, broadcast sowing, manual harvesting and threshing. They have also noticed that this crop has been abandoned in some douars close to urban areas such as Demnate and Kelaât Sraghna.

5. Anti-nutritional and toxic properties of *Lathyrus*:

Currently, there has been an increased interest regarding nutrition among a wider segment of society. Despite good nutritional composition of legumes for human consumption, they are often associated with a series of compounds known as antinutrients, which generally interfere with the assimilation of some nutrients (Hailu *et al*., 2015).

Anti-nutrients have been defined as substances, which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Francis *et al*., 2001).

In some cases, antinutrients can be toxic or cause undesirable physiological effects (e.g. flatulence). However, recent epidemiological studies have demonstrated that many antinutrients may be beneficial, in small quantities, in the prevention of diseases like cancer and coronary diseases. For this reason, they are now often called non-nutritive or bioactive compounds; although they may lack nutritive value, they are not always harmful (Muzquiz *et al*., 1999).

The most frequently occurring anti-nutritional substances in grass pea are protease and amylase inhibitors, lectins, tannins, saponins, alkaloids, phytates, and lathyrogens that causes a disease called “lathyrism” (Ramachandran and Ray, 2008).

5.1 Lathyrism or neurolathyrism:

Lathyrus species, particularly *L. sativus*, have been known since classical times to be implicated in a paralysis of humans and animals (Hugon *et al*., 2000) known as "lathyrism" or more specifically "neurolathyrism". This disease has been observed since antiquity, with the earliest surviving description by the Greek physician Hippocrates (460-377 BCE), followed by other ancient sources including the Greek physician Galen, the Roman naturalist Pliny the Elder and the Persian Polymath ibn Sīnā (lat. Avicenna) (Hendley, 1903; Dastur and Iyer, 1959).

Chemotaxonomic studies which was established in the early 1960s found the presence of toxic amino acids named as ODAP (*β-N*-oxalyl-*L-α*, *β*- diaminopropionicated) in 1964 (Murti *et al*., 1964; Rao *et al*., 1964). There are two forms of lathyrism, neurolathyrism and osteolathyrism.

Osteolathyrism is characterized by skeletal deformities and can be caused by consumption of the species *L. odoratus* (sweet pea), *L. hirsutus*, *L. pusillus* and *L. roseus* (Roy, 1981). Osteolathyrism has been recorded experimentally in a wide range of animals (Barrow *et al*., 1974). The principal compound responsible was found to be b-aminopropionitrile (BAPN; Figure 5), although the

related nitriles aminoacetonitrile (AAN) and methylene aminoacetonitrile (MAAN) also have some osteolathyritic activity (Barrow *et al*., 1974). Although BAPN is not found in either *L. sativus* or *L. cicera* (Bell, 1964), there is evidence that a BAPN precursor (2-cyanoethyl-isoxazolin-5-one) is present in *L. sativus* seedlings but not in seed (Lambein *et al*., 1993). Consumption of *L. sativus* seedlings and shoots as vegetables has been blamed as the cause of osteolathyritic symptoms found in a small proportion of people with chronic neurolathyrism (Haque *et al*., 1997). Incidents of osteolathyrism from feeding of *L. sativus* or *L. cicera* have not been reported in animal studies, either under natural grazing or experimental conditions, and consequently the following discussion will focus on neurolathyrism.

Neurolathyrism is the term used to describe the symptoms shown after heavy consumption of several different *Lathyrus* species and some *Vicia* species. The symptoms are weakness of the hind limbs and paralysis or rigidity of the muscles. Within the *Lathyrus* genus, the category of neurolathyrism has been further divided into two sub-categories. One is caused by the compound L-2,4-diaminobutyric acid (DABA; Figure 5), primarily in the perennial species *L. sylvestris* (Foster, 1990) and *L. latifolius* (Barrow *et al*., 1974).

However, DABA is not found in *L. cicera* or *L. sativus* (Bell, 1964; Padmanaban, 1980). The form of neurolathyrism most pertinent to this discussion is that caused by the non-protein amino acid 3-(-N-oxalyl)-L-2,3-diamino propionic acid (ODAP, also referred to as b-N-oxalylamino-L-alanine or BOAA; Figure 5): which has been recorded in humans and animals following consumption of *L. sativus*, *L. cicera*, *L. ochrus* and *L. clymenum* (Barrow *et al*., 1974; Franco Jubete, 1991; Padmanaban, 1980).

The seed of a number of other uncultivated *Lathyrus* species have been found to contain ODAP (Bell, 1964). Historically the consumption of *L. sativus* has been most often linked with lathyrism in humans and animals, primarily because of all *Lathyrus* species it is the most widely utilized as grain and fodder. Lathyrism is the term mostly used to refer to neurolathyrism caused by ODAP, therefore this term will be used in this review from this point onward.

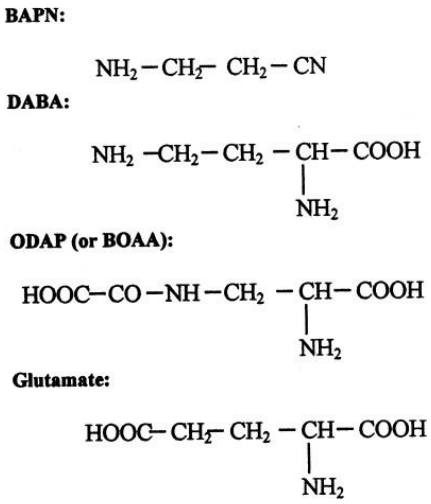


Figure 5: Chemical diagrams of b-aminopropionitrile (BAPN), L-2,4-diaminobutyric acid (DABA), 3-(-*N*-oxalyl)- *L*-2,3-diamino propionic acid (ODAP or BOAA) and glutamate (Hanbury *et al.*, 2000).

Lathyrism in humans has received more attention than that in animals, due to the social cost. Symptoms in humans are most often initial painful spasms in the muscles of the lower limbs with accompanying weakness, followed by chronic spastic paraplegia of various degrees (Spencer *et al*., 1986), and can lead to total loss of use of the legs (Attal *et al*., 1978). The paralysis is rarely reversible (and then only in early stages of the symptoms; Hugon *et al*., 2000) and the consequences for poor communities who depend upon *L. sativus* as a primary food source at times of food scarcity can be devastating.

Socio-economic factors are clearly important in controlling the incidence of lathyrism. Dwivedi (1989) clearly demonstrated that in social classes I (richest) to V (poorest) the syndrome did not occur in classes I and II and in a season of high incidence (1981) increased rapidly through social classes III (5.65%) and IV (26.5%) to an incidence of 67.8% in one sample of social class V. A similar distribution by occupation was also apparent with the highest incidence (72.31%) among unskilled laborers. There is also a very marked difference in incidence between the sexes, males are more susceptible than females, who do not apparently develop the condition between puberty and the menopause. Among males the incidence declines markedly after 35 years of age. The onset of the disease may be insidious (chronic) or sudden (acute). It can be progressive but the progression from mild to more severe forms is not rapid (Kaul *et al*., 1989). Patients can be

classified in four categories, depending on the extent of the disability and resultant loss of mobility, these are (1) "non-stick", (2) "one-stick", (3) "two-stick", and (4) "crawler" (Dwivedi, 1989). The condition of patients who seek treatment in the majority of cases does not improve, but in about 25% there may be no further deterioration in their condition. While some also reported improvement in their condition none have been reported as making a complete recovery. The onset of the disease is indicated by a feeling of heaviness and pain in the lower limbs. Although that part of the nervous system controlling the movement of the legs is impaired other parts of the nervous system are apparently not affected; there is, for example, no loss of mental faculties (Smartt *et al*., 1994).

Although comprehensive survey has never been done, in Asia and Africa there may be several hundred thousand people affected by this disease that crippling not only their legs but also their livelihoods grounding their socio-economic functioning in the already poor communities of farmers surviving in a hand to mouth economy (EIAR., 2007).

Lathyrism still occurs, with a 1997 outbreak during food shortages in Ethiopia crippling 2000 people (Getahun *et al*., 1999). Lathyrism is endemic to the areas of the world which have significant areas of *L. sativus* cultivation; India, Bangladesh, Ethiopia and Nepal. However, in the 20th century outbreaks were also reported in Afghanistan, Algeria, China, France, Germany, Italy, Pakistan, Romania, Russia, Spain and Syria (Barrow *et al*., 1974; Hugon *et al*., 2000).

5.2 Neurotoxins *β-L*-ODAP:

The compound *β-N*-oxalyl-*L-α, β*-diaminopropionic acid (*β*-L-ODAP) has been identified as the causative agent of neurolathyrism (Murti *et al*., 1964; Rao *et al*., 1964; Spencer *et al*., 1986).

Following its isolation and identification (Murti *et al*., 1964; Rao *et al*., 1964) the neurolathyritic action of ODAP was soon demonstrated in adult monkeys (*Macaca radiata*; Rao *et al*., 1967). Cheema *et al*., (1969) administered ODAP intraperitoneally to rats. Young rats showed lathyrism symptoms and had 0.11 μmol g^{-1} ODAP in the brain, adult rats showing trace or nil ODAP and no symptoms. Olney *et al*., (1976) found some indication of exclusion of ODAP by the blood-brain

barrier in mice. Padmanaban (1980) suggested that the hypothesis of less ODAP exclusion by the blood-brain barrier in young animals should be re-examined, as greater excretion of ODAP by older animals may be an important factor. Spencer *et al*., (1986) showed unequivocally that ODAP, either naturally present in *L. sativus* or when added to other food sources, was the cause of corticospinal dysfunction in monkeys (*Macaca fascicularis*), with symptoms of hind limb motor difficulties.

Neurotoxins

- A neurotoxin is a substance that is poisonous or destructive to nerve tissues.

Neurotoxic exposures that threaten human health can be the result of a wide range of factors, including: air pollution (e.g. ultrafine particulate matters, ozone); toxic heavy metals in drinking water (e.g. as a result of soil acidification or industrial runoffs); medical or recreational drug use (e.g. opioid abuse); venomous exposures (e.g. snake bites) and dietary uptake (including plant-derived neurotoxins such as cyanogens from cassava or *β-L*-ODAP from grass pea, microbial products such as ethanol or botulinum toxin and animal-derived neurotoxins such as pufferfish tetradotoxin) (Tshala-Katumbay and Spencer, 2007; Tshala-Katumbay *et al*., 2015).

β-L-ODAP appears to act as an analogue to glutamate, which serves as a common neurotransmitter in animals. This causes *β-L*-ODAP to interfere with the transport of glutamate and its decarboxylation into γ-aminobutyric acid (GABA) (Ross *et al*., 1985), which may explain the excitotoxic (convulsive) effects observed in some experimental animals, although these symptoms are not apparent in humans. *β-L*-ODAP also acts as a competitive antagonist to glutamate on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, causing neurotoxic effects in rat brains (Ross *et al*., 1989; Kusama-Eguchi *et al*., 2010). An isomer of *β-L*-ODAP, *α-L*-ODAP is also found in grass pea tissues, but makes up only around 5 % w/w of total ODAP (Roy and Rao, 1968; Arentoft and Greirson, 1995). The *α*-isomer does not appear to be neurotoxic (Chase *et al*., 1985).

The ultimate fate of ODAP and the distribution in the brain and spinal cord is not known (Hugon *et al*., 2000). ODAP was not detected in pig loin tissue following feeding for an extended period (Castell *et al*., 1994).

In human populations young men are widely reported as the most susceptible to lathyrism (McCarrison, 1926; Shourie, 1945; Attal *et al*., 1978; Hamid *et al*., 1986; Getahun and Tekle Haimanot, 1997) although the reasons for this are not understood (Hugon *et al*., 2000). The production and susceptibility to ODAP may be linked to Zn deficiency in plants and humans, respectively (Lambein *et al*., 1994; Lambein and Kuo, 1997). ODAP has also been found to inhibit growth of some insects and yeasts (Rao *et al*., 1964; Mehta *et al*., 1972), and so may have a plant protective role.

The level of ODAP in *Lathyrus sativus* seeds is very variable, due to the high variability of the species, and high levels of ODAP are generally blamed for the occurrence of human lathyrism. Kumar (1997) revealed that the consumption of *Lathyrus* in different countries is due not only to genetic variability but also to climate change on the toxin level. When some environmental effects such as drought stress were studied, it was found the level of ODAP increased under drought conditions in hydrophonic cultures, or could decrease by irrigation in the field.

5.2.1 Biomedical potential of *β*-ODAP:

The consequence of the overconsumption of grass pea in an unbalanced diet for a longer period, causes a neurolathyrism (Rao *et al*., 1964). Despite the presence of this disease, grass pea has a many physiological functions with therapeutic potential (Lambein *et al*., 2019).

One of the physiological functions with possible therapeutic potential of *β*-ODAP is the activation of protein kinase C, which adds a new dimension to explore grass pea potential in the treatment of Alzheimer's disease, hypoxia, and long-term potentiation of neurons essential for memory (Singh and Rao, 2013). In addition to *β*-ODAP, grass pea grains also contain L-homoarginine (Rao, 2011), which is a modulator of the biosynthesis of nitric oxide which, in turn, reduces the excitation of neuronal receptors (Bell, 2003).

However, *β*-ODAP with low dosage is also reported as neuro-protective. It has been reported as a neuro-excitatory amino acid also known as dencichine found in *L. sativus* and occur in several Panax species known for its antihemorrhage property. So, this "neuro-excitatory amino acid" may be acceptable as described in Vaz Patto and Rubiales (2014), Lambein *et al*., (2019), and several others. Thus, a dietary intake of grass pea grains could be valuable for human health and deserves to be studied further (Barpete *et al*., 2021).

β-L-ODAP has known homeostatic properties, which appear to be due to a vasoconstrictive effect, rather than any effect on blood clotting. To capitalize on this bioactive property, the Chinese company Yunnan Baiyao (Kunming, Yunnan Province) is developing band-aids containing *β-L*-ODAP to reduce blood flow (Wang *et al*., 2014).

5.2.2 Physiological breakdown of *β-L*-ODAP:

There appears to be a catabolic pathway for the breakdown of *β-L*-ODAP in humans, which is less efficient or non-existent in other animals. Human volunteers who consumed cooked grass pea seeds or controlled amounts of pure *β-L*-ODAP excreted less than 1 % of it in their urine, but increased their excretion of oxalic acid (Pratap Rudra *et al*., 2004).

In mice, rats and chicks, much greater proportions (between 21.1 % and 75.2 %) of orally or intra-peritoneally administered radiolabelled *β-L*-ODAP were excreted in urine.

The breakdown of *β-L*-ODAP in humans may provide an explanation for the typically observed low incidence of neurolathyrism in human populations relying heavily on grass pea (Dufour, 2011; Girma *et al*., 2011).

It is also possible that there is genetic variation in the susceptibility to neurolathyrism among the human population (Pratap Rudra *et al*., 2004), but no study to identify genetic markers associated with susceptibility to neurolathyrism has been conducted.

5.3 Trypsin inhibitors:

Trypsin inhibitors (TI) have a wide distribution in the plant kingdom and are present in most legume seeds and cereals (Francis *et al*., 2001). In comparison with most edible legumes, trypsin inhibitor activity (TIA) in grass pea is high (Aletor *et al*., 1994).

The major physiological effect of trypsin inhibitor is to cause the enlargement of the pancreas and secretion of excessive amounts of pancreatic enzymes much of which is lost to the animal in feces (Urga *et al*., 2005; Oke, 2007). The study of Urga *et al*., 2005 have shown Trypsin inhibitor activity (TIA) values varied from 15.53 to 18.99TIU/ mg. However, the variation in TIA was within narrow ranges as investigated by area of cultivation.

5.4 Phytates (Phytic acid):

Phytic acid, myo-inositol-(1, 2, 3, 4, 5, 6) hexakis-phosphate and its salts are the major sources of phosphorus in legume seeds (Urbano *et al*., 2000). Phytic acid content in grass pea can vary with genotype, climate, type of soil and year.

Phytic acid has been considered an antinutrient as it binds with other nutrients and makes them indigestible. Excessive phytic acid in the diet can have a negative effect on mineral balance by forming a complex with mono, di- and trivalent mineral ions such as Na^{+}, K^{+}, $Ca^{2+,}$ Mg^{2+}, Zn^{2+}, Cu^{3+} and Fe^{3+} resulting in these ions becoming unavailable for body utilization or causes poor mineral bioavailability (Urbano *et al*., 2000; Urga *et al*., 2005).

Phytic acid is also able to make phytate–protein complexes, decreasing protein solubility, thus reducing the availability of dietary protein (Francis *et al*., 2001). The ability of phytic acid to bind with minerals, proteins or starch, directly or indirectly, may alter solubility, functionality, digestibility and absorption of these nutrients.

5.5 Tannins:

Tannins are secondary metabolites of various chemical structures widely occurring in plant kingdom (Francis *et al*., 2001). They are defined as high-molecular-weight polyphenolic compounds that have the ability to bind with protein and preserve animal hides.

The anti-nutritional effects of tannin include interference with the digestive processes either by binding the enzymes or by binding to food components like proteins or minerals. Tannins also reduce the absorption of vitamin B12 (Hailu *et al*., 2015). Contrary to condensed tannins, the hydrolysable tannins are easily degraded in biological systems, forming smaller compounds that can enter the blood stream and over a period of time causing toxicity to the organs (e.g., liver and kidney).

Tannins are also known to interact with other anti-nutrients. For example, interaction between tannins and lectins removed the inhibitory action of tannins on amylase, and interactions between tannins and cyanogenic glycosides reduced the deleterious effects of the latter (Francis *et al*., 2001). Condensed tannin levels in grass pea lines ranged from zero to 4.38 g/kg (Urga *et al*., 2005).

Tannins are strongly astringent (owing to their protein-binding properties), a depression of feed intake, which lowers animal productivity, would be expected. Although astringency seems to be the major cause of lower feed intakes, several other factors may contribute to the lower feed efficiency ratios of tannin-containing diets. These include the formation of tannin/protein complexes that make the protein unavailable, inhibition of the digestive enzymes, increased synthesis of digestive enzymes due to inadequate enzyme digestion, and increased loss of endogenous proteins such as the mucoproteins of the gastrointestinal tract (Campbell, 1997).

6. Effects of processing methods on nutritional composition of grass pea:

Food can be processed at different levels including home-based food processing and at industrial level. The processing of grass pea into the many food and feed products can cause changes in their nutritive value. However, despite the changes in nutritive value, the changes that are known to occur during normal thermal processing may not in any measurable way adversely affect the nutritive value of grass pea.

Any processing attempt on grass pea food contributes to toxicity reduction even if one mechanism is more effective than the other. Raw grass pea is the most toxic causing and consumption should be avoided by all means. Children are most exposed as they eat the green seeds as snack during field stay with the herd or walking. Despite any grass pea food processing attributes to decrease

the toxin level by certain amount, pre-processing like long soaking (>18hrs) and fermentation have significance role (Hailu *et al*., 2015). When legumes are properly soaked and germinated, their nutritive value increases greatly and the anti-nutritional factors such as enzyme inhibitors, toxic amino acids and other anti-nutrients are greatly decreased to insignificant levels. Mixing grass pea food preparation with condiments such as onion, ginger as in gravy and gravy sauce or keep in proportion below 1/3 in cereal mix, richer in essential amino acids, are among outweighing principles to use the crop safely (Haileyesus *et al*.,2005).

6.1 Soaking and Boiling:

Different seeds are soaked in water for different periods of time. Soaking in water allows the seeds to absorb water, to decrease and eliminate anti-nutritional factors in legumes. However, soaking for long periods of time has been found to reduce nutritional quality of legumes through leaching of nutrients into the soaked water (Taiwo, 1998).

Shiwani Srivastava *et al*., (1999) reported that soaking of *lathyrus sativus* seeds in various media reduced the contents of *β*-ODAP to a varying and significant extent; losses of *β*-ODAP were higher after soaking in boiled water, alkaline and tamarind solutions but less after soaking in drinking water. The highest losses in boiled water (65–70%) were observed for *β*-ODAP, followed by trypsin inhibitors (42–48%) and polyphenols (30–37%).

Also ordinary cooking and pressure cooking of pre-soaked seeds were found to be most effective in reducing the levels of all the natural toxicants examined, while fermentation and germination were more effective in destroying both of the enzyme inhibitors (amylase inhibitors by 69–71%; trypsin inhibitors by 65– 66%) than either phytates or polyphenols.

Boiling improves the appeal and sensory properties of legume. Boiling is usually at 1000C for some minutes. It tenderizes the seeds through water absorption. Traditionally, cooking of beans can be done using firewood. Pressure cooking pots allows legumes to be cooked under pressure and it reduces cooking time. This process eliminates heat labile antinutritional factors such as trypsin inhibitors (Bishoi and Khetarpaul, 1993).

Haileyesus *et al*., (2005) reported that soaking grass pea in water before cooking roughly halved the risk of neurolathyrism but cooking in clay utensils more than quadrupled it. Also, they concluded that consumption of grass pea in the green unripe and boiled forms increased the risk 10 times or more. However, mixing the food with gravy that contains condiments with antioxidant activity reduced it by a factor of 4 and consumption of grass pea mixed with cereals rich in sulphur amino acids was also highly protective, but the magnitude of the effect depended on the grass pea preparation consumed.

A reduced risk of paralysis was associated with soaking *L. sativus* seeds in water before preparation, fermentation, mixing the seeds with gravy that contains condiments with antioxidant activity or mixing with cereals rich in sulphur amino acids is shown to be protective (Akalu *et al*., 1998). Boiling has been found to reduce ODAP levels in several cases.

Padmajaprasad *et al*., (1997) reported that boiling grain and discarding the water reduced ODAP levels by up to 90%. Cooking for 10, 20 and 30 minutes had variable effects. Cooking for 20 minutes resulted in clearly better reduction in ODAP content compared to the shorter duration of 10 minutes or the longer period of 30 minutes for all temperature intensities. There was also a trend of increasing reduction in ODAP content with increasing temperature days of age (Tadelle *et al*., 2003).

6.2 Roasting:

Legumes are roasted on the open frying pan in the presence or absence of salts or ash. Roasting improves the taste and edibility of legumes. It is important also in reducing and eliminating anti-nutritional factors. Roasted legumes are characterized by unique flavours which can increase their sensory appeal. Roasting resulted in only marginal reduction of ODAP content (20.61%) (Tadelle *et al*., 2003).

6.3 Fermenting:

The process increases the digestibility of plant proteins and also reduces the anti-nutritional factors. Fermentation enhances flavour, colour and texture of legumes. Changes in these attributes are major stimuli in development of legume fermented products. It reduces heat stable anti-

nutritional factors such as phytate. Solid state fungal fermentation on pure grass-pea using the fungal strains *Rhizopus oligosporous* and *Aspergillus oryzae* in succession has shown that the neurotoxin *β-N*-oxalyl-*α*, *β*-diaminopropionic acid (*β*-ODAP) in grass pea has been removed by 80% on average for the high-toxin variety and by up to 97% for the low-toxin variety (Yigzaw *et al*., 2004). Solid state fermentation of several low-toxin varieties of grass pea (*Lathyrus sativus* L) seeds with *Aspergillus oryzae* and *Rhizopus microsporus* var *chinensis* removed the neurotoxin *β*-ODAP (3-*N*-oxalyl-*L*-2,3- diaminopropanoic acid) to a considerable degree from the seed meal (Lambein *et al*., 2009).

6.4 Germinating:

Germination enhances desired qualities such as improved digestibility, reduced antinutrients like trypsin inhibitors (Ahmed *et al*., 1995). It improves nutritional quality of the proteins by hydrolyzing them into absorbable polypeptides and essential amino acids. Germinated or malted legumes are eaten in form of sprouts and are better than ungerminated ones. Sprouting improves the availability of vitamins B and C. It also reduces polyphenols content. Chicken pea and broad beans are commonly germinated before eating, cooking or use in salad dressing.

6.5 Extrusion:

Extrusion cooking is a high-temperature, short-time process in which moistened, expansive, starchy and/or proteinacious food materials are plasticized and cooked in a tube by a combination of moisture, pressure, temperature and mechanical shear, resulting in molecular transformation and chemical reactions (Castells *et al*., 2005). Parallel to the increased applications, interest has grown in the physico-chemical, functional and nutritionally relevant effects of extrusion processing. It also significantly reduces antinutrients present in legumes (Abd El Hady and Habiba, 2003). Nutritional concern about extrusion cooking is reached at its highest level when extrusion is used specifically to produce nutritionally balanced or enriched foods, like weaning foods, dietetic foods, and meat replacers (Plahar *et al*., 2003). Also, extrusion technology causes substantial viscosity reduction in cereal gruels and enhances its nutrient densities (De Muelenaere, 1989). Thus, extrusion technique allows broadening the product range, and increasing the consumption of leguminous seeds product matrix.

6.6 Autoclaving:

According to study found by Girma *et al*., (1998), roasting and autoclaving of the milled grass pea caused significant reduction in the content of *β*-ODAP up to 30% and 50%, respectively, compared to that of raw whole seeds.

7. Nutritional values of *Lathyrus*:

The legume is nutritious, rich in protein (28-32%) and contains good quantities of essential amino acids (Urga *et al*., 2005). Availability of nutritional data is important both to understand the possible beneficial effects of *L. sativus* seeds consumption on human health and to support their possible production and marketing. Grass pea seeds are rich in proteins, macro-micronutrients and essentials amino acids.

Tamburino *et al.,* (2012) have investigated nutritional values and metabolic profile of *Lathyrus sativus* L. seeds. The results shown that these seeds contain high levels of proteins (25.6±0.20 g/100 g) and essential amino acids (7.92 g/100 g). Different unsaturated fatty acids contribute to the total lipids amount (1.67±0.18 g/100 g); among them, the essential PUFA or linoleic acid and γ-linolenic acids (GLA) are the most abundant. Ascorbic acid (13.50±0.30 mg/100 g) and glutathione (15.90±0.10 mg/100 g) are also present and, the folic acid content (206.70±8.30 μg/100 g) represents 50% of the vitamin RDA (Recommended Dietary Allowance). Total phenolic content (174.91±8.39/100 g) have been estimated.

In addition to being important source of protein and calories, grass pea is rich in minerals (Urga *et al.,* 2005). The seeds have a higher concentration of magnesium and phosphorus followed by calcium. The ratio of calcium to magnesium ranges from 1:0.53 to 1:1.02 (mg/100g) and that of calcium to phosphorus ranges from 1:0.22 to 1: 0.43 (mg/100g). This study has also investigated the important quantities of carbohydrates and crude fiber in grass pea seeds.

The total seed protein in grass pea varies between 18.2% and 34.6% (Aletor *et al*., 1994; Bisignano *et al*., 2002; Tadesse and Bekele, 2003; Urga *et al*., 2005; Barpete *et al*., 2012; Girma and Korbu, 2012; Tamburino *et al*., 2012; Kumari *et al*., 2018) (Table 6). The grass pea seed protein is composed of globulins (66%), albumins (14%), glutelins (15%) and prolamins (5%) (Chandna and

Matta, 1994). The large differences in total seed protein content between *Lathyrus* species including *L. sativus*, *L. cicera*, *L. maritimus*, *L. tingitanus*, *L. amphicarpos*, *L. angulatus*, *L. annuus*, *L. aphaca*, *L. clymenum*, *L. fliformis*, *L. hirsutus*, *L. latifolius*, *L. ochrus*, *L. pratensis*, *L. setifolius* and *L. sphaericus* have been observed (Cavada *et al*., 2011; Grela *et al*., 2012; Kumari *et al*., 2018). The total seed protein content ranged from 10.7% to 28.0% in *L. maritimus* (Dnyanu, 1998) and 21.5% to 27.4% in *L. Cicera* (Cavada *et al*., 2011; Grela *et al*., 2012) (Table 6).

The matured grass pea leaves also contain 17% protein content (Rizvi *et al*., 2016). Grass pea contains higher protein content in seeds than other cool-season food legumes like field pea (23%), faba bean (24%), chickpea (22%) and lentil (27%) (Ravindran and Blair, 1992; Petterson *et al*., 1997).

Data of micronutrient profile of *Lathyrus* species are limited (Table 6). Grass pea has a good quantity of iron in its seeds that ranged from 4.11 mg to 8.74 mg/100 g (Duke 1981; Urga *et al*., 2005; Grela *et al*., 2012), whereas iron content in *L. cicera* and *L. maritimus* contained 4.11–5.28 mg/100 g (Grela *et al*., 2012) and 9.4 mg/100 g (Dnyanu, 1998), respectively.

The zinc content of *Lathyrus* genus in seeds ranged from 2.46 to 4.52 mg/100 g in *Lathyrus sativus* (Urga *et al*., 2005; Grela *et al*., 2012), 1.96 to 2.77 mg/100 g in *Lathyrus cicera* (Grela *et al*., 2012), and 3.0 mg/100 g in *Lathyrus maritimus* (Dnyanu, 1998).

Recently, ICARDA breeding program has analyzed 485 germplasm accessions representing many species (personal communication, under publication) and found large genetic variability for micronutrient concentration in grass pea (Table 7) which can be used for mainstreaming biofortifcation in grass pea.

More such efforts are needed to phenotype *Lathyrus* germplasm for Fe and Zn concentration to find out spectrum of genetic diversity for various micronutrients existing within cultivated species (Sen Gupta *et al*., 2021).

Table 6: Protein, iron, zinc ranges and ODAP content of *Lathyrus* species (Sen Gupta *et al*., 2021).

Trait	Species	Ranges	References
Protein	*L. sativus* L.	30%	Aletor *et al*. (1994)
		25.60%	Tamburino *et al*. (2012)
		23.93–31.94%	Tadesse and Bekele (2003)
		23–29.9%	Bisignano *et al*. (2002)
		27.29–31.98%	Urga *et al*. (2005)
		18.2–34.6%	Girma and Korbu (2012)
		22.4–28.2%	Barpete *et al*. (2012)
		8.6–32.2%	Kumari *et al*. (2018)
	Lathyrus cicera L.	21.50%	Cavada *et al*. (2011)
		23.8–27.4%	Grela *et al*. (2012)
	Lathyrus maritimus L.	10.7–28.0%	Dnyanu (1998)
	L. tingitanus	25.60%	Cavada *et al*. (2011)
	L. amphicarpos	19.90%	Cavada *et al*. (2011)
	L. angulatus	23.80%	Cavada *et al*. (2011)
	L. annuus	21.00%	Cavada *et al*. (2011)
	L. aphaca	20.80%	Cavada *et al*. (2011)
	L. clymenum	21.60%	Cavada *et al*. (2011)
	L. filiformis	21.20%	Cavada *et al*. (2011)
	L. hirsutus	25.10%	Cavada *et al*. (2011)
	L. latifolius	24.40%	Cavada *et al*. (2011)
	L. ochrus	20.20%	Cavada *et al*. (2011)
	L. pratensis	23.20%	Cavada *et al*. (2011)
	L. sphaericus	23.50%	Cavada *et al*. (2011)
	L. setifolius	22.70%	Cavada *et al*. (2011)
Zinc	*L. sativus* L.	2.74–4.52 mg/100 g	Urga *et al*. (2005)
		2.46–36.7 mg/100 g	Grela *et al*. (2012)
	Lathyrus maritimus L.	3.0 mg/100 g	Dnyanu (1998)
	Lathyrus cicera L.	1.96–2. 77 mg/100 g	Grela *et al*. (2012)
Iron	*L. sativus* L.	4.64–8.74 mg/100 g	Urga *et al*. (2005)
		4.11–5.48 mg/100 g	Grela *et al*. (2012)
		7.3 mg/100 g	Duke (1981)
	Lathyrus maritimus L.	9.4 mg/100 g	Dnyanu (1998)
	Lathyrus cicera L.	4.11–5.28 mg/100 g	Grela *et al*. (2012)
ODAP	*L. sativus* L.	0.02–2.40%	Abd El Moneim *et al*. (2001)
		0.14–0.91%	Tadesse and Bekele (2003)
		0.50–2.50%	Xiong *et al*. (2015)
		0.02–0.54%	Fikre *et al*. (2008)
		0.15–0.95%	Kumar *et al*. (2011b, 2013)
		0.13–0.44%	Girma and Korbu (2012)
		0.21–0.55%	Shiferaw and Porceddu (2018)
	Lathyrus cicera L.	0.09–0.13%	Grela *et al*. (2012)
		0.03–0.22%	Abd El Moneim *et al*. (2001)
	Lathyrus ochrus	0.46–2.5%	Abd El Moneim *et al*. (2001)
		1.01%	Aletor *et al*. (1994)

Table 7 : Genetic variability for micronutrient and macronutrient concentration in 485 grass pea accessions (Sen Gupta *et al*., 2021).

Nutrient	Range	Minimum (ppm)	Maximum (ppm)	Mean (ppm)	Standard deviation
P	2830	953.74	3784.12	2249.84	721
K	2513	1478.90	3992.29	2901.25	543
Zn	31	20.39	51.05	32.81	6
Ca	709	688.45	1397.54	1028.39	182
Mg	1125	554.00	1679.12	986.03	236
Mn	38	0.12	38.29	6.82	8
Fe	46	12.98	59.35	29.68	9
Se	0.58	0.01	0.59	0.18	0.14

8. Prospecting, collecting and conservation:

Two major types of conservation were defined: ecological and genetic. Ecological conservation attempts to preserve an ecological niche, rather than just concentrating on protecting a single or groups of species. Genetic conservation, however, focuses on preventing the genetic erosion of a single species and the component of its genepools (Maxted *et al*., 1997b).

In fact, there are two techniques of biodiversity conservation; ex situ and in situ conservation which are defined by CBD (1992) as: “In-situ conservation means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species (on-farm conservation), in the surroundings where they have developed their distinctive properties; “Ex-situ conservation means the conservation of components of biological diversity outside their natural habitats” (CBD definition, UNCED, 1992).

Both strategies are equally important and should be regarded as complementary (Maxted *et al*., 1997a; Dulloo *et al*., 1998; Engelmann and Engels, 2002; Thormann *et al*., 2006). Figure 6 presume the technique of conservation by Maxted *et al*., 1997a.

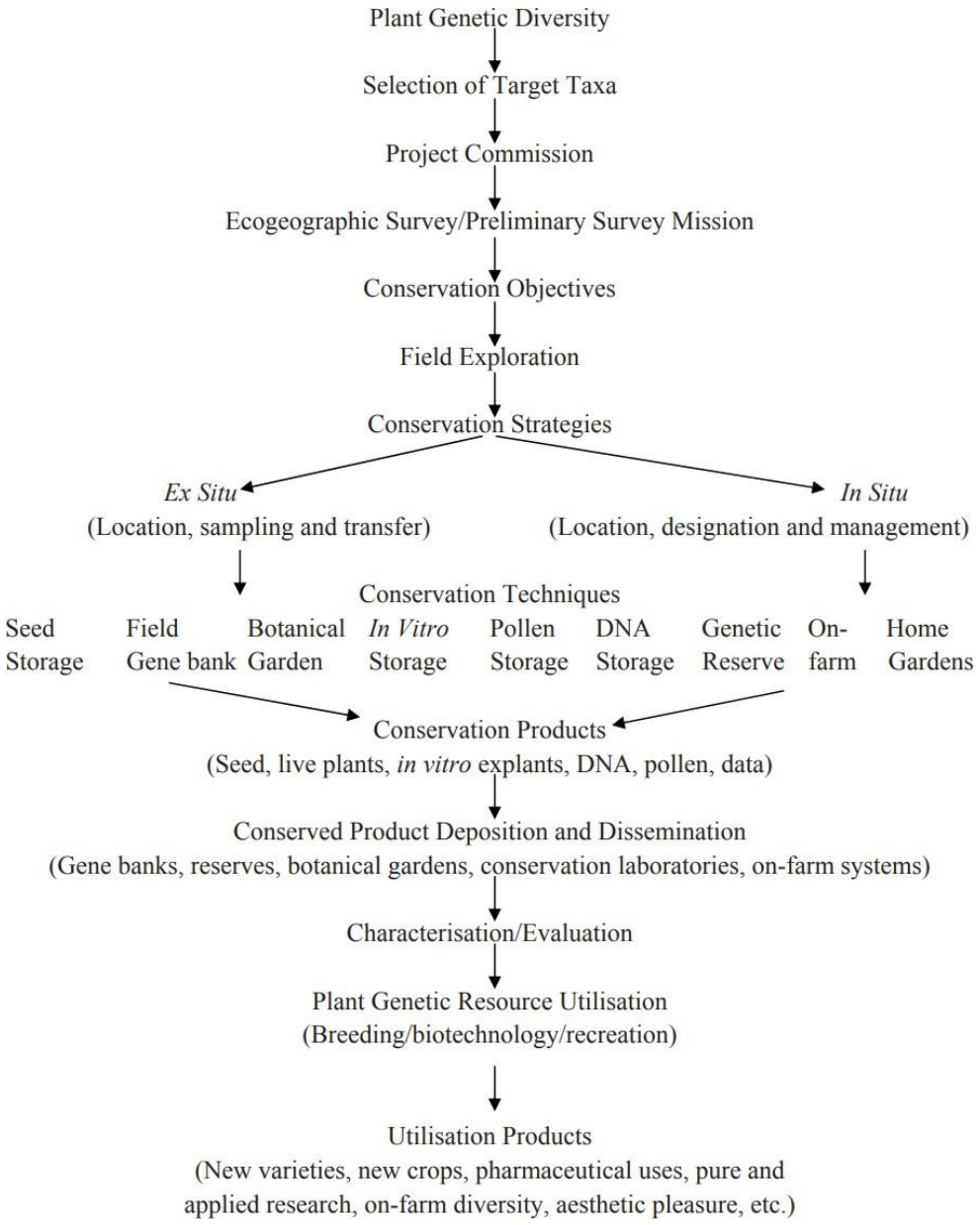


Figure 6 : A model of plant genetic conservation (Maxted *et al*., 1997a).

8.1 Global ex-situ conservation:

In regard to conserve the genetic diversity of many species, many genotypes collection has been assembled around the world.

Lathyrus genetic material is collected across the world and conserved in many different countries (India, Pakistan, Bangladesh, Nepal, China, Australia, Ethiopia, France, Turkey, Spain, Canada, Chypre, Allemande, Greece, Italy, Bologna, Portugal, Russia, Syria, UK and USA), denoting the importance of these species worldwide (Robertson and Abd-El-Moneim, 1995). The collection is often conducted by national institution's in collaboration with international center such as ICARDA, IPGRI and with institutions in developed countries.

The germplasm collection of *Lathyrus* maintained by ICARDA contained 44 species from 46 countries with a total of 3038 accessions. These accessions are held in-trust under the auspices of the Food and Agriculture Organization (FAO) under a CGIAR agreement with FAO. In WANA region, the most accessions of *Lathyrus sativus* are from Morocco, Turkey and Syria. In 1993-95, a series of target missions were conducted in Morocco to fill a significant gap representation of *Lathyrus* species in the ICARDA and Moroccan collections. Germplasm collection of *Lathyrus* accessions (1992-97), consists a large number of *L. sativus*, the rest being mainly *L. cicera*, *L. articulatus* and *L. ochrus* (a potentially important species in Morocco) (Robertson and Abd-El-Moneim, 1997).

Russia holds 1240 accessions, Australia more than 1000 accessions. In Europe, Allemande, Spain and Hungary contains a collection of 300 to 500 entries. The biggest collection of *Lathyrus* in Africa is situated in Ethiopia with 96 accessions. As for *Lathyrus sativus*, France has the largest collection, with 4387 accessions. Indian National Genebank includes 2720 accessions of grass pea and Bangladesh has 2078 accessions (Campbell, 1997; Mathur *et al*., 1998; Kumar *et al*., 2011).

Germplasm constitution by international institutions and scientists began a long time ago. Over the past twenty years, national programs in different countries have paid particular attention to genetic resources, especially fodder and pastoral species. In Algeria, Libya, Morocco, Tunisia, Italy, France, Spain and Portugal, herbaceous species were most widely collected (Abdelguerfi and Abdelguerfi-Laouar, 2004).

Some important collections of wild and cultivated *Lathyrus* species have been assembled and maintained ex situ in a number of institutes around the world.

The available collections of *Lathyrus* are mainly found in INA, ICARDA, INRA and ITGC. It is recognized that plant genetic resources are largely the main raw material of farmers and scientists, and they also constitute a genetic adaptability reserve, a guarantee against the threat of environmental modification.

Many grass pea collections have been evaluated in Morocco. Abdallah *et al.,* (2020) have studied the resistance against broomrape species (*Orobanche crenata* and *Orobanche foetida*) in the field and controlled conditions. The results shown that *L. aphaca, L. articulatus, L. blepharicarpus, L. cassius*, *L. pseudocicera*, and *L. ochrus* with <1 PI against *O. crenata* offer scope to develop introgression linesfor their use in mainstream grass pea breeding. The resistant accessions are included in the crossing block to strengthen pre breeding efforts in grass pea.

The studies of Ashilenje *et al.,* (2023) have shown the 50–50% barley, grass pea mixture as the most promising in sustaining mechanisms of soil rejuvenation from salinity based on vibrant soil microbial activities along a gradient of increasing soil salinity.

Barpete *et al.,* (2021) have indicated that method of preparation and cooking can improve protein availability and make grass pea food safe to consume. In general, the magnitude of cooking effect was genotype specific. Among cooking treatments, boiling grains was superior followed by autoclaving and microwaving. This study showed significant genetic variability for hydration and swelling capacity, and β-ODAP concentration in grass pea germplasm.

Alsamman *et al.,* (2023) have used grass pea seeds obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA) genebank. They have studied the complete set of NBS-LRR genes in the grass pea genome and explore their functionality and evolutionary path through various bioinformatics and laboratory techniques. Further research could focus on the functional analysis of these genes, and their potential use in breeding programs to improve the salinity, drought, and disease resistance of this important crop.

El Fatehi *et al*., (2021) have confirmed a diversity between *L. cicera* ecotypes in Morocco. They suggest that the characterization is the first step towards conservation. Subsequently, in a region where culture continues to decline, we must highlight the urgency of putting in place strategies to encourage the conservation of this heritage. The best strategy seems to us to be in situ conservation in a peasant environment by encouraging the maintenance of traditional cultures.

Detailed information about the current conservation status of *Lathyrus* species is documented by WIEWS (World Information and Early Warning System on Plant Genetic Resources), which contains information on national PGR holdings (www.fao.org/ag/agp/pgr/wiews/) and SINGER (System-wide Information Network for Genetic Resources), which contains information on CGIAR holdings (http://www.cgiar.org/singer).

Table 8 lists the main collections of *Lathyrus* around the world, together with an indication of the composition of each collection in terms on the percentages of accessions of wild relatives, landraces and breeding materials (breeders advanced lines etc.), as well as the percentage of the collection that originated in the country concerned (GCDT, 2009).

Table 9 gives a breakdown of the collections in terms of species richness. The two largest collections (Pau, France and ICARDA) both comprise about 50% *L. sativus*. The collections in the main grass pea producing countries all have high percentages of *L. sativus*: those in Bangladesh, Ethiopia, India and Nepal all comprise at least 70% *L. sativus*.

Based on all accumulated information, important gaps were identified in the coverage of global genetic diversity in existing collections (Table 10). But a more detailed analysis is needed on the conservation status, species richness coverage, geographic coverage, arrangements made for regeneration. The efforts of collection, conservation and characterization should be extended to the associated *Rhizobium* strains (GCDT, 2009).

Table 8: Major *Lathyrus* collections in the world (data collected from 2005 and 2007).

No	Country	Genebank /institutes	TOTAL No of *acces.*	Wild relatives	Landraces	Breeding material	Origin collected in country
1	GLOBAL	ICARDA	3327	45%	54%	0.1%	17%
2	France	Université de Pau, IBEAS	4477	n.a.	n.a.	n.a.	34%
3	India	NBPGR	2619	3%	85%	12%	94%5
4	Bangladesh ***	GRC BangladeshAgric. Res. Inst.	1841	-	100%	-	100%
5	Chile	Centro Reg. De Inv. Carillanca	1424	n.a.	n.a.	n.a.	n.a.
6	Australia ***	Australian Temp. Field Crops Coll.	986	28%	39%	19%	0.6%
7	Russia ***	VIR	848	43%	30%	18%	40%
8	Canada	PGRC, Canada	840	10%	90%	-	n.a.
9	USA	Western RegionalPlant IntroductionStation, USDA, Pullman, Washington	669	n.a.	n.a.	n.a.	7%
10	Ethiopia ***	BCRI	588	2%	75%	25%	98%
11	Germany***	IPK	568	40%	n.a.	n.a.	5%
12	Spain ***	Fernando Franco Jubete	543	n.a.	n.a.	n.a.	n.a.
13	Algeria	Institute National Agronomique	437	n.a.	n.a.	n.a.	n.a.
14	Hungary ***	Research Centre for Agrobotany	394	1%	22%	n.a.	22%
15	Spain ***	INIA	377	14%	86%	-	100%
16	Bulgaria***	Institute for PGR "K.Malkov"	368	1.6%	80%.	n.a.	5.4%
17	Turkey	AARI	363	94%	n.a.	n.a.	100%
18	Nepal***	Nepal Agricultural Research Council	164	-	100%	-	100%
19	Armenia ***	Institute of Botany, National Academy of Sciences of Armenia	157	98%.	1%	1%	99%
20	Pakistan	Plant geneticResources Institute	130	n.a.	n.a.	n.a.	n.a.
21	Portugal***	Genebank,, Braga	199	5%.	30%	n.a.	45%
22	China	CAAS	80	n.a.	n.a.	n.a.	100%
23	Azerbaijan ***	Genetic Resource Institute, NationalAcademy of Science	66	47%	33%.	20%.	100%.
24	Czech Republic ***	Research Instituteof Crop Production	52	75%	-	25%	56%.
25	Greece ***	Greek Genebank,Agricultural Center of Mecedonia and Thrace	47	-	2%	98%	100%
26	Slovakia ***	Research Instituteof Plant Production	47	-	87%	13%	87%.
27	Cyprus ***	Agricultural Research Institute	31	-	100%	-	100%
28	Poland ***	PGR Laboratory, Research Instituteof Vegetable Crops	10	-	-	100%	30%.
TOTAL			**21652**				

n.a. Detailed information not available/ * ECPGR/Pau database/ ** EURISCO database/ *** Accession-level ICARDA (2007)

Table 9: Number of accessions for the three major *Lathyrus* species.

Country	Genebank / institutes	No of acc. *L. sativus*	No of acc. *L. ochrus*	No of acc. *L. cicera*	Total No of acc. All *Lathyrus*
GLOBAL	ICARDA	1756	137	208	3327
France	Université de Pau, IBEAS	2382	0	789	4477
India	NBPGR	2561	0	1	2619
Bangladesh ***	GRC Bangladesh Agric.Res. Inst.	1841	0	0	1841
Russia ***	VIR	632	21	195	848
Canada	PGRC, Canada	781	0	0	840
Chile	Centro Reg. de Inv.Carillanca				1424
Australia***	Aus. Temp. Field CropsColl.	592	51	302	985
USA	Western Regional Plant Introduction Station, USDA	242	25	33	669
Ethiopia***	BCRI	435	151	0	588
Spain *	Fernando Franco Jubete	108	0	328	543
Germany***	IPK	254	48	266	568
Algeria	Institute NationalAgronomique	10	0	16	437
Hungary***	Research Centre forAgrobotany	296	3	58	394
Spain***	INIA	157	7	179	377
Bulgaria***	Institute for PGR"K.Malkov"	213	38	44	368
Turkey	AARI	22	0	35	363
Greece *	Agricultural Center of Mecedonia and Thrace	208	0	112	320
Portugal	Genebank, Braga	136	0	116	256
Nepal***	Nepal Agricultural ResearchCouncil	164	0	0	164
Pakistan	Plant genetic ResourcesInstitute	11	0	0	130
Armenia ***	Institute of Botany, NationalAcademy of Sciences	3	0	154	157
China	CAAS				80
Czech Republic	Research Institute of CropProduction	3	0	0	52
Slovakia **	Research Institute of PlantProduction	47	0	0	47
Cyprus *	Agricultural ResearchInstitute	44	0	0	44
Azerbaijan ***	Genetic Resource Institute,National Academy of Science	29	0	37	66
Cyprus **	Agricultural ResearchInstitute	19	12	0	31
Poland **	Plant genetic Resource Laboratory, Research Institute of Vegetable Crops	16	0	0	16
Total					**21652**

* From ECPGR/Pau database/ ** From EURISCO database/ *** From accession-level data sent to ICARDA in April 2007

Table 10: Possible gaps in global *Lathyrus ex situ* conservation (GCDT, 2009).

Country	***L. sativus***	***L. cicera***	***L. ochrus***
Egypt	+	+	
Iraq	+	+	
Iran	+	+	
Tunisia		+	+
Greece			+
Turkey			+
Russia	Black Sea Coast and Volga-Kama region		
Iraq	Kurdish area		
Bangladesh	Syleth area (high altitude)		
India	Northeast and Eastern parts		
Ethiopia	High altitude areas, recently opened area by roads.		
Afghanistan	Northeast and Central part		
Spain	Almeria (Andalucía) and Murcia		

Blank: No gaps identified in the collections

\+ : Gaps identified

8.2 ICARDA genebank holdings of *Lathyrus*:

ICARDA has the second largest collection of *Lathyrus* germplasm for the Mediterranean region after Pau-France. At present ICARDA is concerned with collection and conservation for *Lathyrus* species in the Mediterranean region and other *Lathyrus*-growing areas of the world (Maxted *et al*., 2009).

ICARDA holds in-trust 3327 *Lathyrus* germplasm from 50 countries under the auspices of the International Treaty on Plant Genetic Resources for Food and Agriculture of the United Nations Food and Agriculture Organization (FAO) (Table 11). While the emphasis at ICARDA for genetic resources and improvement of *Lathyrus* is for three species (*L. sativus*, *L. cicera* and *L. ochrus*), a sizeable collection of 50 other species is being maintained (Table 11).

The majority of accessions of all species of *Lathyrus* held at ICARDA genebank, except *L. sativus*, are from the West Asia and North African region. The collections have been collected from cultivated or from naturally occurring populations, found mostly in disturbed habitats such as roadsides, crop fields and orchards.

The *L. sativus* accessions in the ICARDA collection are from Ethiopia and the Indian sub-continent and are local landraces. Besides, expeditions within the Mediterranean, expeditions from ICARDA have been made to Bangladesh, Ethiopia, India, Nepal and Pakistan, primarily to collect genetic resources of *L. sativus*. The majority of accessions of all species of *Lathyrus* held in the ICARDA genebank, except *L. sativus*, are from the West Asian and North African as well as Central Asian regions (CWANA). ICARDA focused on three species of *Lathyrus* (*L. sativus*, *L. cicera* and *L. ochrus*) but holds also around 50 other species (Table 12).

Around 60 % of these accessions are georeferenced, 65% are characterized and about 30% are evaluated for at least *ß*-ODAP content. Some accessions are characterized using DNA molecular techniques.

Table 11: ICARDA holdings of *Lathyrus* species in ex-situ conservation by origin.

Country of Origin	**Number of species**	**Number of priority species***	**Accessions of priority species*/Total number of accessions**
Afghanistan	2	0	0/25
Algeria	9	6	25/32
Armenia	11	5	25/52
Australia	3	2	2/3
Azerbaijan	13	4	20/57
Bangladesh	3	2	2/1114
Bulgaria	2	0	0/19
Canada	1	0	0/5
Cyprus	3	2	25/46

Czech Republic	2	1	1/3
Denmark	1	1	1/1

Ecuador	2	1	½
Egypt	2	1	½
Ethiopia	1	0	0/176
France	4	1	¼
Georgia	8	2	8/27
Germany	7	3	3/11
Greece	7	4	78/110
Hungary	1	0	0/5
India	3	2	3/10
Iran	6	3	3/22
Iraq	5	4	6/9
Italy	3	3	6/6
Jordan	9	5	21/39
Kazakhstan	1	0	0/1
Lebanon	8	6	14/23
Moldova Republic	2	1	½
Morocco	10	5	50/148
Nepal	2	0	0/86
Norway	1	0	0/1
Pakistan	5	2	2/89
Palestine	3	2	3/6
Portugal	5	3	16/28

Russian Federation	4	3	4/69
Slovakia	1	0	0/9
Spain	5	4	7/9
Switzerland	1	0	0/1
Syrian Arab Republic	27	16	327/560
Tajikistan	7	2	2/36
Tunisia	6	4	32/38
Turkey	21	13	170/364
Turkmenistan	7	3	9/25
Ukraine	1	0	0/32
United Kingdom	1	1	1/1
USA	1	0	0/1
Unknown	4	4	7/7
Uruguay	1	0	0/1
Uzbekistan	3	1	1/12
Total			**878/3327**

* Priority species which included only those species in crop gene pool GP1B and GP2 or taxon groups TG1b and TG2 (Maxted *et al.*, 2009

Table 12: ICARDA's holdings by *Lathyrus* species (Maxted *et al*., 2009).

Taxa_name	Countries represented	Number of accessions
Lathyrus amphicarpus	2	3
Lathyrus annuus	11	79
Lathyrus aphaca	18	304
Lathyrus articulatus	9	101
Lathyrus basalticus	1	5
Lathyrus belinensis	1	1
Lathyrus blepharicarpus	6	47
Lathyrus cassius	4	11
Lathyrus chloranthus	3	8
Lathyrus chrysanthus	1	7
Lathyrus cicera	24	208
Lathyrus cilicicus	1	6
Lathyrus ciliolatus	1	2
Lathyrus clymenum	7	17
Lathyrus cyaneus	1	2
Lathyrus digitatus	1	1
Lathyrus gloeospermus	1	2
Lathyrus gorgoni	5	67
Lathyrus hirticarpus	1	1
Lathyrus hierosolymitanus	4	112

Lathyrus hirsutus	9	43
Lathyrus inconspicuous	16	191
Lathyrus laxiflorus	2	2
Lathyrus marmoratus	5	28
Lathyrus nissolia	7	15
Lathyrus occidentalis	1	1
Lathyrus ochrus	16	137
Lathyrus odoratus	2	4
Lathyrus pallescens	1	1
Lathyrus pseudocicera	7	77
Lathyrus rotundifolius subsp. *Miniatus*	1	2
Lathyrus sativus	33	1756
Lathyrus setifolius	4	8
Lathyrus sp.	8	30
Lathyrus sphaericus	7	27
Lathyrus stenophyllus	1	2
Lathyrus sylvestris	1	1
Lathyrus tingitanus	7	13
Lathyrus tuberosus	2	2
Lathyrus vinealis	1	4
Total		**3327**

9. Bibliographic data on *Orobanche*:

In the Mediterranean region, broomrapes are considered plants formidable parasites particularly on legumes. The danger comes from the long viability of seeds, which exceeds ten years, and from their very high multiplication rate (Cubano, 1984).

They are holoparasites without chlorophyll completely dependent on their hosts, causing a significant loss of yield. This loss can be total on some crops, in case of heavy infestation.

9.1 Family of Orobanchaceae:

The family of Orobanchaceae contains 15 obligate parasitic genera with 250 species and is considered closely related to the Scrophulariaceae (Plaza *et al*., 2004; Pusch and Günther, 2009).

The Orobanchaceae constitute a family of dicotyledons, gamopetalous of the order Tubiflorales, located between the Scrophulariaceae and the Labiates (Aber, 1984). They are parasitic phanerogams, with leaves reduced to the state of bracts devoid of chlorophyll.

The whitish, brownish or bluish flowers are arranged in a simple or compound terminal cluster. The calyx is often reduced to two lobes, the lateral sepals being more or less divided. The corolla is bilabiate tubular. There are four stamens and the unilocular ovary with 2-3 carpels is extended by a style with a bilobed stigma (Quezel and Santa, 1963).

The Orobanchaceae family constitutes the end of the evolutionary cycle reached by holoparasites, which probably begins with Rhinantheae and Gerardieae (Cubero, 1983).

This family brings together holoparasitic plants widespread especially in the hemisphere boreal, in warm and temperate regions.

9.2 Genus of *Orobanche*:

The genus *Orobanche* comprises approximately 170 species that inhabit mainly the northern hemisphere (Uhlich *et al*., 1995).

Orobanche species parasitize and cause invasive damage to a wide range of economically important plants, e.g. legumes (faba bean, lentil, grass pea (Figure 7), sunflower, tomato and tobacco (Parker and Riches, 1993).

Orobanche comprises holoparasitic herbs, with alternative scale like leaves and flowers arranged in spike. Calyx campanulate, 4(-5)-toothed, or split into two lateral segments, each entire or 2-toothed, shorter than the corolla. The corolla is sub-zigomorphic bilabiate; upper lip more or less 2-lobed, often erect; lower lip 3-lobed (Saeidi Mehrvarz *et al*., 2010).

Species of the *Orobanche* genus exclusively parasitize roots of angiosperms, very often herbaceous dicotyledons. They bring together plants without chlorophyll, formed of an erect stem, emerging from the ground, they can be terminated by a single flower or in the majority of cases, by an inflorescence more or less elongated in a spike, more or less dense, sometimes in clusters, with scale-like bracts at the base of each flower. The fruits are capsular and contain numerous tiny black seeds (Punia, 2014). The underground part, generally bulged bulb-shaped, is attached to the roots of the host plant (Ait Abdallah, 2012).

Figure 7 : General appearance of *Orobanche crenata* in grass pea in the field (A) and in the glasshouse (B) at ICARDA, Morocco (Abdallah F., 2018-2019).

9.2.1 Botanical classification:

According to APG III simplified, 2009, *Orobanche* is classified taxonomically as:

Apogy: Vegetal

Phylum: Spermatophytes.

Subphylum: Angiosperms.

Class: Eudicotyledones.

Subclass: Evolved Eudicotyledons called Eu Asteridae I.

Order: Lamiales.

Family: Orobanchaceae.

Genus: *Orobanche*.

As stated by Joel *et al*., 2013, the classification of broomrape is described in the following figure:

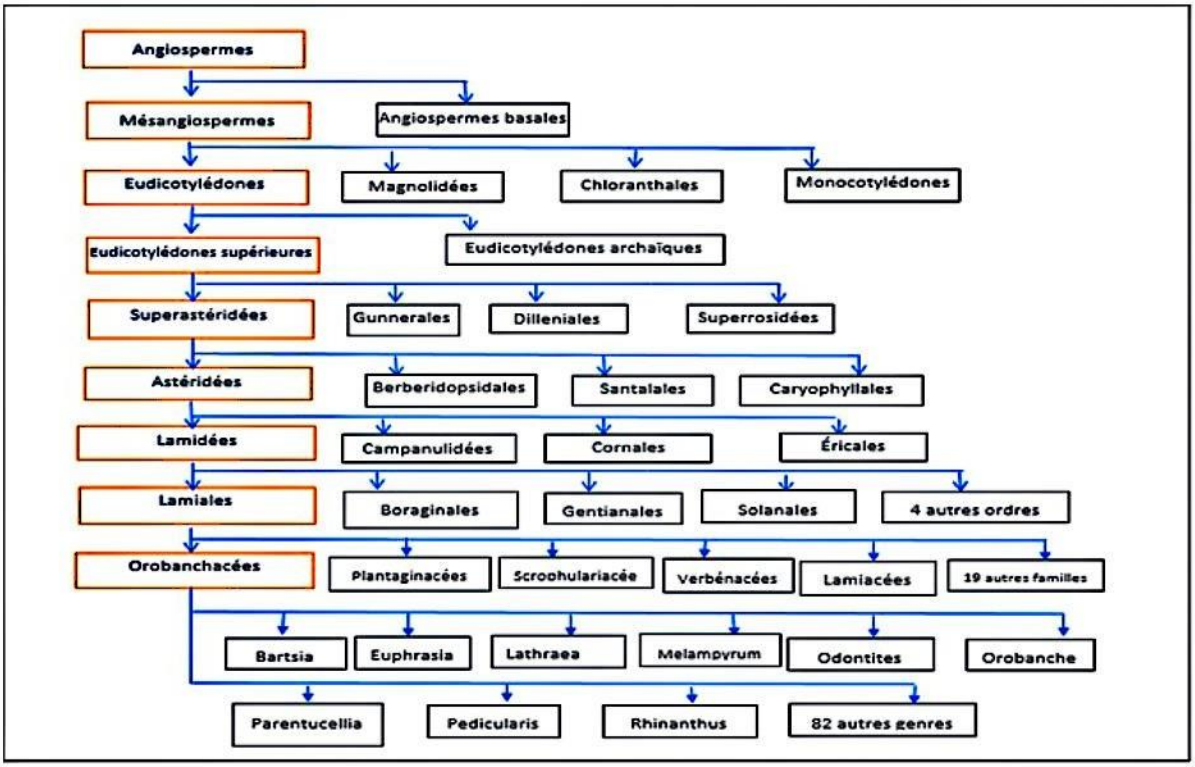


Figure 8 : *Orobanche* Classification (Joel *et al*., 2013).

9.2.2 Geographic distribution of *Orobanche* species in the world:

It is mainly distributed throughout the subtropical and temperate regions of the northern hemisphere, and the Mediterranean region is one of the most important centers of its diversity (Plaza *et al*., 2004).

The centers of origin of *Orobanche* species are thought to be Italy, Spain, Turkey and Morocco (Sauerborn, 1991). Then different species achieved broader distribution through international trade and commercial exchange of contaminated seeds which contributed to the worldwide spread of these species.

Out of the 140 known species of *Orobanche* (Kroeschel and Klein, 2002), about ten species are of economic importance, and represent a serious threat to a wide range of cultivated hosts, namely; *Orobanche aegyptiaca* Pers./*Orobanche ramosa* L., *Orobanche cernua* Loefl., *Orobanche cumana* Wallr., *Orobanche crenata* Forsk., *Orobanche minor* Sm. and *Orobanche foetida* Poir. The following species have been found in the participating countries (Table 13):

Table 13: *Orobanche* species in selected countries (Barakat, 2007).

Species / Countries	*O. aegyptiaca ramose*	*O. crenata*	*O. cernua*	*O. Cumana*	*O. minor*	*O. Foetida*
Algeria	X	x				
Egypt	X	x	X		x	
Ethiopia	X	x	X			
Morocco	X	x		X		X
Sudan	X	x	X		X	
Syria	X	x				
Tunisia	X	x				X

According to Parker and Riches (1993), the 5 most harmful species are: *O. cernua*, *O. crenata*, *O. ramosa*, *O. aegyptiaca* and *O. minor*.

The main broomrape species of economic importance as well as their geographical distribution in the world are summarized in table 14 and figure 9:

Table 14: The geographic distribution of economically important broomrape species (Foy *et al*., 1989).

Species	Geographic distribution
Orobanche ramosa L.	Central Europe, USSR, Mediterranean basin, all countries between Eastern India and Eastern Africa.
Orobanche aegyptiaca pers.	Introduced in Mexico and the USA, widespread in warm countries
Orobanche crenata Forsk.	Mediterranean Basin
Orobanche cernua Loefl.	Eastern Europe, USSR, Indian subcontinent

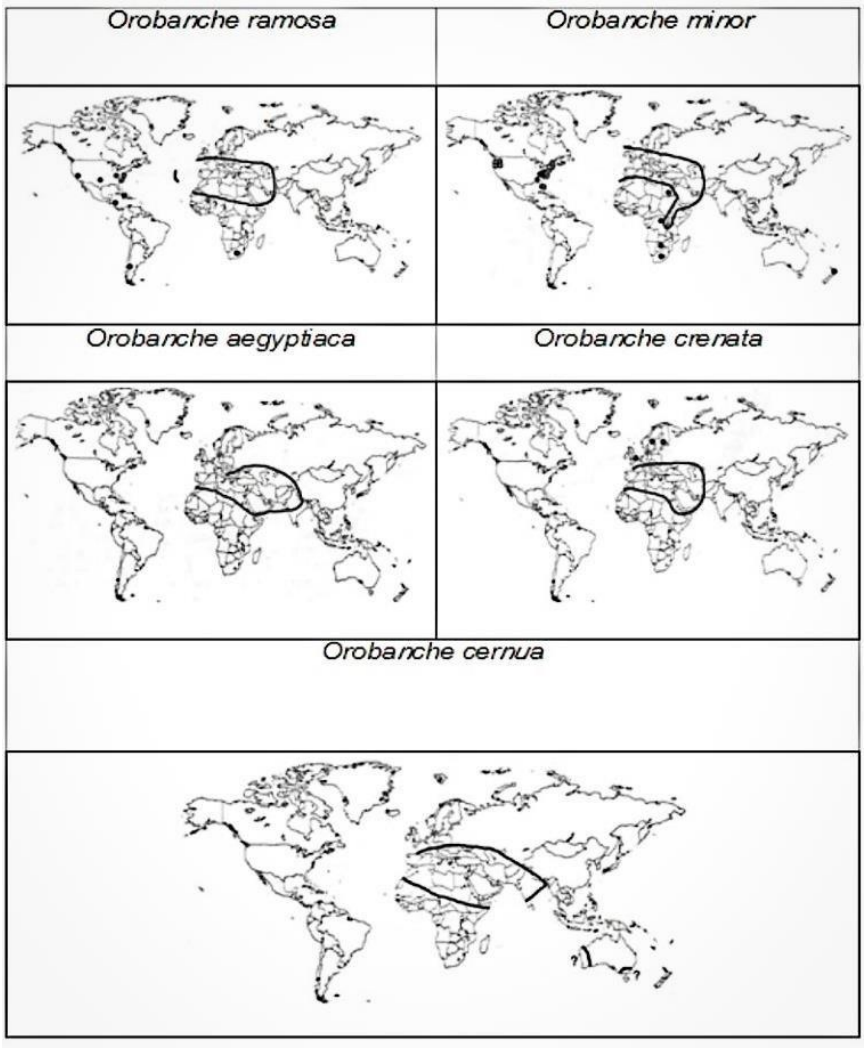


Figure 9 : Distribution of broomrape species with economic impact in the world (Parker, 1994).

9.2.3 Geographic distribution of *Orobanche* in Morocco and Tunisia:

9.2.3.1 In Morocco:

Four botanical families furnished the whole parasitic weeds of Morocco: Lauranthaceae, Cuscutaceae, Santhalaceae and Orobanchaceae. However, Orobanchaceae is the most important based on the distribution of its species, the infested area and the damages caused on host crops. (Bouhache and Dahan., 2007).

According to a recent survey, *Orobanche crenata* Forsk. (on faba bean, lentil, pea, chickpea and carrot); *Orobanche ramosa* L./*Orobanche aegyptiaca* Persl. (on faba bean, lentil, pea, chick pea, alfalfa, rape seed, tomatoes, melon and carrot and *Orobanche foetida* Poir. (patches on faba bean and alfalfa) are considered to be the most damaging to crops in Morocco (Saffour, 2003). Two highly infested regions in Morocco which use broomrape control techniques; Saiss et Pre-Rif (Bouhache and Dahan., 2007). *O. foetida* was identified infecting common vetch (*V. sativa*) on a small farm in Taounate, Saiss Region, 50 km north of Fes. The level of infection was moderate (0.1 to 0.2 broomrape plants per vetch plant) (Rubiales *et al*., 2005).

Orobanche tetuanensis Ball (Orobanchaceae), a problematic taxon initially described from specimens found in the Beni Hosmar Mountains (Tetuan, Morocco), was identified from the original material collected by Ball, 1878.

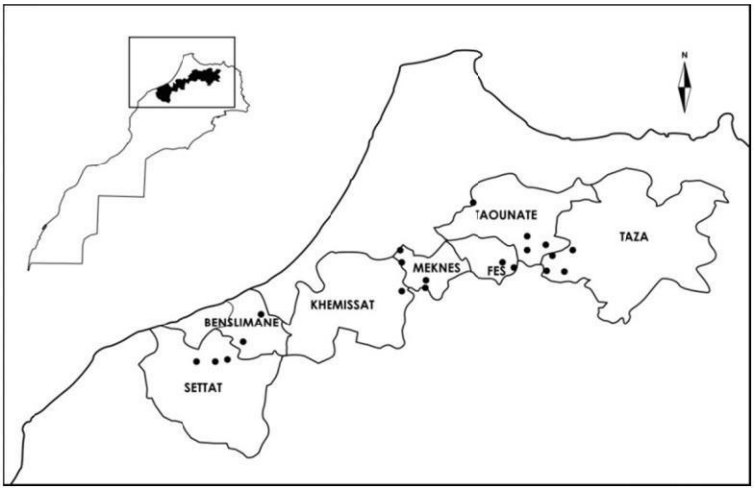


Figure 10: *O. crenata* populations naturally infested faba bean fields from different regions in Morocco (Ennami *et al*., 2017).

9.2.3.2 In Tunisia:

In Tunisia, the dominant broomrape species are *O. crenata*, *O. foetida* and *P. ramosa*, with no accurate estimation of their impact on Tunisian agriculture even though 5000–70,000 (ha) hectares of legume crops could be infected (Amri *et al*., 2019).

In order to overcome this problem, some farmers have been replacing sensitive legume crops with others, such as sunflowers, oilseed rape and garlic (personal observation). However, the above strategy is not sound since the first infestation of *O. cumana* in sunflowers has been reported, particularly in the most infested regions (the Beja region) (Amri *et al*., 2012).

In Tunisia, broomrape is dispersed in the capital Tunis, in the North and the middle regions. Figure 11 revealed the geographic distribution of *Orobanche foetida* and *Orobanche crenata* samples in Tunisia. Currently *Orobanche foetida* is observed mainly in the North-West regions while *Orobanche crenata* is mainly found in northern coastal regions, central Tunisia, the Sahel and the south. *O. ramosa*/*aegyptiaca* is reported in some northern and central regions and currently does not constitute a major problem on crops. Zouaoui (2000), has found the latter in the Cap-Bon region on carrots with a low infestation and on a weed plant near a tomato field. This species was observed on Ariana faba bean (Kharrat, non publied).

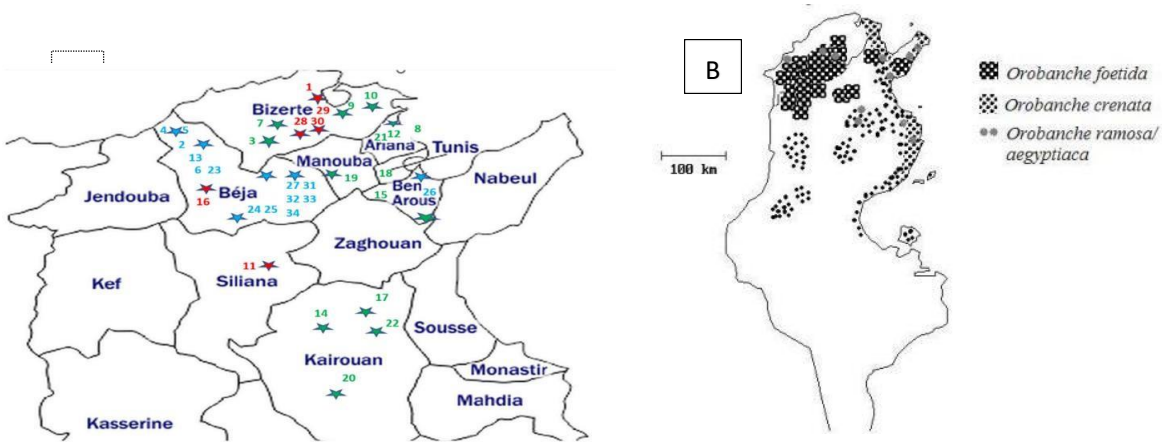


Figure 11: Geographic location map of brommrape in Tunisia. A/ (Parasite samples-blue; *O. foetida*, green; *O. crenata,* red; *P. ramosa;* Khamassi *et al*., 2023)/ B. (Kharrat *et al*., 2007).

9.3 Life cycle of *Orobanche*:

Years of investigation have uncovered an elegant system of chemical signaling by which root parasitic plants (Striga and *Orobanche*) recognize a potential host plant and regulate their development in order to optimize their chances for survival. (Kuijt, 1969; Musselman, 1980; Parker and Riches, 1993). Several mechanisms exist that insure tighter coordination between developmental stages of parasite life cycle and the one of the host plants (Figure 12). The biological cycle of the broomrape takes place in two phases:

- ✓ The underground phase which lasts between 30 and 80 days.
- ✓ an aerial phase which lasts approximately 30 days.

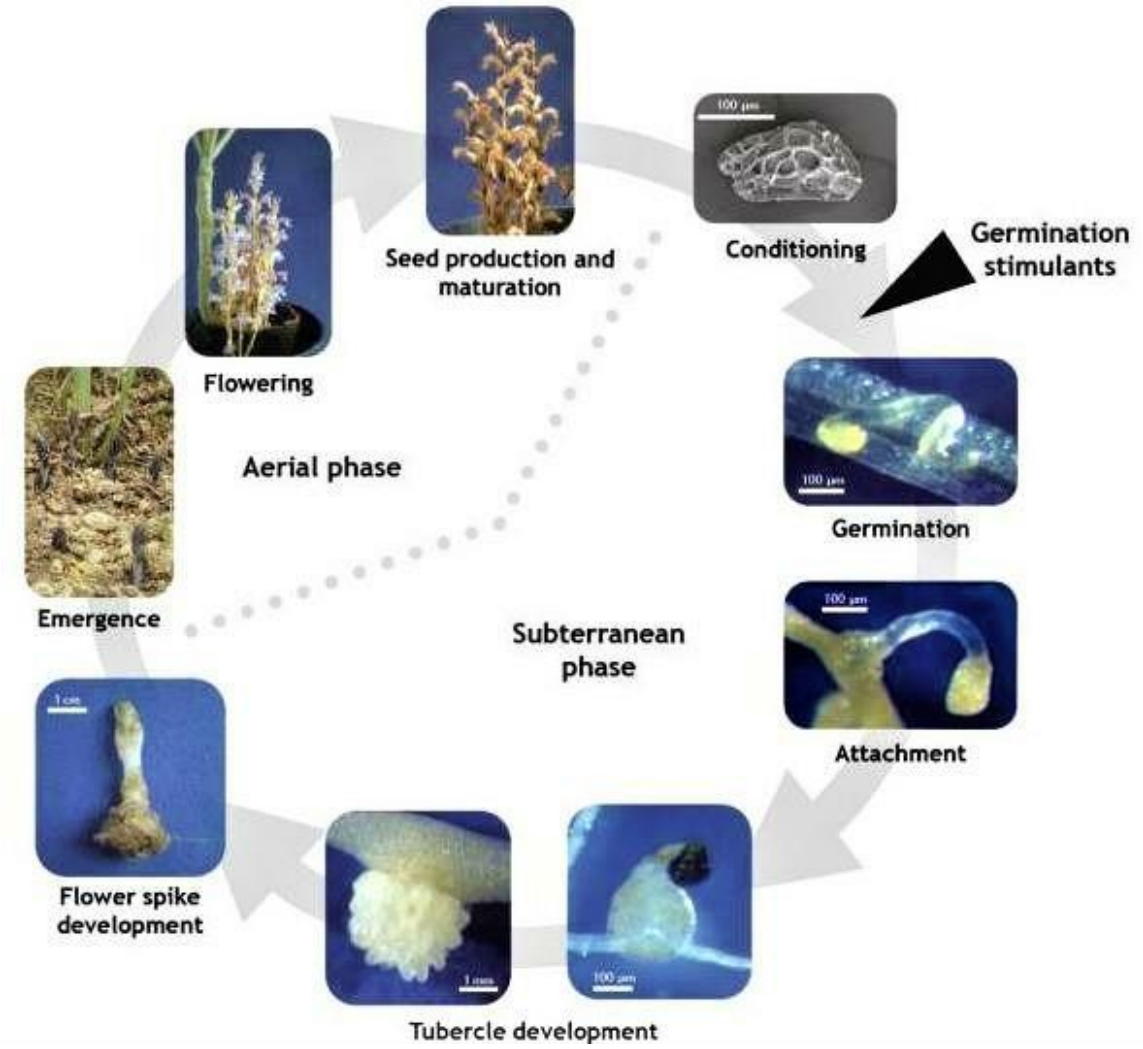


Figure 12: Life cycle of *Orobanche* (Baghyalakshmi *et al*., 2019).

9.3.1 Subterranean phase:

9.3.1.1 Seed production:

Mature broomrape plants produce between 50.000 and 500.000 seeds per generation (Gevezova *et al*., 2012), dust sized weighing 3 to 6 µg (Parker and Riches, 1993) and very difficult to recognize without a magnifying microscope.

Each capsule contains 600-800 seeds and a single plant may produce more than one lakh seeds depending upon species. Seeds have a pattern of raised ridges (rough surface) and hardened testa, surrounding a fatty endosperm that has an undifferentiated embryo at one end (Kadry and Tewfic, 1956). Once ripe, some seed may remain in the capsule but majority of them fall to the ground. Seeds can emerge from as deep as 15 cm below the soil surface. Seed generally remains viable in soil for 10 to 13 years (Brenchley, 1920) but the viability can be up to 20 years (Puzilli, 1983).

Cubero (1983) estimates that a plant of *O. crenata* can produce between 40.000 and 500.000 seeds. According to Parker and Riches (1993), a capsule of *O. ramosa* contains between 600 and 800 seeds and that of *O. crenata* more than 4000. The average number of seeds per capsule varied from 1000 to 4400 (López-Granados and García-Torres, 1991; Teryokhin, 1997). An average number of 2500 seeds per fertile capsule is assumed in the model and the number of fertile capsules per plant was density-dependent (López-Granados and García-Torres, 1991). An extensive study of *Orobanche* capsules revealed that, with rare exceptions, 10±25% of seeds in each capsule were not developed enough to germinate and were therefore non-viable (Teryokhin, 1997).

9.3.1.2 Seed dormancy and germination:

Seed germination depends on the presence of a suitable host plant (Musselman, 1996) and on prevailing environmental conditions. Seeds need to undergo a pre-conditioning period in the form of warm stratification before they can germinate (Fernández-Aparicio *et al*., 2016). The pre-conditioning period requires moist and warm (59°F [15°C] to 68°F [20°C]) environmental conditions from 5 to 21 days.

According to Uematsu *et al*., (2007), for germination to proceed, the seeds require an after-ripening period and next specific preconditioning, which has been linked with water imbibition, production

of gibberellins and cAMP, which seem to be prerequisites for germination.

The conditioned seed then can germinate in response to a signaling compound (strigolactone, SXSg, and resorcinol released from the host plant root (Butler, 1995; Pierce *et al*., 2003; Joel *et al*., 2007). If conditions remain conducive, multiple flushes of germination can occur within a single season; however, in the absence of stimulants, these preconditioned seeds re-enter dormancy. As the environment becomes drier, the seed's ability to germinate gradually reduces.

9.3.1.3 Attachment:

After germination, the radicle of the broomrape seedling grows a few millimeters in length and attaches to the host plant (Figure 12). If it fails to attach to a host within a few days, the radicle exhausts its food reserves and dies (Fernández-Aparicio *et al*., 2016).

Following attachment to the host plant, the radicle develops into a specialized modified root called a haustorium, a plant organ common to all parasitic plants (Buschmann *et al*., 2005). The haustorium fuses into the vascular system of the host root and serves as the bridge for extraction of nutrients and water from the transport systems (phloem and xylem) of the host (Fernández-Aparicio *et al*., 2016).

Penetration requires the presence of mediators such as exo-enzymes. The process is similar to that of infection by pathogenic fungi (Wegmann, 1996). The hypothesis proposed concerning the connection between the parasite and the host according to Foy *et al*., (1989) is similar to graft fusion, whereas ultrastructural observations on the fusion point do not indicate a direct connection between the phloem of host tissues and the parasite. On the other hand, it is direct between the xylem of the haustorium and the host (Saghir *et al*., 1973; Dörr and Kollmann, 1974; cited by Foy *et al*., 1989).

The haustorium develops when intrusive cells of the parasite penetrate host tissues, eventually reaching the conductive system of the host. The mechanisms of intrusion are only partly understood and probably includes (but are not limited to) mechanical pressure employed by the intrusive cells to force their way through host tissues and secretion of the pectolytic enzymes pectin

methyl esterase (PME) and polygalacturonase to insure a smoother penetration (Ben-Hod *et al*., 1993; Losner-Goshen *et al*., 1998).

9.3.1.4 Tubercle development:

Once connected to a host plant, broomrape grows rapidly, forming a tubercle — a storage organ for nutrients and water extracted from the host -underground (Figure 12). The tubercle grows with age by swelling of the contact zone and formation of adventitious roots (Ait Abdallah, 2012).

9.3.2 Aerial phase:

9.3.2.1 Bud and underground stem:

The tuber turns into a bud, which elongates vertically into a young underground stem and progresses towards the soil surface. From the emergence, an axis devoid of chlorophyll develops near the host plant and marks the beginning of the aerial phase of the parasite cycle.

In a field, the infestation is therefore only visible when the parasite has emerged, which is too late because the damage has already been done (Ait Abdallah, 2012).

Multiple shoots develop from the tubercle and emerge above the soil surface, then grow to stalks from 6 inches (15 centimeters [cm] to 12 inches (30 cm) in height (Figure 12). The shoots, wrapped with alternate bracts, completely lack leaves and chlorophyll. Prior to flowering, young plants look like yellowish spikes (Osipitan *et al*., 2021).

9.3.2.2 Flowering:

Flowering begins within 3 to 7 days after a broomrape shoot emerges above the soil surface (Figure 12). Branched broomrape flowers are spike-like, irregular, bisexual and usually pale white to purple in color. The petals of the flower are merged, tubular and have an upper and lower lip (Osipitan *et al*., 2021).

9.3.2.3 Fructification:

After fertilization, the carpels are usually united to form a single chamber on the upper part of the flower; this chamber matures as a capsule with thousands of very tiny seeds, each smaller (0.2

millimeters to 0.4 millimeters) than a grain of sand. Seed production can occur within 14 days after flowering. A mature broomrape plant can produce hundreds of thousands of tan or brown-colored seeds, which can remain dormant and viable for many years (> 20) in soil. The entire life cycle, from seed germination to seed production, takes place within the March-to-August growing season of processing plants (Osipitan *et al*., 2021).

9.4 Impact of *Orobanche*:

A considerable loss in growth and yield of many food and fodder crops is caused by root parasitic flowering plants. Globally, the damage caused by *Orobanche* on field and vegetable crops is significant in the Near East (Linke *et al*., 1989; Abu-Irmaileh, 1994), South and East Europe and in various republics of the former Soviet Union. It causes yield losses ranging from 5-100 percent (Linke *et al*., 1989).

Thus, *Orobanche* hinders cultivation of many strategic crops including legumes, industrial crops, oil crops, vegetables, and threaten the livelihood of many nations. It attacks fruit trees as well as coffee and Catha plantations. Over one million hectares of broad bean are infested by *Orobanche crenata* or at risk around the Mediterranean region of which: 113 000 ha in Morocco and 5 600 ha in Syria, 10 000 ha in Tunisia (Sauerborne, 1991). *Orobanche* can limit the choice of crop rotation to non-host crops, which in most cases, are less rewarding. Many farmers abandoned cultivation of faba beans, tomato, and tobacco in heavily infested fields. Many others abandoned agriculture altogether, forced to migrate and seek other means of income outside the rural domains.

Recent estimates on the impact of *Orobanche* in the participating countries, as obtained from farmers, officials, and recent literature can be summarized as the following:

9.4.1 Syria:

Heavy infestations with *Orobanche crenata* are encountered in food legume major growing areas (ex. Idlib) in 60 percent of the area causing 30-100 percent yield loss. Farmers were forced to replace lentil, faba bean and vegetables with less profitable crops such as black cumin, coriander, or cereal.

Heavy infestations with *Orobanche ramosa* are common in solanaceous crops in many regions. *Orobanche ramosa* infests over 70 percent of tomato, tobacco and eggplants in Dara'a governorate with infestation ranging between 25-50 percent inflicting 15-50 percent yield losses. It is estimated that *Orobanche* impact accounts for 5-15 percent of the total production management constraints facing agriculture in the country (Barakat, 2007).

9.4.2 Egypt:

Orobanche is second important problem after foliar diseases in faba bean and tomatoes and is number one pest in peas (El Hassanein and Salim, 1999). Once Egypt was exporting faba bean, but *Orobanche* infestations is impacting its production and threatening the livelihood of the nation, forcing the Egyptian government to import faba bean to meet the dietary demand of the population.

Faba bean cultivation was abandoned in Upper Egypt due to either many *Orobanche* or FBNYV infestation. *Orobanche* infestation in the newly reclaimed lands approached 30 percent of the 16 000 ha cultivated in this area (Barakat, 2007).

9.4.3 Sudan:

Orobanche species present in Sudan include: *Orobanche crenata*, *Orobanche cernua* and *Orobanche minor*. The major species is *Orobanche ramosa* infesting tomatoes and potatoes and hosted by many other vegetable crops (Babiker *et al*., 1994). It was also found on onions and many weedy hosts. Heaviest impact of *Orobanche ramosa* is in Khartoum and Al Jezira states on Nile soils. Infestation approached 100 percent of tomatoes and potatoes areas; consequently, large areas were put out of tomato production. Impact of *Orobanche ramosa* on potatoes is somewhat less than in tomatoes. Yield loss in potatoes was estimated by 30 percent.

Infestation with *Orobanche crenata* was only recently encountered, but spreading at a fast pace, in the two main states of faba bean production, threatening food security of the nation.

The first report on the presence of *Orobanche crenata*, was in 2001 in one site of about one hectare. Infestation spread within a 3 years' period to cover over 28 sites in the Nile states with an average infestation of 1 percent from the total 21 000 ha, and 12 percent in the Northern state, with an average yield loss ranging from 6-90 percent (Barakat, 2007).

9.4.4 Ethiopia:

Orobanche surveys indicated that several districts are plagued with *Orobanche* (Gojam and Gondar, Tatch Gayint, south Welo, Kedijo and Flagober). According to farmers, crop loss could reach as high as 75 - 100 percent. Farmers in Kedijo area indicated that the weed appeared for the first time in 1983 in Dehit area (Goshi-locality).

Infestation with *Orobanche ramosa* on tomatoes is concentrated in the River Awash basin of the Central Rift Valley threatening both the large scale commercial farms and the small scale subsistence farming communities. *Orobanche cernua* is serious pest of tobacco in Awara Malka and Nura Era. Due to *Orobanche* problems, some State Farms have had to give up growing tomato. The average yield reduction ranged from 30-40 percent.

The first collection and identification of *Orobanche crenata* was in 1993 in Amhara district - in one site (10 ha), presently infesting 19 locations in two districts.

Average yield loss for the whole farm production is estimated to be 36 percent and under extreme conditions yield losses in severely infested fields can reach up to 62 percent (Barakat, 2007).

9.4.5 Algeria:

The dominant *Orobanche* species in Algeria include: *Orobanche crenata* in northern Wilayats (Sahel) where several farmers abandoned growing legume crops and replaced them with cereals and vegetables; *Orobanche ramosa* on industrial tomatoes in Annaba and Ainn Defleh areas; and *Orobanche cernua* Southern Algeria, Saharan area. It was estimated that 60 percent of the area in the middle coast is infested with *Orobanche crenata*.

The available information indicated that average yield losses in legumes are 25 percent in the Sahel area. *Orobanche ramosa* infests autumn planted vegetable crops (tomatoes, pepper, carrots, lettuce and cucumber). Estimate of infestation in tomatoes in Ain Defla was about 70 percent in Boumadfaa, and about 55 percent in Al-Ataf (Barakat, 2007).

9.4.6 Morocco:

The main species of *Orobanche* in Morocco are *Orobanche crenata* and *Orobanche ramosa.* The more virulent species, *Orobanche foetida* was also reported to occur. Infestation of *Orobanche* in legumes progressed from 12 percent in 1981, 26 percent in 1994, to 30 percent in 2001 (Ben Yassine *et al*., 2002) and reached 51 percent in 2003 growing season. The estimated average yield losses due to *Orobanche crenata* were 37.4 percent in 1998, forming about 55.4 percent of annual local legume consumption. The estimated percent of the impact of *Orobanche* infestation alone amounted to 40 percent of the total impact of all other stresses in crop management. Legume acreage has been declining for the past 30 years for many reasons of which *Orobanche* infestation stands among the major reasons. This forced the government to pass a general regulation, in 1992, preventing the export of faba bean in order to ensure food security.

9.4.7 Tunisia:

The main *Orobanche* species in Tunisia include *Orobanche crenata, Orobanche foetida* and *Orobanche ramosa*. The exact estimates for the impact of *Orobanche* in Tunisia are lacking (Kharrat and Halila, 1994). However, it was indicated that about 5 000 ha out of 70 000 ha planted to food legumes might have *Orobanche* infestation. Some farmers replaced food legumes by sunflower and garlic. Yield losses ranged from 20 to 80 percent. *Orobanche foetida* is common in the Northern parts on large and small seeded faba bean beside other species of different families. Production failure occurs under high infestations. *Orobanche ramosa* was also cited attacking leguminous crops, as well as many vegetables and tobacco.

The estimated levels of *Orobanche* incidence in different countries are not very well quantified. They are approximate estimations in many cases. Yet, these estimates could point out the magnitude of the parasite problem. However, the incidence of parasitism varies within the same location with time according to the prevailing weather conditions, especially temperature and soil moisture and level of soil fertility. Mesa-García and García-Torres (1991) showed that crop rotation, irrigation, fertilization, variety and sowing date affect *Orobanche* infestation affect the severity of infestations.

Research results indicate that there is a great potential for improving the productivity and yield stability if the biotic and abiotic stress factors are effectively controlled and the inherent yield potential of the cultivars is improved. Various workshops on parasitic weeds at large or specifically on *Orobanche* discussed various management methods, claiming various levels of successes. In spite of the diverse methods of *Orobanche* control, the adoption of many methods is hampered by many draw backs in the subsistent farming systems (Barakat, 2007).

9.5 Management strategies against broomrape:

The available methods of control against broomrapes have not proven as effective, economical and applicable as predicted (Goldwasser *et al.,* 2008; Pérez-de-Luque *et al.,* 2010). Although several potential control measures were developed over the past few decades for some crops, any approach applied alone is often only partially effective and the results are sometimes inconsistent due to variable environmental conditions. Therefore, the only effective way to combat weedy root parasite like *Orobanche* to date is through an integrated approach, combining a variety of measures in a concerted manner (Habimana *et al*., 2014).

9.5.1 Preventive measures:

The strength of broomrape lies in its ability to form a bank of seeds in the soil. A management or eradication program must aim at reducing this seed bank, while minimizing the production of new seeds and their dispersal to new sites. Quarantine is therefore an essential element in control or eradication programs (Habimana *et al*., 2014).

The best option for winning against broomrapes is avoiding the fight. It is not possible when the fields are already infested with the seeds, but preventive measures must be taken into consideration to avoid spreading the infestation into neighboring fields (Habimana *et al*., 2014).

The main ways for broomrape seeds dispersal are through machinery and tools, and together with the host seeds; proper phytosanitary measures in and around the field are necessary to reduce the spread of *Orobanche.* Farm equipment and machinery should be cleaned prior to their use in uninfested fields. Special care must be applied to disinfestation and cleaning of field machinery and harvesters, and avoid trucks going from infested to non-infested fields. Containment is a must

to avoid spreading of the infestation and eradication programs should be considered. *Orobanche* shoots should be removed prior to flower opening (Habimana *et al*., 2014).

The collected shoots should be burnt or disposed of properly. Good extension agents could easily convince the farmers to execute such task, especially when they made aware of the tremendous production of *Orobanche* seeds per plant. One important spreading agent of various weeds, including *Orobanche,* is the uncontrolled movement of grazing animals. Grazing animals should be forbidden to enter un-infested fields after grazing infested areas (Panetta and Roger, 2005). Furthermore, farmers should use certified seed in order to insure themselves it is clean of parasite seeds.

Fresh contaminated manure aggravates *Orobanche* problem. Farmers should be instructed to use fermented manure, as fermentation process kills the seeds of the parasite. Pre-plant composting fresh manure under plastic mulch in the planting rows causes *Orobanche* seeds to lose viability within six weeks, and reduces *Orobanche ramosa* infestation on many vegetables. This practice could be a useful asset in high value crops. Fermenting manure in the farm can be easily practiced by subsistent farmer without much input and can aid sustainable farming strategy (FAO, 2008).

Strict quarantine measures, at various levels, national and international, help in preventing the introduction of the parasite into parasite-free areas. Technical inspection of imported agricultural materials should be carried out by a subject matter specialist in parasitic weeds (FAO, 2008).

9.5.1.1 Cultural control:

9.5.1.1.1 Hand weeding:

This technique is quite expensive and is only recommended in the event of a weak infestation or as a complement to other techniques to avoid increasing the seed stock in the plot. *Orobanche* stems should be pulled out no later than the time of the pest's flowering and should be immediately incinerated. Kharrat and Halila (1994) observe that this technique does not significantly increase the faba bean yield in a plot heavily infested by *O. foetida* and attribute this to the damage caused by the parasite during its underground development phase.

Weeding of *Orobanche* is mainly accomplished after the parasitic damage has already been done. It is not likely to show any yield increase in the short term. Weeding is laborious and time-consuming, and not very promising in highly infested areas. However, in combination with other methods, it can reduce the seed bank very efficiently (FAO, 2008).

9.5.1.1.2 Use of trap and captivating cultures:

Rotation with non-host crops is usually suggested. The use of "trap crops" offers the advantage of preferentially stimulating broomrape suicidal germination. Flax, fenugreek and Egyptian clover are established to be successful trap crops for *O. crenata*. There are claims that a reduction in infestation has been reported in rotations with rice, due to water flooding (Sauerborn and Saxena, 1986), however, this has not been substantiated. The incorporation of resistant legumes in crop rotations may also maintain broomrape infestation at low levels.

Captivating culture "Catch crops" allow the parasite to germinate and develop. They are generally sown at high density to allow the germination of a greater number of seeds of the parasite and are destroyed before the flowering of the latter. This technique is practiced in certain countries of the Mediterranean basin using fava beans as a host plant (Ait Abdallah, 2012).

In Tunisia, the results suggested that in fields infested with *O. foetida,* the use of bean, pea, flax and fenugreek in the crop rotation may reduce the *Orobanche* seed bank. This has been confirmed by experiments showing the pea stimulates germination of *O. foetida* seeds but is resistant to infection, therefore, reducing seed bank (Fernández-Aparicio *et al.*, 2011). Whereas in fields infested with *O. crenata,* crops such as bean, flax, alfalfa, wheat and oat used in the crop rotation may reduce the soil seed bank of this broomrape (Abbes *et al*., 2008).

9.5.1.1.3 Fertilization:

Field trials show that nitrogen fertilization could reduce crop infestation by broomrape and striga (Pieterse, 1996). Effectiveness depends on the form and formulation of the nitrogen fertilizer used. In this regard, ammonium sulfate fertilization has given the best results (Jain and Foy, 1987; Pieterse, 1996). Sauerborn (1991) also reported the beneficial effect of manure for broomrape control.

Mesbah *et al*, (2012) reported that with increasing of nitrogen fertilizers concentration, studied indices (bush height of sunflower, fresh weight of stem, leaf and crop of sunflower) were increased. In the other side, with increasing of nitrogenous fertilizer, the amount of broomrape germination was decreased. In interaction of type of nitrogenous fertilizers and amount of nitrogenous fertilizer, the maximum yield of sunflower seed in the concentration of 5 ppm of urea fertilizer was obtained. The minimum amount of broomrape germination also was obtained from the concentration of 5 ppm of urea fertilizer.

Urea at 276 and 207 kg N ha^{-1}, ammonium nitrate, and ammonium sulfate at 207 kg N ha^{-1} and the goat manure at 20 and 30 t ha^{-1} were found to be most effective in reducing parasitism of *Orobanche* and enhancing growth of tomato plants (Haidar and Sidahmed, 2006).

9.5.1.1.4 Sowing date:

Several researchers have found that late sowing helps reduce attacks of *Orobanche* (Nassib *et al*., 1984; Kukula *et al*., 1985; Mesa-Garcia and Garcia Torres, 1986; Arjona-Berral *et al*., 1987; Hezewijk *et al*.,1987). In Tunisia, the results experiments show that late sowing of beans (second half of December) allows a reduction of 46.5 and 54% in the number and dry weight of *O. foetida* emerged stems which leads to an increase in yield of 20% (Kharrat and Halila, 1994). In Algeria, Aït Abdallah and Hamadache (1996) show that late sowing leads to reduction in the number and dry weight of *O. crenata* on bean, as well as an increase in yield.

Germination of *Orobanche crenata* tends to be very much reduced below 8 Â°C and further development is greatly reduced at low temperatures (Habimana *et al*., 2014). Abundant experimental evidence in faba bean shows that shifting sowing from October to November, December or January reduces numbers and dry weight of attached and emerged broomrapes, both *O. crenata* and *O. foetida* (Grenz *et al.*, 2005a). Two factors are known to reduce parasite damage in late-sown crops: (i) decreased *O. crenata* germination due to suboptimal soil temperatures and (ii) obstructed *O. crenata* development during underground stages. Since faba bean development is less susceptible to low temperatures and can be accelerated by increasing day- length, pods enter the critical phase of rapid biomass accumulation relatively earlier than parasites. As a result, more parasites and lesser pods are aborted (Grenz *et al.*, 2005a) observed a

more pronounced effect of late sowing in dry years, which also may indicate the existence of soil moisture-driven effects.

9.5.1.2 Physical Control (Solarization):

Solarization is a fairly effective technique for controlling broomrapes. However, the high cost of the operation does not allow its use on a large scale. Soil solarization is the heating of soil by sunlight trapped under a mulch of black, or more usually clear, polyethylene film. The temperatures of 48-57 Â°C kill *Orobanche* seeds that are in the imbibed state; therefore, soil must be wet at the time of treatment. Seeds of *O. ramosa* can survive 35 days at 50 Â°C in dry air, but are quickly killed by temperatures of 40 Â°C when wet at the time of treatment (Habimana *et al*., 2014).

This technique has been used successfully on cropping land in many countries around the world like Middle East with an endemic *Orobanche* problem, as a pre-planting treatment for tomato, carrot, eggplant, faba beans and lentils. Soil solarization has been proven to be the most effective methods in controlling broomrape in open crops fields (Haidar and Sidahmad, 2000). This approach has attracted the interest in many warm-climate countries because of its effectiveness, simplicity, and safety for humans, plants, and the environment. Solarization entails covering wet soil with transparent polyethylene sheets during the hot season. There are advantages to using solarization: specifically, it is a simple, non-chemical, non-hazardous method that avoids the use of any toxic materials, does not contaminate the site, and is, therefore, suited to organic farming or other low-input agricultural systems. As global environmental quality considerations grow in importance, along with an increasing human population, evolving concepts, such as soil solarization and other uses of solar energy in agriculture, will become more important (Ashrafi *et al.*, 2009).

Solarization for 30 to 50 days during the hot season made it possible to control *O. crenata* and *O. aegyptiaca* on beans and lentils (Sauerborn *et al*., 1989). The reduction in dry weight of *Orobanche* in both bean and lentil fields is more than 90%. The effect of solarization persists for the following two years provided that the soil is not worked after the solarization treatment. Jacobsohn and Kelman (1980) also used this technique to control *O. aegyptiaca* on carrot.

9.5.2 Curative control:

9.5.2.1 Chemical Control:

Suicidal germination by application of synthetic strigolactones to the soil and other germination stimulants: the seeds of broomrape require chemical signals from the host root to germinate, the use of chemicals with the ability to stimulate broomrape seed germination in the absence of a suitable host may lead to a reduction in the *Orobanche* seed bank. This is "suicidal" germination because; once germinated, the *Orobanche* seeds cannot return to dormancy and cannot survive for longer than a few days without nutritional supply from a host. This is an ideal control strategy, but its utility is limited in real-world applications. Initial attempts to deplete broomrape seed banks using synthetic Strigolactone were made by using the synthetic Strigolactone analogue GR7 (Fernández-Aparicio *et al.*, 2011).

The herbicides that are currently in use for broomrape control are glyphosate, and herbicides belonging to the imidazolinones (Eizenberg *et al.*, 2006a) or sulfonylureas. The use of glyphosate on beans makes it possible to fight against *O. crenata* (Schmitt *et al*., 1979; Schluter and Aber, 1980; Mesa-Garcia and Garcia-Torres, 1984; Kharrat and Halila, 1994; Aït Abdallah and Hamadache, 1996) obtained a reduction in the number and weight of *Orobanche* of 98.3% on average. They observed an increase in bean yield of 53.2%, following a double spraying glyphosate at a dose of 70 g.a.m./ha at 15-day intervals from the tuber stage. Arjona-Berral *et al*., (1987) recommended the use of glyphosate at 40 or 60 g.a.m./ha in double applications on peas and lentils and noted better tolerance to the product by the lentil than by the pea. The effect of glyphosate is improved by the addition of ammonium sulfate (Ramirez-Ortega and Garcia-Torres, 1992).

Herbicides from the imidazolinone group have given satisfactory results for the control of broomrape in pre-emergence and post-emergence applications (Garcia-Torres *et al*., 1991). In Tunisia, Kharrat and Halila (1994) obtained good control of *O. foetida* on bean with imazethapyr and imazaquin applied as pre-emergence. The effect of imidazolinones and in particular of imazapyr depends on the edaphic conditions and mainly on the level of organic matter in the soil, its pH and its texture (Ramirez-Ortega and Garcia-Torres, 1992). Coating or immersing pea and

bean seeds in imazethapyr, and lens in Imazapyr provided satisfactory control of *O. crenata* (Garcia-Torres *et al*., 1996).

Chemical control of broomrape has been extensively explored since the 1970s. However, this form of control is complicated by a number of factors including: (i) it is effective only as a prophylactic treatment, since in most cases we do not know the infestation level; (ii) the parasite is directly connected to the host; (iii) if the herbicide is to be applied to the parasite through the conductive tissues of its host, the host must be selective to the herbicide without reducing its phytotoxicity; (iv) the parasite can often continuously germinate throughout the season, developing new infections (Pérez-de-Luque *et al.*, 2010).

9.5.2.2 Crop resistance:

The screening of genetic resources has enabled the identification of a certain number of lines with some resistance to *O. crenata* in broad beans, chickpeas, vetch and peas (Kasasian, 1973; Cubero and Martinez, 1980; Linke *et al*., 1993; Cubero, 1994) and *O. foetida* in broad beans (Kharrat unpublished). In beans, lines of the major type seem to be more sensitive to *Orobanche* than those of the equina or minor type (Cubero, 1973; Ciccarone and Piglionica, 1979; Cubero and Moreno, 1979).

Breeding of legume crop cultivars resistant to *Orobanche crenata* Forsk. was initiated with faba bean improvement in the early 1960s. Only low levels of resistance to *O. crenata* were available in faba bean, until the appearance of the Egyptian line F402 (Nassib *et al*., 1982). Similarly, resistance against *Orobanche foetida* Poir. has also been identified in faba bean germplasm. Some lines (674 ⁄ 154 ⁄ 85 L3-4 and 402 ⁄ 29 ⁄ 84) selected against *O. crenata* have also shown a high level of resistance to *O. foetida*, with a high yield potential (Abbes *et al*., 2008).

Abdallah Fadoua *et al.*, (2020) reported that screening at hot spots in Morocco and Tunisia resulted in the identification of resistant accessions of wild *Lathyrus* species against the parasitic weed. The level of resistance to *O. foetida* was higher among wild species compared with *O. crenata*. Field results showed complete resistance for *O. crenata* and *O. foetida* by *L. articulatus* L. and moderate resistance by *L. aphaca* L. and *L. ochrus* (L.) DC. Resistance to *O. crenata* in *L. sativus* accessions

was validated in a pot experiment under controlled conditions. Two accessions—namely, IG64782 and IG65197—showed complete resistance to *O. crenata*. A moderately resistant accession, IG116989, that revealed low infestation in the field showed high susceptibility in pot experiment. The results indicated that the resistance against *O. crenata* and *O. foetida* was associated with slow development of the established tubercles and low induction of parasite germination.

Resistance to *O. crenata* does not seem to protect against *O. foetida* infection, but combining resistance to both species has been possible, by selecting for *O. foetida* resistance in *O. crenata*-resistant germplasm. Little resistance is available within field pea germplasm against *O. crenata* (Rubiales *et al*., 2003b), but promising sources of resistance have been identified in wild relatives within the genus Pisum (Rubiales *et al*., 2005), which have successfully been hybridised with cultivated pea (Pérez-de-Luque *et al.*, 2005).

Resistance is also very limited in cultivated grass pea (*Lathyrus sativus* L.) and chickling vetch (*Lathyrus cicera* L.) (Fernández-Aparicio *et al*., 2009), but is available in related *Lathyrus* species. Resistance in lentils has only recently been reported (Fernández-Aparicio *et al*., 2008c). However, resistance is frequent in common vetch and chickpea germplasm and cultivars (Gil *et al*., 1987; Rubiales *et al*., 2003c; Fernández-Aparicio *et al*., 2008b), as well as in their wild relatives (Rubiales *et al*., 2004, 2005; Sillero *et al*., 2005). Resistance to *Phelipanche aegyptiaca* Pomel (formerly *O. aegyptiaca*) has been found in *Vicia atropurpurea* Desf. (Goldwasser *et al*., 1997).

Genetic studies using Cavalli and diallel tests concluded that resistance to *O. crenata* in faba bean and common vetch (*Vicia sativa* L.) was controlled by a quantitative genetic system with strong additive effects. Dominance and epistatic effects seemed to depend on the environment or upon the level of infestation. When dominance effects were present, susceptibility was usually dominant over resistance in faba bean, whereas the opposite occurred in common vetch (Gil *et al*., 1987; Cubero and Moreno, 1999).

The mechanisms of resistance to Broomrape are being studied in certain cultivars; it is mainly mechanical. It manifests itself, in resistant cultivars, by necrosis of the cells exposed to parasites (Zaitoun and ter Borg, 1994) or by osmotic pressure high (Wegmann *et al*., 1991).

Nassib *et al.*, (1982) attribute the resistance of the Giza 402 cultivar to the morphology of its root system and Khalaf and El-Bastawesy (1989) explain it by a fairly reduced root biomass. Other authors have concluded that there is no effect of biomass on the resistance and sensitivity (ter Borg and van Ast, 1991; Kharrat *et al*., 1994).

In addition, the secretion of exudates which stimulate seed germination broomrape does not appear to influence the host response (Khalaf and El-Bastawesy, 1989; Van Woerden *et al*., 1994).

9.5.2.3 Biological control:

Biological control involves the use of biological agents or processes to damage seed, kill weedy plant or interfere with parasite-host relationships. A few examples of biological control of broomrapes have been reported in the literature:

- **Mushroom:**

Linke *et al.*, (1992) isolated, from samples of *O. crenata* Forsk. and *O. minor* Sm. collected in Syria, Morocco and France, 36 mushrooms belonging essentially to the genera *Alternaria*, *Fusarium* and *Ulocladium*. Preliminary tests in the laboratory and in the field showed that the fungus U. atrum infects and destroys at 20°C and in relative humidity conditions between 50 and 80%, the tubers and stems of *O. crenata* that have emerged.

- **Bacteria:**

Zermane (2005) evaluated 337 strains of bacteria associated with the rhizosphere of bean-*Orobanche* couple, for their antagonistic activity towards *O. crenata* and *O. foetida* under controlled conditions. The results of this study showed that 70.3% and 83.8% of strains significantly reduced the stages of underground development of *O. crenata* and *O. foetida*, respectively and that rhizobacteria, particularly *Pseudomonas fluorecents* and certain strains of *Ralstonia pickettii* are of certain interest as effective candidates for biological control.

Strains of *Escherchia coli* and *Azotobacter* spp. genetically transformed were tested for their ability to induce germination of *O. crenata* seeds in vitro and in soil (Khalaf *et al*., 1997). Mabrouk *et al.*, (2007) showed that certain strains of *Rhizobium* reduce infestation of pea plants by *O.*

crenata. This reduction in parasitism would be explained by a reduction of parasite seed germination.

- **Pests:**

An insect herbivore, *Phytomyza orobanchia*, is known to be specific for broomrapes and feeds on broomrape ovules and seeds, there by reducing broomrape seed production (Fernández-Aparicio et *al*., 2016).

Linke *et al.*, (1990) showed that the fly *Phytomyza orobanchia* is present in 95% of sites prospected in Syria. Of the 1890 capsules examined, approximately a third was attacked by this fly, which caused a reduction in *Orobanche* seeds of around 29%.

According to Rebouh and Bouznad 2006, garlic and lavender extracts can reduce the germination of seeds of *O. crenata* in vitro between 100 and 91%, respectively.

9.5.2.4 Integrated method:

Total control of *Orobanche* can only be ensured by the integration of certain methods where each of them can contribute to the fight against this parasite, reduce the level of infested plots and prevent the extension of infestation areas. This integrated approach must take into account the production systems of the region and must be economically profitable. Integrated pest management developed in the Mediterranean region mainly concerned the bean (Aït Abdallah and Hamadache, 1996).

In Syria, work on *O. crenata* on lentils, chickpeas and peas made it possible to develop an integrated control approach based on the use of an herbicide from the group imidazolinones with late sowing (Linke, 1992; Pieterse *et al*., 1994).

Addition of an imidazolinone herbicide application such as imazapic or imazamox 63-70 days after planting prevents almost completely broomrape shoot emergence and seed setting of inflorescences present in the field during herbicide application (Hershenhorn *et al.*, 2006).

Mohammed *et al.*, (2012) conducted experiment to assess the effect of combination of bacterial strains and chicken manures on broomrape on faba bean, the results displayed that among all treatments faba bean inoculated with TAL 1399 alone or in combinations with *Bacillus megatherium var Phosphaticum* (BMP) or *Azospirillum brasiliense* (Ab) plus chicken manure at 35 g pot^{-1} displayed no *Orobanche* emergence (above the ground) until the end of experiment. Furthermore, crop treated with TAL 1399 plus chicken manure at 35 g pot^{-1} was significantly higher in root, shoot and total dry weight as compared to control and other treatments.

Three isolates of *Trichoderma* species including *T. harzianum* T1, *T. harzianum* T_3 and *T. viride* T_2 were tested for control of *Orobanche* species in peas, faba bean and tomatoes under field conditions in Egypt. Results of field studies showed that soil treatment with these three fungal agents alone or soil treatment with fungal agents plus aerial spray of glyphosate (50 ppm) was efficient and cost-effective method in reducing infection, minimizing the number of spikes parasitic on host plants and increasing yields of peas, faba bean and tomatoes (Mokhtar *et al.*, 2009).

Integrated control of broomrape weeds means to combine and to integrate different preventive measures and control instruments into the given farming system (Table 15).

Table 15: Promising combinations for integrated *Orobanche* control in legume crops (Habimana *et al*., 2014).

Lentil	Delayed sowing (Dec./Jan.) + genotype, adapted for late sowing (+ herbicide, depending on severity of field infestation)
Chickpea	Less infected variety (ILC 482); herbicide (imazethapyr 50g a.i./ha post-sowing) only if infestation of the site is heavy
Faba bean	Less infected or resistant variety (Spanish material) + imazethapyr 2 x 30g a.i./ha; imazethapyr 2 x 20g a.i./ha), or delayed sowing + herbicide
Narbon vetch	Less infected genotype (e.g. ICARDA acc. 578) + sowing delayed for two weeks (under heavy infestation: imazaquin 2 x 20g a.i./ha) in spring)
Vetch	Less infected genotype (e.g. ICARDA acc. 715) + sowing delayed for two weeks
Forage legumes	Less/not infected species (*Vicia dasycarpa*, *Lathyrus ochrus*), or delayed sowing + herbicide, or mowing for hay production followed by soil tillage
Field pea	Delayed sowing + herbicide (imazaquin 2 x 20g a.i./ha in spring)

10. Genetic diversities:

Several methods have been used to study the phylogenic relationships among different *Lathyrus* species including morphological traits (Shehadeh, 2011), crossability (Yunus, 1990), karyotype analysis (Murray *et al*., 1992; Battistin and Fernández, 1994; Schifino-Wittman, Lan, and Simioni, 1994), chromosome banding and in situ hybridization (Murray *et al*., 1992; Unal *et al*., 1995) and molecular markers (Croft *et al*., 1999; Badr *et al*, 2002; Ceccarelli *et al*., 2010). Different regions have been claimed as the primary centres of diversity of grass pea, in particular South Asia (Chowdhury and Slinkard, 2000; Wang *et al*., 2015) and the Ethiopian highlands (Vavilov, 1927).

10.1 Analysis of morphological diversity:

These measurements have the advantage of being immediately available, of not requiring sophisticated equipment and of being the most direct measurement of the phenotype, therefore being available for immediate use, which is an important asset. However, morphological determinations must be carried out by an expert in the species, they are subject to changes due to environmental factors and can vary according to the stages of development and their number is limited.

Diversity can be observed for many phenotypes; flower colour, seed size, seed colour and patterning and biomass production (Tadesse and Bekele, 2003a; Polignano *et al*., 2005; Vaz Patto and Rubiales, 2014). This diversity is partially correlated with the geographic spread of grass pea cultivation. While most European accessions produce white flowers and large, flattened, cream coloured seeds, most South Asian accessions produce blue flowers and smaller, more rounded, dark and speckled seeds (Campbell, 1997). The species of Mediterranean/European origin were systematically more productive, with larger and later seeds (Hanbury *et al*., 1999). These species had a low ODAP content (Abd-El-Moneim *et al*., 2001).

According to Ulloa and Mera (2010), the environment has a strong effect on the average weight of pea seeds and the effect is stronger as the seed weight increases.

Pandey *et al.*, (1995) reported that wide variability in plant height (15 to 68 cm), number of branches per plant (1.8 to 28.40), number of pods per plant (2.4 to 59), pod length (1.88 to 5.18), width of pods (0.26 to 1.3 cm), number of seeds per pod (1.6 to 4.6), biological index (0.4 to 51 g), the seed yield per plant 0.62 to 19.81 g and the ODAP content 1.28 to 0.872% in *Lathyrus sativus*. Similarly, Dixit *et al*., (1996) observed the highest coefficient of variation for pods per plant followed by grain yield and weight of 100 seeds in this crop.

Malek (1998) assessed a wide range of high genotypic variation for days to maturity, the number of seeds per pod and ODAP content in 1821 *Lathyrus* accessions collected from three different agro-climatic regions of Bangladesh.

Sixty-six accessions were evaluated by Sammour *et al*., (2007a) representing 18 species of the genus *Lathyrus sativus* collected in different geographical regions on variations in quality traits (100 pea seeds, ash, total proteins and ODAP content contained in seeds).

Sedehi *et al.*, (2008) evaluated the morphological characters of local varieties of *Lathyrus sativus*. Analysis of variance indicated highly significant differences between 20 local accessions.

Lioi *et al.*, (2011) reported the genetic relationships among 13 *Lathyrus sativus* peas grown in Southern Italy, using agronomic characters. The data obtained provided useful information for the choice of the best local pea varieties for the marginal areas of Southern Italy.

10.2 Analysis by molecular markers:

Molecular approaches have been increasingly applied to plant systematics and phylogenetics to elucidate relationships between allied taxa (Soltis, Soltis, and Doyle, 1992a). Molecular diversity analysis supported a close phylogenetic proximity between *L. sativus* and *L. cicera* based previously on morphological and hybridization studies (Kupicha, 1983; Jackson and Yunus, 1984).

Chtourou-Ghorbel *et al.*, (2001) concluded that random amplified polymorphism DNA markers (RAPDs) are equivalent to restriction fragment length polymorphisms (RFLPs) in assessing the genetic diversity of *Lathyrus* species belonging to the sections *Lathyrus* and *Clymenum*.

Recently, six amplified fragment length polymorphism (AFLP) markers along with 47 morphological characters were used to clarify the taxonomic and phylogenetic relationships within and between the sections and the species of the genus *Lathyrus*, subjecting 184 accessions belonging to 9 predefined sections and 144 originating from the Mediterranean basin and Caucasus, Central and West Asia regions (Shehadeh, 2011). The results showed that the sections *Aphaca*, *Clymenum*, *Lathyrostylis* and a large part of the *Lathyrus* section could be differentiated either by using morphological characters or AFLP markers.

Croft *et al.*, (1999) studied the intraspecific genetic diversity of 8 *Lathyrus* accessions using RADP analysis, while Belaid *et al*., (2006) proved it by ISSR method only in 5 populations.

Ambade *et al*., (2015) studied the genetic diversity of 48 accessions with 10 parents using ISSR markers which showed polymorphism. Among 48 genotypes, Bio-L-203 and RLK120 are the least similar with a similarity coefficient of 0.63.

Wang *et al*., (2015) employed 30 SSR loci to assess the genetic diversity and structure of individuals from wild and domestic populations in Africa, Europe, Asia and ICARDA. The results showed that the cultivated species wee quite disctinct from released species, however a low level of differenciation was revealed between their geographical origins.

Genetic diversity of numerous *Lathyrus* species has been assessed with DNA markers in addition to morphological analyses (Croft *et al*., 1999; Chtourou-Ghorbel *et al*., 2001; Belaid *et al*., 2006; Lioi *et al*., 2011; Shehadeh, 2011).

Marghati *et al.*, (2016) used Sequences related amplified polymorphism (SRAP) markers to evaluate the interspecific variations in 40 genotypes of *Lathyrus* (four species) (Fabaceae). Ten SRAP primer combinations were obtained with high interspecific variability.

Different levels of diversity have been detected in different species using isozymes (Ben-Brahim *et al*., 2002), RFLPs (Chtourou *et al*., 2001), RAPDs (Croft *et al*., 1999; Barik *et al.*, 2007), chloroplast DNA restriction sites (Asmussen and Liston, 1998) and AFLPs (Badr *et al*., 2002).

Isozyme patterns on 18 accessions of five *Lathyrus* species allowed an unexpected grouping between *L. pubescens* and *L. sativus* (Schifino-Wittmann, 2001). By using the storage protein gene sequences, de Miera, Ramos, and Pérez de la Vega (2008) showed that *L. sativus*, *L. annuus*, *L. cicera* and *L. tingitanus*, all belonging to section *Lathyrus*, formed a monophyletic group, while *L. latifolius* of the same section is included in the group formed by *L. clymenum* and *L. ochrus* of the section *Clymenum*.

Chowdhury and Slinkard (2000) studied genetic diversity in 348 accessions and subaccessions of *L. sativus* from 10 geographical regions using polymorphism for 20 isozymes. They observed the closest genetic distance between populations from the Near East and North Africa. Populations from South Asia and Sudan–Ethiopia, though geographically widely separated, exhibited a closer genetic distance from each other than from other regions.

10.3 Breeding of low-ODAP varieties:

Breeding grass pea for low ODAP content has *been* full of uncertainties. These uncertainties were of global nature, and the primary challenge was fixing the low level of ODAP content obtained through years of research. In India, a grass pea cultivar P-24 with an ODAP content of 0.3% was developed. However, the instability of the low ODAP content has been indicated as a major bottleneck of this cultivar (Mehta and Santha, 2008). Several germplasm collections and introductions were screened at various agricultural research centres in Ethiopia. However, varietal performances were inconsistent with regard to ODAP content.

This implies that improvement in ODAP content has been achieved through breeding. However, further studies indicated that most of the varieties were unable to retain their low level of ODAP content when tested across different years. Although not surprising, the ODAP content of the officially released grass pea variety Wasie has increased from 0.2% when tested in some lowland areas (Fikre, 2008).

Analysis of a large number of *L. sativus* accessions revealed that samples from Bangladesh, Ethiopia, India, Nepal and Pakistan have a high *β*-ODAP content in dried seeds, in a range varying from 0.7% to 2.4%, while samples from North Africa, Syria, Turkey and Cyprus have a lower β-ODAP content, ranging from 0.02% to 1.2% (Abdel Moneim *et al*., 2000).

In Turkey, the studies carried out showed that genotypes 452, 508 and 519 have low ODAP content and low variability between winter and spring sowing (Karadag *et al*., 2010).

Among 66 accessions representing 18 species of the genus *Lathyrus* collected in different geographical regions, the Tunisian accessions present a low ODAP content and a high protein content (Sammour *et al*., 2007b).

Interspecific hybridization with wild *Lathyrus* species lacking or with very low ODAP content could be a solution. Moreover, it would make it possible to study the biochemical and genetic mechanisms which control the ODAP biosynthesis (Boukecha, 2019).

Narayan and Addis (2000) crossed *L. sativus* with 12 wild *Lathyrus* species. Only crosses between *L. sativus* and *L. pseudocicera* were successful; other crosses failed to produce viable seeds. In several interspecific crosses, pod development was observed but the embryo aborted during early stages of development.

A population of 64 single-seed selections from Pusa-24 were grown at Morden, Manitoba, Canada. These plants were selfed and seeds produced from offspring plants were assayed for ODAP. One plant was selected as having a very low ODAP content. Seed from this plant was bulked to give rise to LS8246 (Campbell and Briggs, 1987). LS8246 was crossed with the high-ODAP Indian landrace a-60 tolerant to powdery mildew to give rise to the variety Prateek (Sastri, 2008).

Low-toxin varieties have also been released in Bangladesh under the names BARI Khesari 1, 2 and 3 (Akter *et al*., 2015). One low-toxin variety, Ceora, has been developed in Australia (Siddique *et al*., 2006). Only one improved variety of grass pea (Wasie) has been released in Ethiopia to date (Kumar *et al*., 2011a).

10.4 Genetic mapping (QTL):

The development of genetic maps and QTL analyses were major breakthroughs in the characterisation of quantitative traits, enabling the identification of genomic regions associated with quantitative traits and their contribution to phenotypic variation. In addition, the mapping of QTLs is a useful tool to identify molecular markers linked to the resistance genes that could be used to assist breeding (Das *et al*., 2021).

In the absence of a sufficient number of robust marker systems for grass pea, the numbers of densely saturated linkage map have become limited for this crop in compared with those for other well-studied legumes. The availability of a densely saturated genome linkage map is expected to detect QTLs controlling the polygenes throughout the genome and subsequently tagging the QTL region with molecular markers for future MAS or map-based cloning of the underlying genes toward expediting the breeding programs (Das *et al*., 2021).

The DNA markers provide the necessary tools to determine the number, position and individual effects of genes/quantitative trait loci (QTLs) controlling interesting traits, such as genes for disease or insect resistance, reduced ODAP concentration, increased protein content, and other characters of agronomic importance (Campbell *et al*., 1994), via, for instance, genetic mapping and QTL analysis.

The first linkage map constructed for *L. sativus* was based on morphological traits (Sen and Ghosh, 1962). However, two *L. sativus* linkage maps have been developed using molecular markers to identify regions in the *Lathyrus* genome linked to desirable traits (Chowdhury and Slinkard, 1999). Limitation of this linkage map was that ~12% of the markers utilized in this study exhibited segregation distortion which increased the rate of false linkages in F2 populations and subsequently lead to the unreliable estimation of map distances. For studying the genetics of tendril trait, bulked segregant analysis was performed on the F2 population of sweet pea (*L. odoratus*) derived from a cross between parents with tendril (“Grace”) and without tendril (“Snoopea purple”) followed by detection of the RAPD marker (WB32a) linked with the tendril gene (Hanada and Hirai, 2003).

In 2004, another group of researchers constructed a genetic linkage map from a population of 92 backcrossed individuals derived from a cross between an accession "ATC 80878" resistant to ascochyta blight (*M. pinodes*) and a susceptible accession "ATC 80407" (Skiba *et al*., 2004a). Two QTLs associated with ascochyta blight resistance of stem of grass pea were identified (Manly et *al*., 2001).

RNA sequencing-derived marker (SSRs, SNPs) systems were deployed for the construction of linkage map of *L. cicera* (Santos *et al*., 2018). Comprehensive research is thus needed to map agronomically important as well as climate- resilient traits to further facilitate mapping and tagging of molecular markers linked to genes conferring these traits for achieving success in MAS for accelerating the breeding process of grass pea.

Table 16: Summary of molecular mapping studies carried out in grass pea (Das *et al*., 2021).

S No.	Population	Trait	Markers	Linkage group	Average marker density (cM)	Mapped loci	No. of QTLs	Reference
1	Four F_2 populations derived from eight sub accessions (PI 283564c.3 × PI 426885.2, PI 358601.5 × PI 173714.5, PI 426891.1×PI 172930.4, and PI 283549a.6 × PI 202803a.3)		11 isozymes (ACO-1, ACO-2, AAT-1, AAT-2, EST-3, EST6, FDH, LAP-1, PGD-2, SKDH, and TPI-1)	2	-	11	-	Chowdhury and Slinkard, 2000
2	F2 progenies from seven accessions of cultivated grass pea	-	9 isozymes	3	-	6	-	Gutiérrez et al., 2001
3	Eleven parents among which four were diploid ($2n$ = 14) and rest seven were primary trisomic ($2n$+1 = 15) types and M4 mutant populations	Flavonoid deficiency	Isozymes of enzyme aconitase (ACO) and S-nitrosoglutathione reductase (GSNOR)	-	-	4	-	Talukdar, 2012
4	F_2 population (Blue flowered: PI42689.1.1.3 × white flowered PI 283564c.3.2)	Flower color	71 RAPDs, 3 isozymes and 1 morphological marker	14	898cM	69	-	Chowdhury and Slinkard, 1999
5	Sweet pea (*Lathyrus odoratus*) F2 derived from cross between (Grace × Snoopea BSA) in sweet pea (*Lathyrus odoratus*) F2 population (Grace × Snoopea purple)	Tendril	302 RAPD markers	-	-	2	-	Hanada and Hirai, 2003
6	Backcross population (ATC 80878 × ATC 80407)	Aschochyta blight resistance	47 RAPD, 7 EST-SSR, 13 STS/ CAPS	9	803.1 cM	64	QTL1 (12%) QTL2 (9%)	Skiba et al., 2004a
7	F_5RIL (BGE008277 × BGE023542)	Rust (*Uromyces pisi*) resistance	189 SNP, 113 EST-SSR, and 5 Intron Targeted Amplified Polymorphism (ITAP) markers	9	724.2 cM	307	-	Santos et al., 2018

Chapter II: Wild Lathyrus species as a great source of resistance for introgression into cultivated grass pea (Lathyrus sativus L.) against broomrape weeds (Orobanche crenata Forsk. and Orobanche foetida Poir.).

I. Introduction:

Grass pea is a vital crop with significant potential for sustainable agriculture, particularly in regions vulnerable to climate change. While challenges such as broomrape infestation pose serious threats to its cultivation, ongoing research and breeding efforts aim to harness genetic diversity and develop resistant varieties, ensuring the continued relevance of this legume in global food systems.

In the Mediterranean region, grass pea faces significant challenges from parasitic broomrape weeds, particularly *Orobanche crenata* Forsk. in Morocco and *O. foetida* Poir. in Tunisia, which can severely impact yield and biomass (Fernández-Aparicio *et al*., 2016; Parker, 2009). A wide variety of approaches—physical, cultural, chemical and biological—have been explored against root parasites, but most of them are not effective, or not selective to the majority of susceptible crops. The above-mentioned control methods have not proven to be as effective, economical and applicable as desired (Parker, 1991; Joel, 2000; Joel *et al*., 2007). In order to achieve efficient control, broomrape parasitism should be targeted at different fronts by an integrated management strategy (Kebreab and Murdoch, 2001). Although *O. crenata* has a broad host range infecting most legume crops, levels of infection vary between the different species and between accessions within species (Rubiales *et al*., 2003b). No complete resistance to *O. crenata* is available in any legume so far, but existing levels of incomplete resistance in some accessions (Rubiales *et al*., 2006). Resistance against *Orobanche foetida* Poir. has also been identified in faba bean (Pérez-de-Luque *et al*., 2007).

This research was covered by a Crop Trust-funded project aimed at enhancing prebreeding in barley and grass pea, this study was conducted to screen 285 accessions representing 13 *Lathyrus* species against *O. crenata* Forsk and *O. foetida* Poir. under highly infested field conditions during two years 2017-2018. The field trials were conducted in two locations, Marchouch in Morocco and Beja in Tunisia. The goal is to identify potential sources of resistance and to confirm some resistance in pot experiment in the glasshouse at ICARDA, Morocco. Key data collected included the severity of broomrape infestation, aboveground biomass, seed yield and other agronomic traits.

II. Scientific contribution:

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Wild *Lathyrus* species as a great source of resistance for introgression into cultivated grass pea (*Lathyrus sativus* L.) against broomrape weeds (*Orobanche crenata* Forsk. and *Orobanche foetida* Poir.)

Fadoua Abdallah[1,2] | **Shiv Kumar**[1] | **Ahmed Amri**[1] | **Rachid Mentag**[4] | **Zakaria Kehel**[1] | **Rajia Kchaou Mejri**[5] | **Zine El Abidine Triqui**[2] | **Kamal Hejjaoui**[1] | **Michael Baum**[1] | **Moez Amri**[1,3]

[1] International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat, Morocco

[2] Faculty of sciences, Univ. Mohammed V, Rabat, Morocco

[3] AgroBioSciences, Univ. Mohammed VI Polytechnic (UM6P), Ben Guerir, Morocco

[4] National Institute of Agricultural Research (INRA), Rabat, Morocco

[5] Regional Field Crop Research Center (CRRGC), Beja, Tunisia

Correspondence
Shiv Kumar, ICARDA, Rabat, Morocco.
Email: sk.agrawal@cgiar.org
Moez Amri, AgroBioScience, UM6P, Ben Guerir, Morocco.
Email: moez.amri@um6p.ma

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Abstract

Broomrape weeds (*Orobanche* spp.) are root holoparasitic plants that cause serious damage to a range of legume crops in the Mediterranean and sub-Saharan African regions. Grain yield of cultivated species of grass pea (*Lathyrus sativus* L.) is almost negligible at the highest infection severity of *Orobanche*. Crop wild relatives (CWRs) have emerged as a novel source for many traits in diverse crops. *Lathyrus* is one of the largest genera with more than 160 species. In the present study, we screened 285 accessions representing 13 *Lathyrus* species for their reactions against two common broomrape species, *Orobanche crenata* Forsk. and *O. foetida* Poir, under field conditions. Screening at hot spots in Morocco and Tunisia resulted in the identification of resistant accessions of wild *Lathyrus* species against the parasitic weed. The level of resistance to *O. foetida* was higher among wild species compared with *O. crenata*. Field results showed complete resistance for *O. crenata* and *O. foetida* by *L. articulatus* L. and moderate resistance by *L. aphaca* L. and *L. ochrus* (L.) DC. Resistance to *O. crenata* in *L. sativus* accessions was validated in a pot experiment under controlled conditions. Two accessions—namely, IG64782 and IG65197—showed complete resistance to *O. crenata*. A moderately resistant accession, IG116989, that revealed low infestation in the field showed high susceptibility in pot experiment. The results indicated that the resistance against *O. crenata* and *O. foetida* was associated with slow development of the established tubercles and low induction of parasite germination.

1 | INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is a climate-resilient, nutrient-rich legume crop grown mainly in fragile agroecosystems since its domestication in the Balkan Peninsula (Kumar et al.,

Abbreviations: β-ODAP, β-N-oxalyl-l-α,β diamino propionic acid; CWR, crop wild relative; EODW, dry weight of emerged *Orobanche* per plant; EON, emerged *Orobanche* spikes per plant; ICARDA, International Center for Agricultural Research in the Dry Areas; PI, parasitism index; MR, moderately resistant.

2013). The crop holds immense value in human food, animal feed, and ecosystem services in South Asia (Kumar, Bejiga, Ahmed, Nakkoul, & Sarker, 2011), sub-Saharan Africa (Girma & Korbu, 2012), and the Mediterranean region (Vaz Patto et al., 2006). It is a promising crop for adapta- tion under climate change and variability because of its toler- ance to drought, waterlogging, and salinity (Jiang et al., 2013; Kumar et al., 2011; Lambein, Travella, Kuo, Van Montagu, & Heijde, 2019). With emphasis on plant-based protein diets and adaptation to climate change, grass pea has emerged as a focused crop for research, as well as for cultivation in difficult regions, in recent years (Sarkar et al., 2019). Despite the presence of a plant toxin called β-N-oxalyl-l-α,β diamino propionic acid (β-ODAP), the potential of grass pea as a health-promoting nutraceutical has recently been recognized (Lambein et al., 2019). The renewed interest in grass pea is also due to emphasis on animal feed and fodder, and diversification of cereal-based cropping systems, which occupy large areas under cultivation globally (Dixit, Parihar, Bohra, & Singh, 2016). The crop is almost free from major diseases and insect pests except some occasional appearance under favorable environmental conditions (Kumar et al., 2011). However, in the Mediterranean region of southern Europe and North Africa, the crop encounters the infestation of parasitic broomrape weeds, mainly *Orobanche crenata*, causing severe yield and biomass losses (Fernández-Aparicio, Flores, & Rubiales, 2016; Parker, 2009).

Broomrape weeds are root holoparasitic plants that attack a range of legume crops (Fernández-Aparicio et al., 2016; Rubiales et al., 2009; Trabelsi, Abbes, Amri, & Kharrat, 2015). Two species of broomrapes, *Orobanche crenata* Forsk. and *O. foetida* Poir., are reported to cause serious damage to grass pea crop (Fernández-Aparicio et al., 2016; Vaz Patto & Rubiales, 2009). Although the infestation of *O. foetida* is confined to Tunisia, *O. crenata* is a serious production constraint across the Mediterranean countries (Fernández-Aparicio et al., 2016). These parasitic weeds are completely dependent on the host for water and nutrients (Abbes, Kharrat, Delavault, Chaïbi, & Simier, 2009; Trabelsi et al., 2015). The germination of *Orobanche* seed occurs at the host roots in response to specific chemical compounds or germination stimulants released by the host plant (Abbes, Trabelsi, Kharrat, & Amri, 2019; Fernández-Aparicio et al., 2016; Trabelsi et al., 2015). Severe effects of *O. crenata* parasitism in the host crop, and high persistence of parasitic seedbank in agricultural soils, have led to abandonment of legume cultivation in major legume growing areas (Rubiales et al., 2009). The detrimental effect of broomrape parasitism in crop yield can reach up to 100% depending on infection severity and the broomrape–crop association (Fernández-Aparicio et al., 2016). In grass pea, reduction in aboveground biomass in both vegetative and reproductive organs was observed starting even at low infection severity, and half maximal inhibitory performance was predicted as 1.5 parasites per grass pea plant with almost zero grain yield at severities higher than four parasites per plant (Fernández-Aparicio et al., 2016).

Core Ideas

- Crop wild relatives are great source of novel traits/alleles
- Complete resistance to parasitic weeds Orobanche exists in wild relatives of grass pea
- Underground Orobanche attachments needs to be included inthe screening for Orobanche resistance

Several options including physical, mechanical, chemical, biological, and cultural practices have been suggested and tested to control *Orobanche* but none of them proved effective due to technical challenges, economic constraints, and the complexity of host–parasite interactions (Grenz, Manschadi, DeVoil, Meinke, & Sauerborn, 2005; Rubiales et al., 2009). Moderate and incomplete resistance has been reported in the cultivated species *L. sativus* (Fernández-Aparicio & Rubiales, 2010; Fernández-Aparicio, Flores, & Rubiales, 2012). Limited screening of crop wild relatives (CWRs) of grass pea, including *Lathyrus ochrus* (L.) DC. and *L. clymenum* L. (Sillero, Cubero, Fernández-Aparicio, & Rubiales, 2005), and *L. cicera* L. (Fernández-Aparicio & Rubiales, 2010; Fernández-Aparicio, Flores, & Rubiales, 2009), has shown some resistance to broomrape, indicating scope for introgression breeding. *Lathyrus* is a large genus containing >160 species (Lewis, Schrire, Mackinder, & Lock, 2005). Its center of diversity is located mainly in the Mediterranean and Irano-Turanian regions (Kupicha, 1983). The International Center for Agricultural Research in the Dry Areas (ICARDA) genebank holds 4,457 accessions of 45 *Lathyrus* species (Shehadeh, Amri, & Maxted, 2013). This collection also includes 1,555 accessions of wild species, which are recognized as an excellent source of novel traits or alleles due to their historical record of adaptation to a diverse range of habitats. This collection is unique because 35 and 64% of the accessions are respectively wild relatives and landraces, mainly of *L. sativus*, followed by *L. cicera* and *L. ochrus* (Kumar et al., 2013). Screening of this wild gene pool has emerged as a rich reservoir for traits of breeders' interest such as β-ODAP content, disease resistance, and so on (Aletor, El-Moneim, & Goodchild, 1994; Kumar et al., 2013; Patto et al., 2011). Therefore, efforts have been made to evaluate wild relatives to identify zero ODAP germplasm. Assessment of ODAP in wild relatives indicated that none of the species is free from ODAP. Under the Crop Trust-funded project on enhanc- ing prebreeding in barley (*Hordeum vulgare* L.) and grass pea (GS16005), the present study was undertaken to screen 285 accessions representing 13 *Lathyrus* species against *O.*

TABLE 1 *Lathyrus* species, number of accessions, and their origin screened for *Orobanche crenata* and *O. foetida* in field experiment during the 2017–2018 cropping season

Species	No. of accessions	Origin
L. annuus	2	Syria
L. aphaca	1	
L. articulatus	7	Bulgaria, Czech Republic, Morocco, Portugal, Syria
L. blepharicarpus	1	
L. cassius	1	
L. cicera	54	Australia, Greece, India, Norway, Syria, Turkey
L. gorgoni	5	Syria, Turkey
L. hierosolymitanus	2	Syria, Turkey
L. inconspicuus	4	
L. ochrus	44	Cyprus, Czech Republic, Germany, Greece, India, Italy, Russia, Syria
L. pseudocicera	2	
L. sativus	161	Afghanistan, Bulgaria, Canada, Cyprus, Ethiopia, Germany, Greece, Iran, Moldova, Turkey
L. tingitanus	1	Bangladesh

crenata and *O. foetida* under highly infested field conditions to identify potential sources of resistance for their use in breeding programs.

2 | MATERIALS AND METHODS

2.1 | Plant material

A total of 285 accessions representing 13 *Lathyrus* species were obtained from the ICARDA genebank and purified under cages during the preliminary screening in the 2016–2017 crop season. Single plant derived seeds of *L. annuus* L. (2), *L. aphaca* L. (1), *L. articulatus* L. (7), *L. blepharicar- pus* Boiss. (1), *L. cassius* Boiss. (1), *L. cicera* L. (54), *L. gor- goni* Parl. (5), *L. hierosolymitanus* Boiss. (2), *L. inconspicuus* L. (4), *L. ochrus* (44), *L. pseudocicera* Pamp. (2), *L. sativus* (161), *and L. tingitanus* L. (1) were evaluated for resistance to *O. crenata* and *O. foetida* under field conditions. These accessions are originated from different geographical regions (Table 1).

2.2 | Experimental details and site description

Field experiments were carried out following an augmented design with repeated susceptible checks of the faba bean (*Vicia faba* L.) cultivar 'Aguadulce' and grass pea (IG90174, IG147607, and IG147655) at two locations: Marchouch (33.56˚ N, 6.63˚ W; 392 m asl) in Morocco and Oued-Beja (36.44˚ N, 9.13˚ E; 150 m asl) in Tunisia during the 2017–2018 cropping season. The Marchouch experimental station of ICARDA represents a Mediterranean semiarid environment with ~400 mm annual rainfall (El-haddad et al., 2020). The Beja agricultural experimental station located in northwest Tunisia is characterized by a subhumid climate with moderate winters and ~600 mm average annual rainfall (Trabelsi et al., 2015). Field screening was taken up in a field plot with a known history of a high and homogeneous infes- tation of *O. crenata* at Marchouch, Morocco, and of *O. foetida* at Beja, Tunisia. There were 19 blocks, each consisting 15 plots of test entries, three plots of faba bean, and one plot each of three grass pea checks. Each accession was planted in a 1-m single-row plot, with 60-cm distance between rows. In each row, 20 seeds were sown by hand at a 5-cm depth maintaining a 5-cm space between plants. Every tenth row in each block was planted with highly susceptible faba bean cultivar Aguadulce. Our previous studies indicated that faba bean served as a good indicator on *Orobanche* infestation and homogeneity within a sick plot and a good facilitator of *Orobanche* infestation on companion crops (Ennami et al., 2017, 2020). In fact, among all the cool-season legume crops, faba bean is considered as the most susceptible crop to broomrapes and was used in our trial in order to monitor the uniformity of *Orobanche* infestation. To increase the infestation, early planting in the first week of December was conducted. Care was taken to raise a successful crop with recommended doses of fertilizers, and need-based irrigation using a sprinkler system. The field was kept weed free by manual uprooting of weeds other than broomrape, and no herbicide was applied to prevent interference with broomrape development.

2.3 | Weather conditions

Daily minimum and maximum temperatures (˚C), precipitation (mm), and relative humidity (%) were recorded throughout the crop season at the experimental sites. A daily mean temperature between 10 and 28 ˚C was recorded at Marchouch and Beja research stations during the crop season (Figure 1). At Marchouch and Beja stations, the crop received 504 and 495 mm of well-distributed rainfall, about 106 mm more water than the average, which augured well for a uniformly high infestation of *Orobanche*.

2.4 | Data recording

Observations were recorded on days to 50% flowering, maturity, and appearance of the first *Orobanche* shoot on a plot

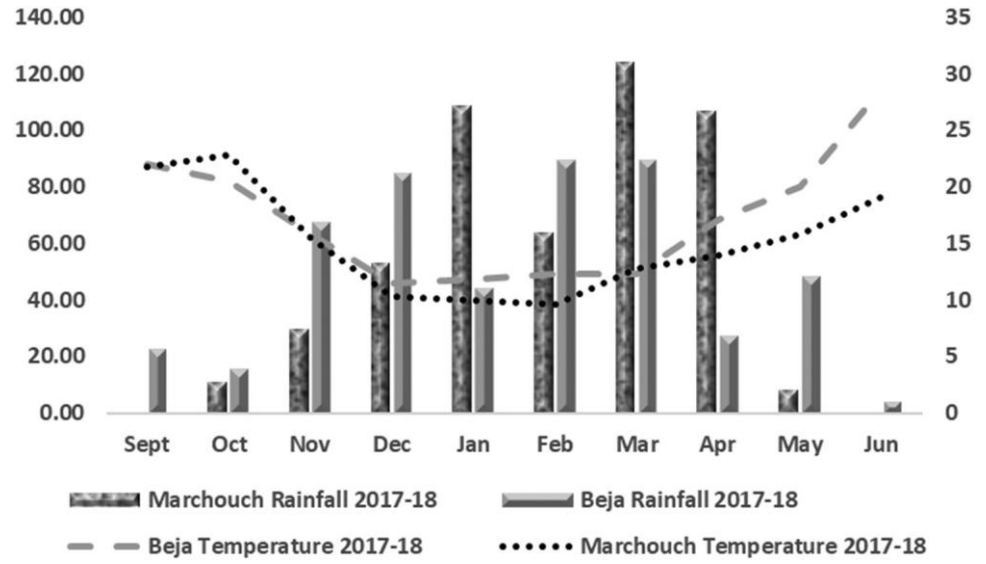

FIGURE 1 Rainfall (mm) and monthly air temperature (˚C) at Marchouch experimental station, Morocco, and Beja research station, Tunisia, during the 2017–2018 cropping season

TABLE 2 Incidence, severity, and parasitism index (PI) of different *Lathyrus* species tested against *O. crenata*- and *O. foetida*-infested plots in Morocco and Tunisia during the 2017–2018 cropping season

	O. crenata			*O. foetida*		
***Lathyrus* species**	**Incidence**	**Severity**	**PI**	**Incidence**	**Severity**	**PI**
	%	0–9 scale		%	0–9 scale	
L. annuus	95.00	6.00	5.85	0.00	1.00	0.00
L. aphaca	60.00	1.00	0.60	0.00	1.00	0.00
L. articulatus	0.00	1.00	0.00	0.00	1.00	0.00
L. blepharicarpus	30.00	1.00	0.30	0.00	1.00	0.00
L. cassius	15.00	1.00	0.15	0.00	1.00	0.00
L. cicera	74.70	1.64	1.19	0.19	1.02	0.00
L. gorgoni	35.00	3.67	1.81	0.00	1.00	0.00
L. hierosolymitanus	100.00	9.00	9.00	0.00	1.00	0.00
L. inconspicuus	85.00	5.00	4.85	0.00	1.00	0.00
L. ochrus	2.39	6.64	0.20	0.00	1.00	0.00
L. pseudocicera	75.00	1.00	0.75	0.00	1.00	0.00
L. sativus	54.88	8.21	4.48	0.47	1.09	0.01
L. tingitanus	100.00	9.00	9.00	5.00	2.00	0.10
Checks	77.28	8.89	6.75	50.88	5.09	4.52

basis. In addition, plants were harvested at maturity for recording biological and grain yield. *Orobanche* infestation level was estimated on each accession by counting the number of emerged *Orobanche* spikes per plant (EON) and by measuring the dry weight (EODW) (Amri, Trabelsi, Abbes, & Kharrat, 2019). This was done by dividing the number of emerged broomrapes by the number of host plants per row. The incidence (percentage of host plants showing emerged spikes on a 0–100% scale), severity on a 1–9 scale, and parasitism index (incidence × severity/100) were measured following the standard procedures suggested by Abbes, Kharrat, Delavault, Simier, and Chaïbi (2007).

2.5 | Pot experiment

Based on the field results of 2017–2018, eight accessions of *L. sativus* selected for their resistance to both *O. foetida* and *O.*

TABLE 3 Number (EON) and dry weight (EODW) of emerged Orobanche per plant along with seed yield of different *Lathyrus* species in field uniformly infested with *O. crenata* in Morocco and *O. foetida* in Tunisia during the 2017–2018 cropping season

	O. crenata			*O. foetida*		
Species	**EON**	**EODW**	**Seed yield**	**EON**	**EODW**	**Seed yield**
		g			g	
L. annuus	2.74	0.38	3.13	0.03	0.13	6.86
L. aphaca	0.38	0.15	21.14	0.00	0.00	0.50
L. articulatus	0.00	0.00	29.68	0.00	0.00	9.08
L. blepharicarpus	0.60	0.25	20.13	0.00	0.00	6.22
L. cassius	1.36	0.14	10.47	0.00	0.00	5.23
L. cicera	2.91	0.60	16.54	0.00	0.00	5.71
L. gorgoni	0.13	0.13	3.43	0.00	0.00	9.93
L. hierosolymitanus	2.83	0.44	0.00	0.00	0.00	1.35
L. inconspicuus	3.96	0.65	6.08	0.00	0.00	6.00
L. ochrus	0.07	0.00	31.17	0.00	0.00	5.72
L. pseudocicera	2.57	0.39	10.97	0.00	0.01	9.43
L. sativus	1.91	1.00	1.14	0.00	0.00	9.93
L. tingitanus	3.77	0.38	0.08	0.02	0.10	9.06
Susceptible checks	19.45	1.97	0.00	0.25	0.75	2.50

crenata were further evaluated along with two moderately and high susceptible checks, respectively, under controlled conditions in a glasshouse at ICARDA-Rabat facilities during 2018–2019. To evaluate the resistance level and the impact of the parasite on the host plant development, two treatments were applied: (a) infested and (b) noninfested plants or pots. The experimental design was a randomized complete block with three replications. Three pots per accession were used for each treatment. Pots were filled with soil–peat (2:3, v/v). Soil in infested pots was artificially inoculated with 20 mg of *O. crenata* seeds per kilogram of soil. Plants were watered when necessary, and at maturity, they were uprooted from soil and roots and were carefully washed with running tap water. Host plant parameters—namely, total attachment (TAN), underground (UON), and emerged (EON) *Orobanche* number per plant—were recorded. Root (RDW) and shoot (SDW) dry weight per plant, number of pods per plant (PDN), dry weight of emerged (EODW) and underground (UODW) *Orobanche* per plant were determined after drying in oven at 70 ˚C for 72 h. The broomrape attachments were classified according to the stage of development (S), following S1 to S5 stages, where S1 = small tubercle (1–3 mm), S2 = crown roots start to develop, S3 = bud ≤1 cm, S4 = first development of spike below ground surface, and S5 = emergence of spike (Labrousse, Arnaud, Serieys, Bervillé, & Thalouarn, 2001; Sillero et al., 2005).

2.6 | Statistical analysis

Statistical analysis was carried out with the R statistical software (RStudio Team, 2016). Analysis of variance (ANOVA) was conducted using a randomized complete block design, to test for the significance of differences among accessions for the host and parasite variables. Treatment means were compared by Duncan test. Pearson correlations were calculated among the host and parasite variables on the basis of mean values. The statistical model for pot experiments involved a completely randomized design with three replicates, in which the host genotype was the unique fixed factor. For shoot dry weight, root dry weight, and pod number, comparison includes the genotype with and without *Orobanche* infection. To get a genotypic variance component, a linear mixed model with genotypes considered to be random was performed for both designs. The Lme4 package (Bates, Mächler, Bolker, & Walker, 2015) in R was used for these analyses.

3 | RESULTS

3.1 | Identification of resistance sources

Field screening showed significant differences ($P \leq .05$) among *Lathyrus* species against *O. crenata*. However, no significant difference was observed against *O. foetida*. Among 13 *Lathyrus* species tested against *O. crenata*, the incidence of *Orobanche* infestation ranged from 0 to 100% and severity ranged from 1 to 9 as compared with 77.3% and 8.9 score in susceptible checks, respectively (Table 2). The incidence of *Orobanche* was zero in *L. articulatus* and 2.39% in *L. ochrus*, with a severity score of 1 in *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius*, and *L. pseudocicera*. Based on the parasitism index (PI), *L. aphaca*, *L. articulatus*, *L.*

FIGURE 2 Infestation of *O. crenata* at Marchouch in Morocco ([a] resistant, [b] moderately resistant, and [c] susceptible) and (d, f) *O. foetida* at Beja in Tunisia in grass pea germplasm during the 2017–2018 cropping season

blepharicarpus, *L. cassius*, *L. pseudocicera*, and *L. ochrus* showed overall high resistance to *O. crenata* parasitism with <1 score as compared with a 6.75 score in susceptible checks. Two species, *L. cicera* and *L. gorgoni*, showed a moderately resistant (MR) reaction with PI score between 1 and 2 while cultivated species *L. sativus* and *L. inconspicuous* recorded PI score between 4 and 5, suggesting their moderate susceptibility to *O. crenata*. The remaining three species (*L. annuus*, *L. hierosolymitanus*, and *L. tingitanus*) recorded >5 PI score, showing high susceptibility to *O. crenata*. All wild species including cultivated one showed less than 1 PI score as compared with 4.5 PI of susceptible checks, indicating high resistance to *O. foetida*.

Table 3 presents the number (EON) and dry weight (EODW) of emerged *Orobanche* spikes per host plant along with seed yield per plant to assess the performance of wild species under *Orobanche* infestation. Among the 13 *Lathyrus* species, the number of emerged *Orobanche* spikes (EON) varied from 0 to 19.45 for *O. crenata* and from 0 to 0.25 for *O. foetida*. Four species—namely, *L. articulatus* (0), *L. ochrus* (0.07), L. gorgoni (0.13), *L. blepharicarpus* (0.6), and *L. aphaca* (0.38)—were compared with 19.45 EON for checks (Figure 2). Dry weight of emerged *O. crenata* (EODW) on these species was <0.25 g as compared with 1.97 g on checks. For *O. foetida*, only three species (*L. annuus*, *L. tingitanus*, and *L. pseudocicera*) showed very low EON and EODW. Seed

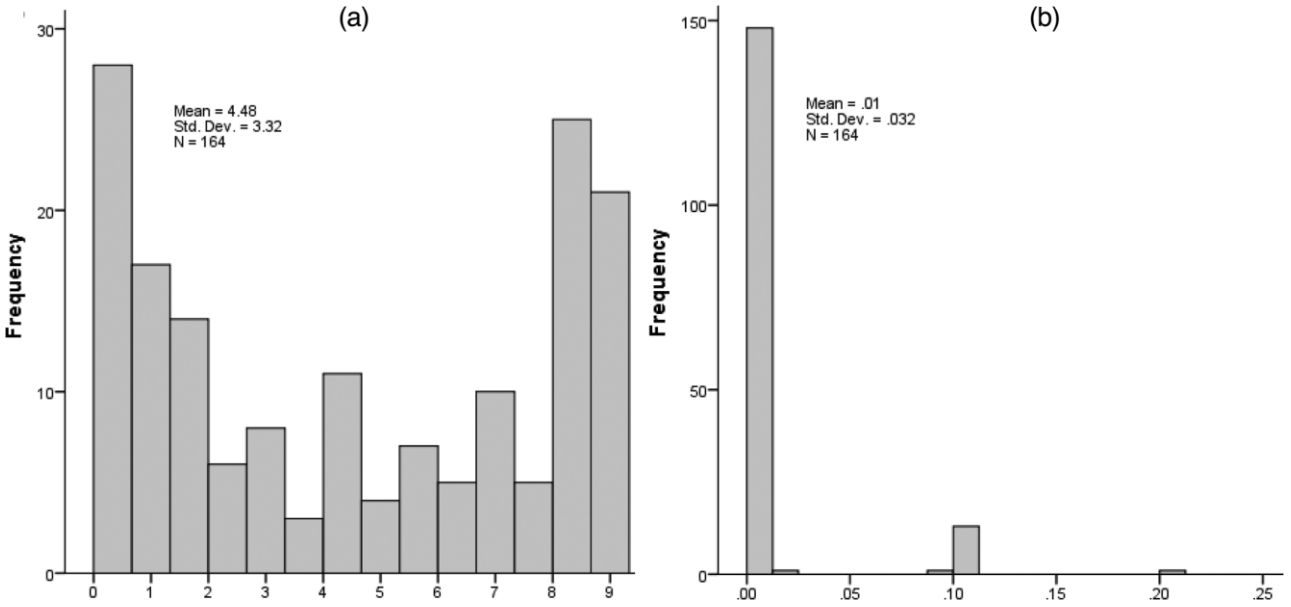

FIGURE 3 Frequency distribution of 162 *L. sativus* accessions based to the parasitism index for (a) *O. crenata* tested in Morocco and (b) *O. foetida* in Tunisia

yield per plant was significantly higher in *Lathyrus* species in Morocco as compared with Tunisia. The highest seed yield was recorded in *L. ochrus* (31.17 g), *L. articulatus* (29.68 g), *L. aphaca* (21.14 g), *L. blepharicarpus* (20.13 g), *L. cicera* (16.54 g), *L. pseudocicera* (10.97 g), and *L. cassius* (10.47 g) as compared with no seed yield in susceptible checks. In Tunisia, the highest seed yield was recorded in *L. gorgoni*, *L. sativus*, and *L. pseudocicera*. Based on the low score of EON and EODW and high seed yield, four species—namely, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, and *L. ochrus*—qualified as highly resistant against *O. crenata* in the present study. The frequency distribution of 162 cultivated accessions showed significant variation for PI ranging from 1 to 10 (Figure 3). Based on low PI and performance, one accession each of *L. aphaca* (IG65053) and *L. gorgoni* (IG65375), four accessions of *L. cicera* (IG64844, IG64867, IG64873, and IG 64864), seven of *L. articulatus* (IG64733, IG64735, IG64786, IG64787, IG64795, IG64798, and IG64800), and 13 of *L. ochrus* (IG62140, IG64731, IG64801, IG64804, IG64807, IG64808, IG64809, IG64810, IG64812, IG64813, IG64814, IG64816, and IG64823), and 10 of *L. sativus* (IG64782, IG64875, IG65187, IG65197, IG116989, IG65204, IG64951, IG116997, IG117544, and IG65149) were identified as resistant against *O. crenata* and *O. foetida* (Table 4). These results were validated in the same field at Marchouch in Morocco during the 2018–2019 cropping season.

3.2 | Result validation

Resistance to *O. crenata* in *L. sativus* accessions was validated in pot experiment during 2018–2019 using 10 grass pea accessions representing resistant, MR, and susceptible reactions in field screening at Marchouch in Morocco during 2017–2018.

The ANOVA (Table 5) showed significant differences among the selected accessions for emerged Orobanche number per plant (EON), emerged Orobanche dry weight per plant (EODW), underground *Orobanche* number per plant (UON), and underground Orobanche dry weight per plant (UODW). The results showed no significant differences between the studied accessions for the average attachments per plant, which varied from 0 to 16.67 in *O. crenata*-inoculated pots (Table 6). Significant differences were observed for the average of emerged and underground *Orobanche* number and dry weight per plant. The results showed that *O. cre- nata* parasitized only eight accessions. Two accessions— namely, IG64782 and IG65197—confirmed their complete resistance to *O. crenata* in the pot experiment. Among the six accessions with MR reaction in the field experiment, four accessions—namely, IG65204, IG64875, IG64951, and IG65187—confirmed their MR reaction in pot experiments. The remaining two accessions (IG116989 and IG116997) that showed low infestation in field experiment revealed high susceptibility in the pot experiment. The numbers of underground *Orobanche* in IG116989 (13.3) and IG116997 (9.3) were higher than the number of emerged ones (3), resulting in the susceptible reaction in the pot experiment. In addition, dry weights of emerged and underground *Orobanche* were significantly higher in two MR lines (IG64951 and IG116997) in the pot experiment. These accessions were more susceptible than the checks because the underground *Orobanche* number per plant were not counted in the field experiment, followed by the susceptible check IG65042 (11.33). The low number of underground *O. crenata* per plant were recorded for MR line IG65187 (1.33) and susceptible check IG64908 (3.00).

TABLE 4 Germplasm accessions of 13 *Lathyrus* species tested and their reactions to *Orobanche crenata* and *O. foetida* in field experiment during the 2017–2018 cropping season

Species	IG name
L. annuus	65353, 65463
L. aphaca	65053
L. articulatus	**64733, 64735, 64786, 64787, 64795, 64798, 64800**
L. blepharicarpus	64865
L. cassius	65277
L. cicera	64831, 64832, 64833, 64836, 64837, 64838, 64839, 64842, **64844**, 64845, 64846, 64848, 64849, 64851, 64855, 64857, 64858, 64859, 64861, 64862, 64863, 64866, **64867**, 64868, 64869, 64870, 64872, **64873**, 64876, 64878, 65103, 65281, 65823, 64843, 64734, 64841, 64745, 64830, 64834, 64840, 64853, 65046, 64879, 64977, 64852, 64856, **64864**, 64854, 64860, 65252, 65820, 64847, 65074, 65949
L. gorgoni	65330, **65375**, 65750, 63034, 64989
L. hierosolymitanus	65735, 65494
L. inconspicuus	65279, 65519, 64983, 65500
L. ochrus	**62140, 64731, 64801, 64804, 64807, 64808, 64809, 64810, 64812, 64813, 64814, 64816,** 64817, 64818, 64819, 64820, 64821, 64822, **64823**, 64824, 64825, 64827, 64828, 64850, 64950, 65075, 65221, 65222, 65224, 65225, 65226, 65227, 65228, 65229, 65230, 65237, 65242, 65310, 65340, 65373, 65376, 65390, 117945,65235
L. pseudocicera	65065, 65543
L. sativus	**64782**, **64875**, 64882, 64886, 64900, 64908, 64909, 64915, 64918, 65136, 65184, **65187,** 65195, **65197**, 65210, 65211, 65212, 65213, 65223, 116890, 64935, 65186, 116889, 117496, 65040, 65179, 117178, 65178, 65183, 65189, 65194, 65200, 116893, 117117, 65039, 117003, 117333, 65017, 65112, 65192, 117121, 117122, 117331, 117493, **116989**, 65108, 65201, 117065, 117369, 117548, 117551, 117553, 64975, 64993, 117113, 117504, 117528, 117022, 65176, 65232, 65240, 65248, 116992, 117012, 117018, 117034, 117047, 117053, 117064, 117110, 117115, 117119, 117175, 65143, 65174, 65175, 65162, 117365, 117598, **65204**, 64723, 65104, 65231, 65244, 116913, 117171, 117224, 117511, 117531, 117543, 64970, 65157, 117352, 64961, 117515, 117535, 117546, 65147, **64951,** 64958, 64960, 64962, 64963, 64968, 65042, 65153, 65154, 65156, 65160, 65163, 65170, 65171, 65173, 65193, 65205, 65233, 65234, 65236, 65245, 65246, 65247, 116818, **116997**, 117004, 117060, 117095, 117182, 117196, 117222, 117225, 117229, 117255, 117277, 117283, 117328, 117332, 117334, 117358, 117368, 117373, 117506, 117507, 117509, 117530, 117533, **117544,** 117604, **65149**, 117118, 117197, 117220, 117223, 117279, 117336, 117540, 64964, 116949, 117204, 117211, 117510, 117526
L. tingitanus	116891

Note. IG numbers in **bold** are highly resistant accessions against *O. crenata* and *O. foetida.*

TABLE 5 Mean squares for five infestation parameters recorded in the pot experiment

Source	df	TAN	EON	EODW	UON	UODW
				g		g
Corrected model	7	51.18ns[a]	14.90*	75.43*	3.95*	30.47*
Genotypes	7	51.18ns	14.90*	75.43*	3.95*	30.47*
Error	12	44.56	7.56	34.5	1.86	24.22

Note. TAN, total attachment number per plant; EON, emerged *Orobanche* number per plant; EODW, emerged *Orobanche* dry weight per plant; UON, underground *Orobanche* number per plant; UODW, underground *Orobanche* dry weight per plant.

[a]ns, nonsignificant.

*Significant at the .05 probability level.

However, dry weight of underground *Orobanche* per plant was highest in MR accession IG116997 (9.73 g), with IG64875 having the lowest value (0.5 g).

Root and shoot dry weight and pod number per plant of tested grass pea germplasm were determined in infested and noninfested pots (Figure 4). The ANOVA analysis showed significant differences among the tested germplasm accessions for shoot dry weight, root dry weight, and pod num- ber per plant under both treatments (Table 7). Performance of the test entries differed significantly under infested vs. noninfested conditions with significant interaction effects. The root dry weight of host plants was higher in infested than noninfested pots (Table 8), whereas the opposite holds true for shoot dry weight and pod numbers. High shoot dry weight per plant in infested pots were observed in resistant accessions IG64782 (20.6 g) and IG65197 (10.7 g), whereas low values were recorded for IG116989 (1.17 g) and susceptible check IG64908 (1.43 g). No pod development was observed on two MR accessions (IG64951 and IG116989) and susceptible accessions (IG65042 and IG64908) in infested pots, whereas

TABLE 6 Total attachment number (TAN), and number (EON, UON) and dry weight (EODW, UODW) of emerged and underground *Orobanche crenata* per plant in selected grass pea accessions in the pot experiment under controlled conditions during 2018–2019

Accession	Reaction against *O. crenata* in 2017–2018 test	TAN S1–S5	Emerged *Orobanche* per plant		Underground *Orobanche* per plant	
			EON S5	EODW S5	UON S5	UODW S5
				g		g
IG65197	Resistant	0.00a	0.00b	0.00d	0.00c	0.00b
IG64782	Resistant	0.00a	0.00b	0.00d	0.00c	0.00b
IG65204	Moderate R	5.67a	0.33b	0.10cd	5.33abc	0.83b
IG64875	Moderate R	11.67a	7.67a	2.30bc	4.00abc	0.50b
IG64951	Moderate R	10.00a	7.67a	4.57a	2.33bc	1.07b
IG65187	Moderate R	10.00a	5.33a	1.93bcd	1.33bc	0.60b
IG116989	Moderate R	16.67a	3.33ab	1.07bcd	13.33a	1.47b
IG116997	Moderate R	12.33a	3.00ab	3.27ab	9.33abc	9.73a
IG65042	Susceptible	14.67a	3.33ab	1.80bcd	11.33ab	3.73ab
IG64908	Susceptible	6.67a	3.67ab	2.57ab	3.00abc	0.73b
Mean over all accessions		11.00	3.43	1.76	5.00	1.87

Note. Values with a common letter per column are not significantly different according to Duncan's test ($p = .05$). R, resistant; MR, moderately resistant; S, susceptible.

these lines showed 8–16 pods per plant in non-infested pots. These results indicate that the resistance against *O. crenata* and *O. foetida* could be associated with a low induction of parasite seed germination and/or a slowed development of the established tubercles on host root system.

3.3 | Correlations among parasitism parameters

Correlation coefficients between different parameters of *Orobanche* infestation are presented in Table 9. No correlation was observed between the number of emerged *Orobanche* spikes and the pod number per plant with all the other recorded parameters. The number of emerged *Orobanche* spikes reflects the level of infestation and could be used as a good indicator of resistance but should be used with the number of underground tubercles. The number of pods per plant is one of the determinant yield attributes that can provide a good information on the resistance level against broomrapes. Results also showed a significant positive correlation between total attachment number and underground *Orobanche* number. This positive correlation indicates that number of underground Orobanche is very important parameter to measure during screening.

4 | DISCUSSION

Grass pea cultivation in the Mediterranean region of southern Europe, North Africa, and West Asia has almost been abandoned because of high incidence of *Orobanche* in the region, high susceptibility of existing grass pea varieties, and favorable environments for growth and development of parasitic weeds (Vaz Patto et al., 2006, 2008). Area under grass pea cultivation could be regained in cereal–livestock systems if *Orobanche* resistant varieties with low ODAP content are developed and popularized in these regions. Past efforts in the screening of limited germplasm showed moderate and incomplete resistance in cultivated (Fernández-Aparicio & Rubiales, 2010; Fernández-Aparicio et al., 2012) and wild (Fernández-Aparicio & Rubiales, 2010; Fernández-Aparicio et al., 2009; Sillero et al., 2005) species of grass pea. The present study screened 13 *Lathyrus* wild species against *O. crenata* and *O. foetida* under most congenial environments in Morocco and Tunisia, as indicated by high PI on susceptible check across the blocks.

In the present study, we used number and dry weight of emerged *Orobanche* shoots per plant, seed yield, *Orobanche* incidence (%), severity score, and PI in field trials to assess *Orobanche* infestation in test entries. The number of emerged *Orobanche* shoots per host plant is generally considered the principal parasitism parameter. Nevertheless, because of technical constraints, field trials do not often consider the number and dry weight of underground *Orobanche* tubercles, which could be important under high infested fields and the results solely derived from the emerged shoots could overestimate the resistance level and might lead to wrong inference (Fernández-Aparicio, Flores, & Rubiales, 2012). This was evident in the present study when some of the MR accessions showed highly susceptible reaction in pot experiment under controlled conditions where both emerged and underground shoots were considered for assessing the

FIGURE 4 Validation of the reaction of *Lathyrus sativus* accessions tested on *Orobanche crenata*-infested and noninfested soils in the pot experiment. Reaction of resistant ([a] IG65197 and [b] IG64782), moderately resistant ([c] IG65204, [d] IG116989, and [e] IG64951), and susceptible ([f] IG64908) accessions in *O. crenata*-infested pots. Comparison of performance of resistant ([i] IG65197 and [j] IG64782) and susceptible ([k] IG64908) plants in infested and noninfested pots

TABLE 7 Mean squares for three *Orobanche* infestation parameters recorded on 10 grass pea genotypes in infested and noninfested pot experiments under controlled conditions during 2018–2019

Source	df	RDW	SDW	PDN
Corrected model	15	25.09*	55.81**	73.60*
Treatment (T)	1	58.75*	26.16**	343.14*
Genotypes (G)	9	17.77*	69.29**	66.55**
T × G	5	33.65*	39.40*	29.95**
Error	23	0	0	0

Note. RDW, root dry weight per plan; SDW, shoot dry weight per plant; PDN, pod number per plant.
*Significant at the .05 probability level.
**Significant at the .01 probability level.

resistance level of test entries. The numbers of underground *Orobanche* shoots in IG116989 and IG116997 were much higher than the emerged ones, resulting in the susceptible reaction.

Field trials at hot spots revealed that *O. crenata* was much more parasitic on *Lathyrus* species than *O. foetida*. Simi- lar results were observed for faba bean with regard to parasitism of *O. foetida* and *O. crenata* (Trabelsi et al., 2015) and chickpea (*Cicer arietinum* L.) and pea (*Pisum sativum* L.) (Abbes, Kharrat, & Chaibi, 2008). The present study identified six wild species—namely, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius*, *L. pseudocicera*, and *L. ochrus*—with <1 PI as compared with 6.75 in susceptible checks. These wild species with complete resistance against *O. crenata* parasitism offer scope for introgression breeding. In earlier studies with a limited number of accessions, *L. ochrus* was identified as a source of resistance to *Orobanche crenata* by Sillero et al. (2005). In the past, only few attempts were made for interspecific hybridization among *Lathyrus* species (Davies, 1958; Hammett, Murray, Markham, Hallett, & Osterloh, 1996; Kearney, 1996; Khawaja, 1985, 1988; Trankovskij, 1962; Yamamoto, Fujiware, & Blumenreich, 1986; Yunus, 1990; Yunus & Jackson, 1991). Yunus (1990) crossed 11

TABLE 8 Root (RDW) and shoot (SDW) dry weight and pod number (PDN) of selected *Lathyrus sativus* accessions in *O. crenata*-infested and noninfested pots during 2018–2019

Accession	Reaction against *O. crenata* in 2017–2018 test	RDW		SDW		PDN	
		Infested	Noninfested	Infested	Noninfested	Infested	Noninfested
		g					
IG65197	Resistant	1.97b	1.47a	10.70b	7.37b	6.00b	3.50c
IG64782	Resistant	1.27b	1.67a	20.60a	16.80a	5.00ab	15.00ab
IG65204	Moderate R	0.93b	1.03b	5.30bc	12.40b	14.33a	21.00a
IG64875	Moderate R	2.80b	0.83b	2.00bc	11.53b	4.00ab	14.67ab
IG64951	Moderate R	5.63b	0.63b	1.53bc	8.20b	0.00ab	12.00abc
IG65187	Moderate R	2.63b	0.73b	3.50c	6.13b	9.50ab	15.67ab
IG116989	Moderate R	2.53b	0.73b	1.17bc	7.90b	0.00a	16.00ab
IG116997	Moderate R	13.00a	1.00b	2.60bc	9.83b	2.00ab	17.00ab
IG65042	Susceptible	5.53b	0.77b	1.43bc	8.87b	0.00ab	9.67bc
IG64908	Susceptible	3.30b	1.30ab	1.43b	11.17b	0.00ab	7.67bc
Mean over all accessions		3.96	1.02	5.03	10.02	7.73	13.5

Note. Data with a common letter per column are not significantly different according to Duncan's test ($p = .05$). R, resistant; MR, moderately resistant; S, susceptible.

TABLE 9 Pearson correlations between different parameter combinations of *Orobanche* infestation in the pot experiment

Parameter	EON	EODW	UON	UODW	RDW	SDW	PDN
TAN	.050ns	−.128ns	**.780****	.496ns	.220ns	−.183ns	.015ns
EON		.389ns	−.179ns	−.314ns	−.054ns	−.036ns	.038ns
EODR			.008ns	.373ns	.012ns	.012ns	.281ns
UON				.592ns	.466ns	.271ns	−.267ns
UODR					.109ns	−.233ns	−.060ns
RDW						.431ns	−.204ns

Note. TAN, total attachment number per plant, EON, emerged *Orobanche* number per plant; EODW, emerged *Orobanche* dry weight per plant; UON, underground *Orobanche* number per plant; UODW, underground *Orobanche* dry weight per plant; RDW, root dry weight per plant; SDW, shoot dry weight per plant; PDN, pod number per plant.

**Significant at the .01 probability level. †ns, nonsignificant.

wild species with *L. sativus* and found viable seeds only with *L. cicera* and *L. amphicarpus*. Under the Crop Trust-funded project on enhancing prebreeding in barley and grass pea (GS16005), we investigated crossability of *Lathyrus sativus* with six wild species resulting in viable F_1 plants only with *L. cicera* and to a lesser extent with *L. ochrus*, *L. inconspicuous*, *L. marmoratus* Boiss., and *L. heirosolymitanus* Boiss., but not with *L. articulatus*, indicating the prospects of transferring useful traits from the CWRs. Resistance in *L. articulatus* and *L. ochrus* accessions was due to low incidence, severity, and PI. This resistance acts as an early barrier to the establishment of broomrape, as none or few broomrape tubercles were recorded at early stage in these accessions. This low establishment is due to the low induction of germination of the broomrape seeds. Low levels of induction of germination have been described as a resistance mechanism in other legume species such as chickpea (Sillero et al., 2005).

Results with complete resistance observed for the two accessions IG64782 and IG65197 against *O. crenata* and *O. foetida* under open field conditions were confirmed and validated under controlled conditions in pot experiment. However, there were few mismatches in MR accessions, which showed a highly susceptible reaction in pot experiments. This includes the susceptible reaction of IG116989 with the highest number of emerged *Orobanche* in the pot experiment. This is due to the underground development of *Orobanche* shoots at the host plant root level. Effectively, it makes a false selection of resistant genotypes in field screening (Briache et al., 2019). Other studies showed that in a susceptible genotype, high infestation level could occur, leading to intense uptake of nutrients by *Orobanche* attachment to host plant roots. In such cases, the majority of underground *Orobanche* sprouts might not emerge, judging the test entry erroneously as resistant (Mbasani-Mansi, Ennami, et al., 2019; Rubiales et al.,

2006). The number of emerged *Orobanche* shoots and pod number did not show any correlation with any of the parasitism parameters and, therefore, these two parameters, which are easy to measure, are not good selection criteria for evaluation of *Orobanche* resistance in grass pea. For this reason, the underground number of *Orobanche* shoots should always be considered for identification and classification of test entries correctly. Infestation by *O. crenata* showed low induction of growth and yield in some accessions with significant difference between them. Pod number per plant was more affected than root and shoot dry weights. It could be attributed to host nutrient losses caused by *O. crenata*, which limited the availability of nutrients to the host plant. The latter tends to maintain its vegetative biomass to offset nutrient losses, rather than to produce seeds. Previous studies reported higher reduction in yield than in growth parameters in faba bean (Briache et al., 2019), lentil (*Lens culinaris* Medikus; Mbasani-Mansi, Briache, et al., 2019) and grass pea (Fernández-Aparicio et al., 2016). Thus, resistance against broomrape is particularly a difficult task for evaluation. Thus, there is a need to find sources of true resistance in order to facilitate the development of resistant cultivars.

5 | CONCLUSION

A 2-yr study in field and controlled conditions showed wide variation among crop wild relatives of grass pea for resistance to broomrape species (*O. crenata* and *O. foetida*). Six wild species—namely, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius*, *L. pseudocicera*, and *L. ochrus*—with <1 PI against *O. crenata* offer scope to develop introgression lines for their use in mainstream grass pea breeding. Underground number and weight of the *Orobanche* shoot should always be considered as selection criteria for identification and classification of test entries correctly. The resistant accessions are included in the crossing block to strengthen prebreeding efforts in grass pea.

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AUTHOR CONTRIBUTIONS

F.A., S.K., A.A., and M.A. designed the research. F.A., S.K., R.M., R.K.M., K.H., and M.A. performed the experiments. F.A., S.K., A.A., Z.K., K.H., and M.A, contributed materials and analysis tools. F.A., S.K., M.A., and A.H. wrote the paper. R.M., Z.K., R.K.M., Z.E.T., and M.B. revised the paper. All authors approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

Shiv Kumar https://orcid.org/0000-0001-8407-3562
Michael Baum https://orcid.org/0000-0002-8248-6088
Moez Amri https://orcid.org/0000-0002-4707-0618

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I. Conclusion:

Breeding for *Orobanche* resistance is difficult due to limited sources of resistance, complex nature of the resistance mechanism, and low heritability (Pérez-de Luque *et al*., 2010). Limited to moderate levels of resistance have been reported in *Lathyrus ochrus* (L.) DC. and *L. clymenum* L. (Sillero *et al*., 2005), and *L. cicera* L. (Fernández-Aparicio *et al*., 2009; Fernández-Aparicio and Rubiales, 2010). According to Sillero *et al*., 2005, *L. ochrus* was identified as a source of resistance to *O. crenata*.

The study revealed a wide range of resistance levels among different *Lathyrus* species. Field trials conducted in known hot spots for broomrape infestation showed that *O. crenata* was significantly more parasitic on various *Lathyrus* species compared to *O. foetida*. Six wild species (*L. aphaca, L. articulatus, L. blepharicarpus, L. cassius, L. pseudocicera*, and *L. ochrus*) exhibited particularly low susceptibility to broomrape, with a PI < 1. The pot experiment revealed that two accessions, IG64782 and IG65197, were completely resistant to *O. crenata*. Some accessions that were previously categorized as moderately resistant (MR) displayed unexpected susceptibility under controlled conditions. For this reason, both the underground number and weight of the broomrape shoots are considering as critical selection criteria.

The screening of *Lathyrus* accessions against *Orobanche* species in Morocco and Tunisia represents a critical step in enhancing the resilience of grass pea. The findings from this study will not only inform breeding programs but also contribute to the broader goal of sustainable agriculture in regions challenged by parasitic weeds. Continued research and collaboration will be essential to fully realize the potential of grass pea as a climate-resilient crop.

Chapter III: Genetic variability for resistance to broomrape weeds (Orobanche crenata Forsk. and Orobanche foetida Poir.) in grass pea (Lathyrus sativus L.)

I. Introduction:

In order to develop new varieties of *Lathyrus sativus* L. that are resistant to *Orobanche* parasitism and introduce them into the breeding program, it is necessary to use molecular hybridization techniques which can significantly accelerate the breeding process and improve resistance traits.

In the case of legumes, progress in broomrape resistance breeding has been slow, as they are rather minor crops in which relatively little has been invested in the last half century (Cusworth *et al*., 2021; Rubiales, 2023; Rubiales *et al*., 2023). Most studies on broomrape resistance in legumes have concluded that there is low heritability and that inheritance is complex, highly influenced by the environment (Rubiales *et al*., 2023). Diversity of *Lathyrus* species is found in Europe, Asia and North America, and extended to South America and East Africa, but the main centre of diversity remains primarily in the Mediterranean and Irano-Turanian regions (Kupicha, 1981).

This paper is a continuation of the first article by studying the same collection. In this analyzis, we added some agro-morphological traits in the field experiment, such as days to flowering (D2F), days to *Orobanche* emergence (D2OE), days to maturity (D2M) and Biological yield (BY). Rstudio was used in this research, in addition to SPSS version 25. The objectives of this study are to prove the heritability, the coefficient of variation and genotype-by-environment interaction (G*P), to evaluate the correlation in both experiments against *Orobanche* infestation, to detect the best-performing accessions (resistant or tolerant) which will be added in future breeding program and to identify the criterion for selection under *Orobanche* open infested conditions.

II. Scientific contribution:

Genetic variability for resistance to broomrape weeds (*Orobanche crenata* Forsk. and *Orobanche foetida* Poir.) in grass pea (*Lathyrus sativus* L.)

Abdallah F.[a,b]*, Kehel Z.[a*], El Kalchi M.A.[d], Amri A.[a], Triqui Z.El.A.[b], Shiv Kumar [a*], Amri M.[c*]

[a] International Center for Agricultural Research in Dry the Areas (ICARDA), Rabat, Morocco.
[b] University Mohammed V, Faculty of sciences, Rabat, Morocco.
[c] AgroBioSciences (AgBS), College Mohammed VI Polytechnic (UM6P), Lot 660, Hay Moulay Rachid Ben Guerir, 43150, Morocco.
[d] Agence National des eaux et forêts (ANEF), Agro Paris Tech, Paris, France.

ABSTRACT

Broomrapes (*Orobanche crenata* Forsk. and *Orobanche foetida* Poir.) is reported as a major constraint for grass pea and other grains legumes cultivation in the Mediterranean region. Breeding strategies for developing resistant varieties remains limited due to limited information on genetic variability. A germplasm collection of 285 accessions representing the cultivated and crop wild relatives (CWR) species were subjected to field screening under high infested sick plots in Marchouch – Morocco (*O. crenata*) and Oued Beja – Tunisia (*O. foetida*). A confirmation experiment was also conducted under controlled conditions at ICARDA - Rabat. The results showed significant genetic variability and high heritability for agronomic and physiological traits associated with resistance under highly infested fields. The two accessions of *L. sativus* IG64782 and IG65197 have expressed a complete resistance against *O. crenata* under field conditions and further confirmed by the glasshouse experiment. The results also revealed good resistance level of several accessions of *L. ochrus*, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius* and *L. pseudocicera* mainly originated from Asia, Cyprus and Ethiopia. However, other accessions representing *L. sativus*, *L. cicera*, *L. annuus*, *L. hierosolymitanus* and *L. tingitanus* originating mostly from Ethiopia and North America showed high susceptibility mainly to *O. crenata*. Multiple linear regression and correlation analyses indicated that parasitism index (PI), *Orobanche* incidence (OIN), *Orobanche* severity (OSV), days to *Orobanche* emergence (D2OE) biological yield (BY), and days to flowering (D2F), were strongly and significantly correlated with seed yield (SY). Under controlled conditions, the total attachment number (TAN) showed highly significant correlations with emerged *Orobanche* number (EON) ($r = 0.543^{**}$) and underground *Orobanche* number (UON) ($r = 0.892^{***}$). SY, BY, PI and OIN could be used as selection criteria in field trials.

Keywords: *Lathyrus* spp., *Orobanche crenata*, *Orobanche foetida*, resistance, genetic variation

1. Introduction:

Grass pea (*Lathyrus sativus* L.), an annual pulse crop belonging to the tribe *Vicieae* in the family *Fabaceae* (Biswas and Biswas, 1997), is known by a wide range of common names, including chickling vetch, Indian vetch, *khesari* or *batura*. The genus Lathyrus contains 160 species and 45 sub-species embracing food, forage, and ornamental crops (Abd El-Moneim *et al*., 2010). It is widely cultivated in India, Pakistan, Bangladesh, Ethiopia, China, and Nepal (Hailu *et al*., 2015) and to lesser extent in the Mediterranean countries (Gonçalves *et al*., 2022). Globally, the area under grass pea cultivation is estimated at 1.50 million ha, with annual production of 1.20 million tonnes (Kumar *et al*., 2011). Grass pea is a climate-smart nutritious crop with an ability to perform well in poor soils overcoming harsh conditions imposed by drought, high temperature, waterlogging, and salinity. Unfortunately, this crop has also been associated with lathyrism, a debilitating muscle paralysis caused by a neurotoxin present in the seeds, if consumed excessively for a longer period (Allen, 2013). The most familiar toxic *Lathyrus* species is *Lathyrus sativus* which contains *β-N*-oxalyl-l-*α*, *β*-diamino propionic acid (*β*-ODAP) in its seeds and foliage (Crews and Clarke, 2014). The crop is almost free from major diseases and insect pests except for some occasional appearance of powdery mildew and aphids under favorable environmental conditions in South Asia and Sub-Saharan Africa (Kumar *et al*., 2011). Vascular wilt, caused by the infection of the soil-borne pathogen *Fusarium oxysporum* (Fo), is one of the most destructive diseases of many crops, including legumes such as grass pea (*Lathyrus sativus*), with several *formae speciales* (ff. spp.) defined according to their hosts. Yield losses by *fusarium* wilt reaching 25% were reported in Indian and Ethiopian grass pea growing areas (Campbell, 1997; Talukdar, 2013). However, in the Mediterranean region of southern Europe and North Africa, legume crops including grass pea encounter the infestation of broomrape, mainly *Orobanche crenata*, causing severe yield and biomass losses (Parker, 2009; Abbes *et al*., Nefzi *et al*., 2016; Amri *et al*., 2019; Ennahli *et al*., 2021). This parasitic weed can cause important losses that may reach 90% of Morocco's legume crop production (Torres *et al*., 2006; Ennami *et al*., 2017; Briache *et al*., 2019; Ennahli *et al*., 2023). In Tunisia, faba bean is the most affected crop by broomrape and two species, *O. crenata* and *O. foetida* are considered widespread (kharrat *et al*., 2007; Abbes *et al*., 2014; Amri *et al*., 2021). Several control methods have been developed and employed but most of them have been impractical, uneconomical, hard to achieve, or have resulted in incomplete protection (Abbes *et al*., 2010; Abbes *et al*., 2014; Trabelsi *et al*., 2016; Abbes *et al*., 2019; Abbes

et al., 2020; Amri *et al*., 2019; Amri *et al*., 2021; Briache *et al*., 2023; El Amri *et al*., 2023). The selection and development of resistant varieties through targeted breeding remain the most effective and ecofriendly control methods for successful management of parasitic weeds (Kharrat *et al*., 2010; Amri *et al*., 2019; En-nahli *et al*., 2023). To develop a successful breeding strategy towards resistance to parasitic weeds, a strong emphasis should be placed on investigating and identifying the genetic variability and diversity within *Lathyrus* genus. Such strategy should take into consideration the level of virulence as well as the genetic structure and diversity of different broomrape populations (Martín-Sanz *et al*., 2016; Boukteb *et al*., 2021). Recently, Abdallah *et al*., (2020) have reported moderate and incomplete resistance in the cultivated species *L. sativus* against *O. crenata* and *O. foetida*. The same authors have, also, reported complete resistance in the wild *lathyrus* species. In this study we aim to (i) investigate the genetic diversity, heritability, coefficient of variance and genotype*parasite interaction within a large germplasm collection representing different *Lathyrus* species under field and controlled conditions in response to *O. crenata* and *O. foetida* parasitism (ii) identify correlations in both experiments and (iii) evaluate the selection criteria traits under field infestation conditions.

2. Material and Methods

2.1. Plant material and experimental trials

2.1.1. Field trials

A set of 285 accessions belonging to 13 *Lathyrus* species (*L. annuus* (2), *L. aphaca* (1), *L. articulatus* (7), *L. blepharicarpus* (1), *L. cassius* (1), *L. cicera* (54), *L. gorgoni* (5), *L. hierosolymitanus* (2), *L. inconspicuus* (4), *L. ochrus* (44), *L. pseudocicera* (2), *L. sativus* (161), and *L. tingitanus* (1)) were assessed for resistance to *O. crenata* and *O. foetida* under field conditions. The germplasm collection, with accessions originated from different regions (figure 1), was received from ICARDA gene bank. Different accessions were purified under insect-proof cages during the preliminary screening in the 2016– 2017 cropping season. Field trials were carried out during the cropping season 2017– 2018 according to an augmented design with repeated susceptible checks of the faba bean (*Vicia faba* L.) cultivar 'Aguadulce' and grass pea accessions (IG90174, IG147607, and IG147655) at two locations: Marchouch (33.56˚ N, 6.63˚ W; 392 m asl) in Morocco and Oued-Beja (36.44˚ N, 9.13˚ E; 150 m asl) in Tunisia. Field screening was carried out in a highly *O. crenata* and *O. foetida* infested fields at Marchouch – Morocco and Beja –

Tunisia, respectively. different accessions were planted in 19 blocks, each consisting of 15 plots of test entries, three plots of faba bean, and one plot each of three grass pea checks. Each accession was planted in a 1 m single-row plot, with 60 cm distance between rows. In each row, 20 seeds were sown by hand at a 5 cm depth maintaining a 5 cm space between plants. Every tenth row in each block was planted with highly susceptible faba bean cultivar Aguadulce. Planting was carried out in the first week of December in both locations. In order to prevent interference with broomrape development, no fertilizers neither pesticides were applied and the field trial was kept free of weeds by manual uprooting of all types of weeds other than broomrape. The following parameters were recorded on individual plant basis before and at crop maturity; emerged *Orobanche* shoot number (EON) and emerged *Orobanche* dry weight (EODW) (Amri *et al*., 2019). This was done by dividing the number of emerged broomrapes by the number of host plants per row. The incidence (percentage of host plants showing emerged shoots on a 0 – 100% scale), severity on a 1 – 9 scale, and parasitism index (severity × incidence / 100) were calculated as reported by Abbes *et al*., 2007.

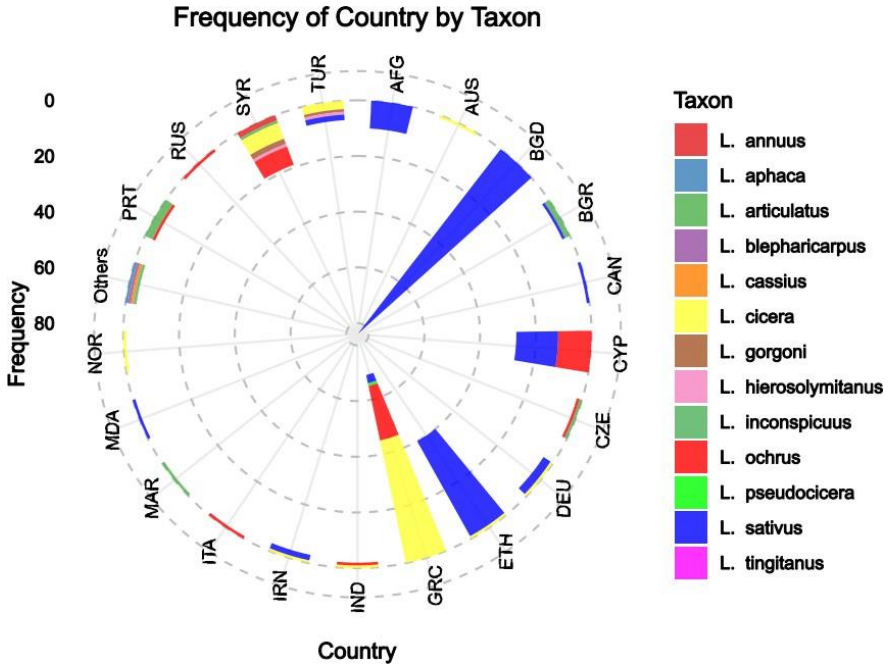


Figure 1: Origin of *Lathyrus* species screened for *O. crenata* and *O. foetida* resistance under field conditions during 2017-2018 cropping season.

2.1.2. Pot experiment

Based on the field screening, eight *L. sativus* accessions (IG65197, IG64782, IG65204, IG64875, IG64951, IG65187, IG116989, IG116997) with moderate to high level of resistance to both *O. foetida* and *O. crenata* were selected and subjected to controlled conditions pot experiment during 2018–2019 at ICARDA-Rabat glasshouse. The two accessions IG65042, IG64908 were used as susceptible checks. The experiment was conducted according a randomized complete block with two treatments (a) infested and (b) non-infested and three replications for each treatment/accession. The pots were filled with soil–peat (2:3, v/v). Twenty mg of *O. crenata* seeds per kilogram of soil was artificially inoculated in infested pots. Plants were watered when necessary, and at maturity, they were uprooted from soil and roots were carefully washed with running tap water. Different parameters related to the host plant growth/development and *Orobanche* infestation were recorded, total attachment (TAN), underground (UON), and emerged (EON) *Orobanche* number per plant. After drying in oven at 70˚C for 72 h, root (RDW) and shoot (SDW) dry weight per plant, number of pods per plant (PDN), dry weight of emerged (EODW) and underground (UODW) *Orobanche* per plant, were determined. The broomrape attachments were classified according to the stage of development (Abbes *et al*., 2011).

2.2. Statistical analysis

Descriptive statistics, analysis of variance (ANOVA), heritability, genotypic variance, coefficient of variation CV (%), R^2 and least significant difference (LSD) for the two experiments were performed using SPSS version 25 (IBM Corporation, New York, NY, United States). All statistical descriptive parameters were tested for significant at $p < 0.05$. Geographical distribution was exploited with the function "ggplot2" and "dplyr" by R Core Team (2023). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/. Two frequencies distributions were carried out in Rstudio with the function "ggplot2" and "ggrigdes" to estimate the distribution and genotype effects on each parameter. Hierarchical Clustering Analysis (HCA) "hclust ()" function was employed to form different groups of accessions based on the field and pot performances, using Ward's method based on Euclidean distance (d^2). Principal component analysis (PCA) was executed using "FactorMineR" and "factorextra" packages in Rstudio software. Two dendrograms resulting from hierarchical clustering analysis were represented using the "ggplot2" and "ggtree" packages in

Rstudio. Person correlations was used to study the relationships between all examined traits with ggpairs and ggcorr function of GGally package. Multiple linear regression was conducted using the package MASS and ggplot with the function lm (), to find the parameter that can predict the seed yield (SY). The general multiple linear regression equation is structured as follows:

$$y_i = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_n x_n + \varepsilon_i$$

where, y_i is the seed yield (SY), β_0 is the constant, $\beta_1, \beta_2 \ldots \beta_n$ are the coefficients of regression corresponding to $x_1, x_2 \ldots x_n$, $x_1, x_2 \ldots x_n$ are the input variables, ε_i is the error term.

Results

1. **Field assessment of agronomic and pheno-physiological parameters against *O. crenata* and *O. foetida* infestation:**

1.1. Descriptive statistics, variance, and frequency distribution:

Results showed significant genotypic variation among all tested *Lathyrus* accessions for different agro-morphological recorded traits in response to *O. crenata* and *O. foetida* parasitism (table 1). The days to flowering (D2F) varied from 0 (IG64723) to 131 (IG64787) days in Marchouch and from 59 (IG117540) to 110 (IG117510) in Beja. The biological yield (BY) ranged from 0 (IG64993, *L. sativus*) to a maximum of 3000 g/m^2 (IG64733, *L. articulatus*), in Marchouch and 1069 g/m^2 (IG64935, *L. sativus*) in Beja. Under *O. foetida* infestation, the seed yield (SY) was lower than the trial infested with *O. crenata*. IG64733 (*L. articulatus*) had the highest value (612.5 g/m^2) of SY under *O. crenata*-infested trial.

The infestation parameters also revealed significant variability for the whole collection (Table 1). IG64821 (*L. ochrus*) had the highest value of 135 days to *Orobanche* emergence (D2OE) at the Marchouch station. However, at Beja station, *L. sativus* (IG117122) exhibited the highest D2OE (140 days). Incidence (OIN) and severity (OSV) values in fields impacted by *O. crenata* were higher than in fields attacked by *O. foetida*. Thus, parasitism index (PI) calculated varied from 0 (IG65376, *L. ochrus*) to 9 (IG65820, *L. cicera*) at Marchouch. While, at Beja station, PI ranged from 0 to 0.2. Under *O. crenata* infested trial, the emerged *Orobanche* number per plant (EON) varied from 0 to 88. The emerged *Orobanche* dry weight per plant (EODW) ranged from 0 (IG64733, *L. ochrus*) to 114.3 (IG117283, *L. sativus*). However, EON and EODW values were

lower at Beja station in Tunisia. IG65494 accession revealed the maximum EON (1.44) while IG117540 accession demonstrated the high value of EODW (2.5).

Table 1: Range, mean and coefficient of variation recorded for different parameters of grass pea accessions under *O. crenata* and *O. foetida* conditions.

	O. crenata				*O. foetida*			
	Min.	**Max.**	**Mean**	**CV%**	**Min.**	**Max.**	**Mean**	**CV%**
D2F	0 **IG64723** *L. sativus*	131 **IG64787** *L. articulatus*	60.7	72.0	59 **IG117540** *L. sativus*	110 **IG117510** *L. sativus*	64.7	40.9
D2OE	93 **IG117022** *L. sativus*	135 **IG64821** *L. ochrus*	88.0	42.1	89 **IG65184** *L. sativus*	140 **IG117122** *L. sativus*	7.1	384.0
D2M	110 **IG65183** *L. sativus*	171 **IG64795** *L. articulatus*	130.1	19.5	119 **IG65226** *L. ochrus*	156 **IG64849** *L. cicera*	127.9	41.1
OIN	0 **IG64795** *L. gorgoni*	100 **IG64858** *L. cicera*	56.3	74.8	0 **IG64822** *L. ochrus*	10 **IG64964** *L. sativus*	14.8	236.6
OSV	0 **IG63034** *L. gorgoni*	9 **IG65820** *L. cicera*	6.9	49.9	1 **IG65226** *L. ochrus*	2 **IG64964** *L. sativus*	2.2	126.2
PI	0 **IG65376** *L. ochrus*	9 **IG65820** *L. cicera*	4.1	93.9	0 **IG65226** *L. ochrus*	0.2 **IG65252** *L. cicera*	1.3	243.2
BY	0 **IG64993** *L. sativus*	3000 **IG64733** *L. articulatus*	255.9	159.5	0 **IG63034** *L. gorgoni*	1069 **IG64935** *L. sativus*	244.7	79.9
SY	0 **IG65227** *L. ochrus*	612.5 **IG64733** *L. articulatus*	64.8	194.3	0 **IG117945** *L. ochrus*	366 **IG64935** *L. sativus*	65.6	92.8
EON	0 **IG64733** *L. ochrus*	88 **IG117283** *L. sativus*	7.2	204.3	0 **IG64993** *L. sativus*	1.44 **IG65494** *L. hierosolymitanus*	0.2	72.3
EODW	0 **IG64733** *L. ochrus*	114.3 **IG117283** *L. sativus*	1.1	542.1	0 **IG64840** *L. cicera*	2.5 **IG117540** *L. sativus*	0.1	464.9

D2F: Days to flowering; **D2OE**: Day to *Orobanche* emergence; **D2M**: Days to maturity; **OIN**: *Orobanche* incidence; **OSV**: *Orobanche* severity; **PI**: Parasitism index; **BY**: Biological yield; **SY**: Seed yield; **EON**: Emerged *Orobanche* number; **EODW**: Emerged *Orobanche* dry weight.

The study revealed significant differences ($p \leq 0.001$) between genotypes of different *Lathyrus* species, except for EODW (table 2). Significant differences ($p \leq 0.001$) were observed between *O. crenata* and *O. foetida* for D2F, D2OE, OIN, OSV, PI, BY, SY and EON. The research revealed significant variance across most genotypes*parasites, with R^2 values oscillates between 0.5 and 0.9. High heritability values were observed for all tested traits ($h^2 \geq 0.5$), specially SY, BY, D2M

(h^2 = 0.9) and PI, OIN, D2OE (h^2 = 0.8). A significant variability was observed in genotypic variance and low significant difference (LSD) for different field parameters.

Table 2: Analyze of variance recorded for different traits of grass pea accessions below *O. crenata* and *O. foetida* field infestation.

	F1 (genotype)	**F2 (parasite)**	**F3 (G*P)**	**R^2**	**Heritability (h^2)**	**Genotypic Variance**	**LSD (95%)**
D2F	19.7***	13.4***	26.6***	0.5	0.6	22.6	1.8
D2OE	26.8***	284.5***	19.4***	0.8	0.8	92.6	1.7
D2M	158.8***	0.2^{ns}	126.1***	0.9	0.9	138.2	1.2
OIN	75.2***	108.3***	17.1***	0.7	0.8	64.4	1.8
OSV	93.1***	111.0***	47.6***	0.8	0.7	119.9	0.3
PI	94.0***	53.9***	15.2***	0.7	0.8	64.9	0.4
BY	35.6***	17.7***	37.1***	0.6	0.9	35.3	21.5
SY	31.2***	7.4***	27.1***	0.5	0.9	28.3	0.8
EON	15.8***	10.6***	16.3***	0.5	0.7	19.9	1.6
EODW	0.8^{ns}	0.5^{ns}	0.4^{ns}	0.1	0.8	0.8	0.7

D2F: Days to flowering; **DOE**: Day to *Orobanche* emergence; **D2M**: Days to maturity; **OIN**: *Orobanche* incidence; **OSV**: *Orobanche* severity; **PI**: Parasitism index; **BY**: Biological yield; **SY**: Seed yield; **EON**: Emerged *Orobanche* number; **EODW**: Emerged *Orobanche* dry weight; **R^2**: coefficient of determination; ******* significant $p \leq 0.001$, **ns** no significant > 0.05.

The frequency distribution was visualized in Figure 3. Among the tested species, a normal frequency distribution was observed for seed yield both for *O. crenata* and *O. foetida.* The frequency distribution of five wild and cultivated species showed a significant variation for PI in *O. crenata* ranging from 0 to 9 (figure 2).

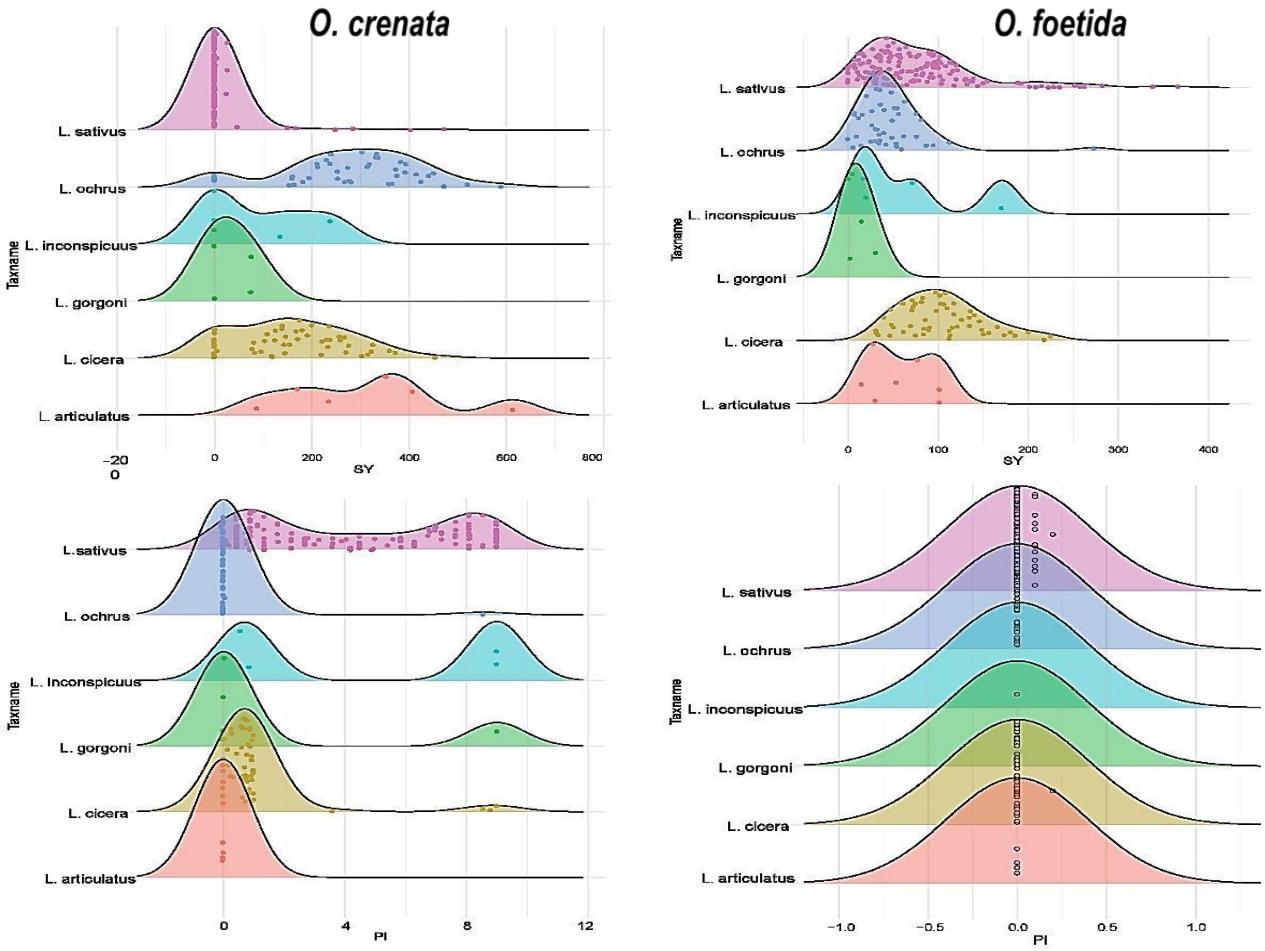


Figure 2: Frequency distribution of six *Lathyrus* species proven by seed yield (SY) and parasitism index (PI) for *Orobanche foetida* examined in Tunisia and *O. crenata* in Morocco.

1.2. Mutivariate analysis

The agronomic performance of different tested accessions and their correlations *Orobanche* infestation parameters were studied using principal component analysis (PCA) (figure 3A). The first two principal components PC1 and PC2 explained 54.9% of the total variation. The PC1 (36.1%) was positively correlated with OIN (r = 0.847), PI (r = 0.737), OSV (r = 0.649) and D2OE (r = 0.593) but negatively correlated with D2F (r = -0.746) and D2M (r = -0.629). The PC2 (18.8%) showed a positive correlation with EODW (r = 0.942) and EON (r = 0.919), and PI (r = 0.423), OSV (r =0.509) and OIN (r = 0.393). Significant positive correlation was observed in PC3 with SY (r = 0.928) and BY (r = 0.897). Further, PCA biplot presented three groups with 85, 20 and 180 accessions in each group. Cluster 1 and cluster 2 revealed almost the same traits values, and

were correlated with BY, SY, D2M and D2F while cluster 3 was more correlated with PI, OIN, OSV, D2OE, EON and EODW.

Cluster 1 mostly grouped the accessions that showed a moderate level of resistance to *O. crenata* and *O. foetida* comprising *L. cicera, L. gorgoni* and some accessions of *L. ochrus* and *L. sativus* (figure 3B) originated from Cyprus, Afghanistan, Iran and Syria (figure 3D). Cluster 2 grouped the accessions that revealed high and moderate resistance to both *Orobanche* species. These accessions belonged to *L. aphaca, L. articulatus, L. blepharicarpus* and *L. ochrus* originating from India, Bangladesh, Portugal and Italy (figure 3D). The third cluster grouped mostly the *O. crenata* and *O. foetida* susceptible accessions. These accessions represent *L. sativus, L. cicera, L. annuus, L. hierosolymitanus,* and *L. tingitanus* and originated mainly from Turkey, Ethiopia, Greece, Germany, Italy, Maldova and Canada. Figure 3C presented the results of the multiple linear regression which is significant at $p < 0.001$ with 73.3% of SY variance explained by OIN, PI and BY. Only three parameters OIN ($\beta = 0.894$, $p < .001$), PI ($\beta = -12.936$, $p < .001$), BY ($\beta = 0.231$, $p < .001$) were significantly associated with SY.

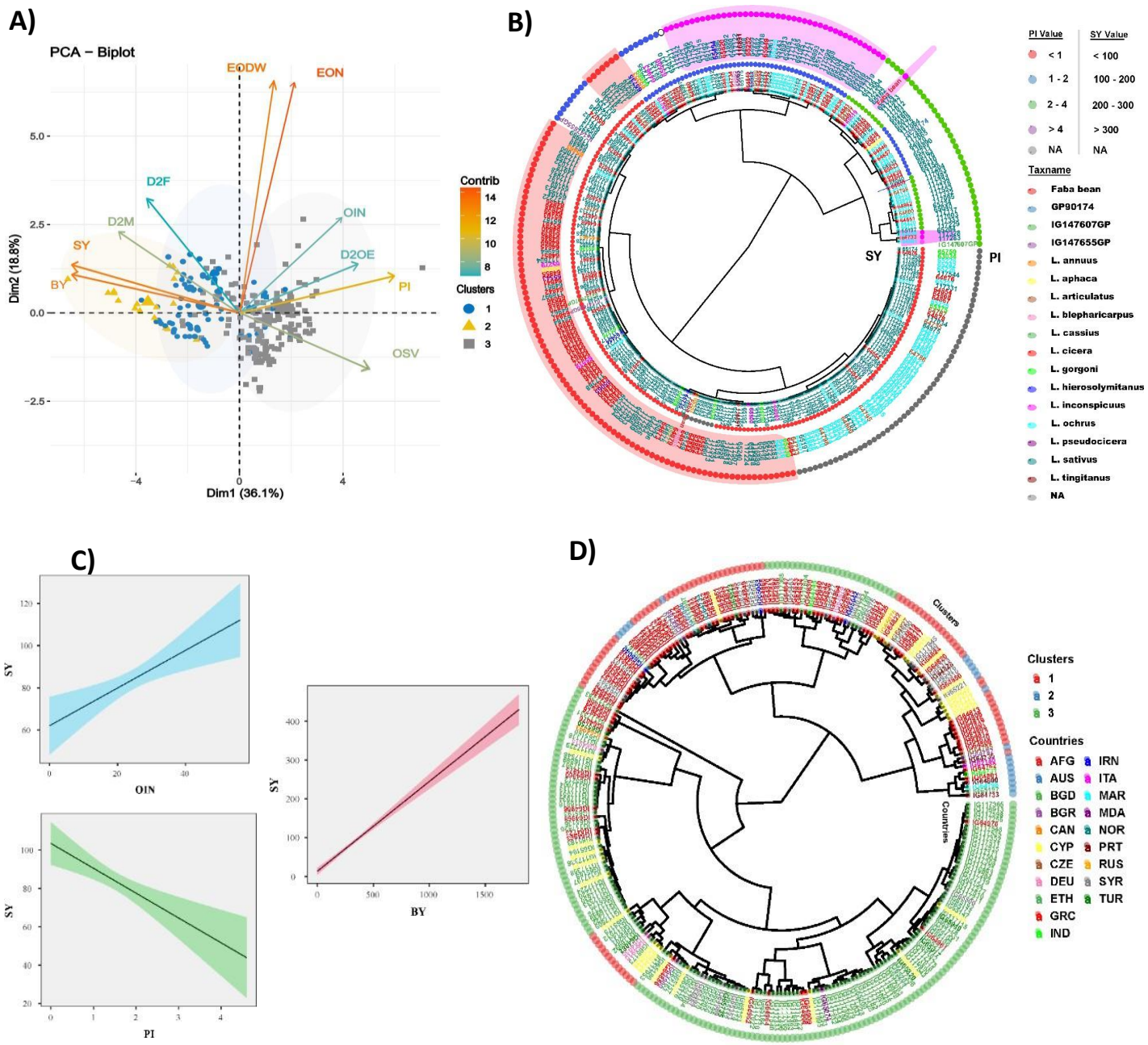


Figure 3: A) Biplot of the two principal components for 285 grass pea accessions consistent with all parameters' performance below *O. crenata* and *O. foetida* infestation under field conditions. B) Dendrogram revealed relationships between SY and PI of global grass pea germplasm. C) Linear regression analysis between SY and OIN, BY and PI in grass pea germplasm accessions. D) Dendrogram revealed relationships between 285 grass pea accessions based on the agronomic performance and their origins.

1.3. Pairwise correlations

The pairwise correlation showed highly positive and significant correlation (r = 0.88) between SY and BY under *O. crenata* infested field at Marchouch (figure 4). *O. crenata* incidence (OIN) was positively and significantly correlated with PI (r = 0.82). D2M of *O. crenata* showed moderate positive correlations with SY, BY of respective values of (r = 0.56) and (r = 0.57). Other moderate and positive correlations were found between OIN and D2OE (0.55) and between PI and OSV (0.53). Days to *O. crenata* flowering (D2F) revealed a low positive correlation with BY (r = 0.41), SY (r = 0.36) and D2M (r = 0.47). D2OE of *O. crenata* showed had a low positive but significant correlations with OSV (r = 0.18) and PI (r = 0.4). Additional, EODW and EON showed a significant low positive correlation with r = 0.47. Negative correlations were registered for *O. crenata* seed yield (SY) and biological yield (BY) with D2EO, OSV and PI. Days to flowering (D2F) and days to *Orobanche* emergence (D2OE) as well as EON and BY showed significant negative correlations (r = -0.17).

Regarding *O. foetida* at Beja station (Tunisia), parasitism index (PI) was highly significant and positively correlated with incidence (OIN) and severity (OSV) along with r = 1 (figure 4). Moreover, *O. foetida* severity was positively correlated with incidence (OIN) (r = 1). Furthermore, D2M and D2F (r = 0.98), SY and BY (r = 0.83), EODW and D2OE (r = 0.77) showed high and significant positive correlations. Both D2M and D2F revealed moderate correlations with EON, SY and BY. Seed yield (SY) showed a positive and moderate correlation with EON (r = 0.62). A low positive and significant correlation was noted among EON and BY with a coefficient of correlation r = 0.37. Both D2M and D2F recorded highly significant but negative correlations with OIN, OSV and PI. Furthermore, these last three parameters had negative and moderate correlations with EON and BY traits and negative and low correlations with seed yield (SY).

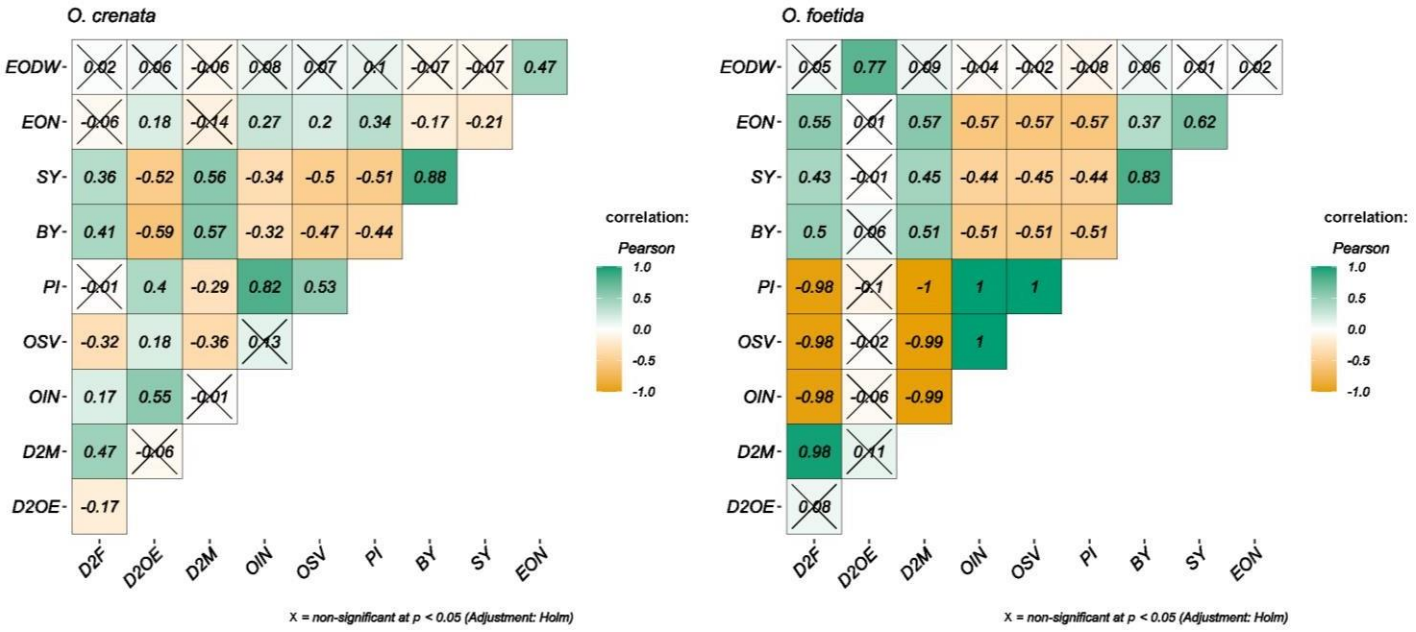


Figure 4: Pairwise correlation between all field parameters contaminated by *O. crenata* in Morocco (left) and *O. foetida* in Tunisia (right).

2.2 Genetic variation in the glasshouse:

2.2.1 Variance and descriptive statistics under non-infested and infested *O. crenata* conditions:

Ten *Lathyrus sativus* accessions (IG65197, IG64782, IG65204, IG64875, IG64951, IG65187, IG116989, IG116997, IG65042, IG64908) were selected from field results to confirm their reaction to *O. crenata* under controlled conditions in pot assay during 2018-2019 (figure 5,6). These accessions have shown resistant, moderate resistant and susceptible reactions under field screening at Marchouch station in Morocco during 2017-2018 cropping season.

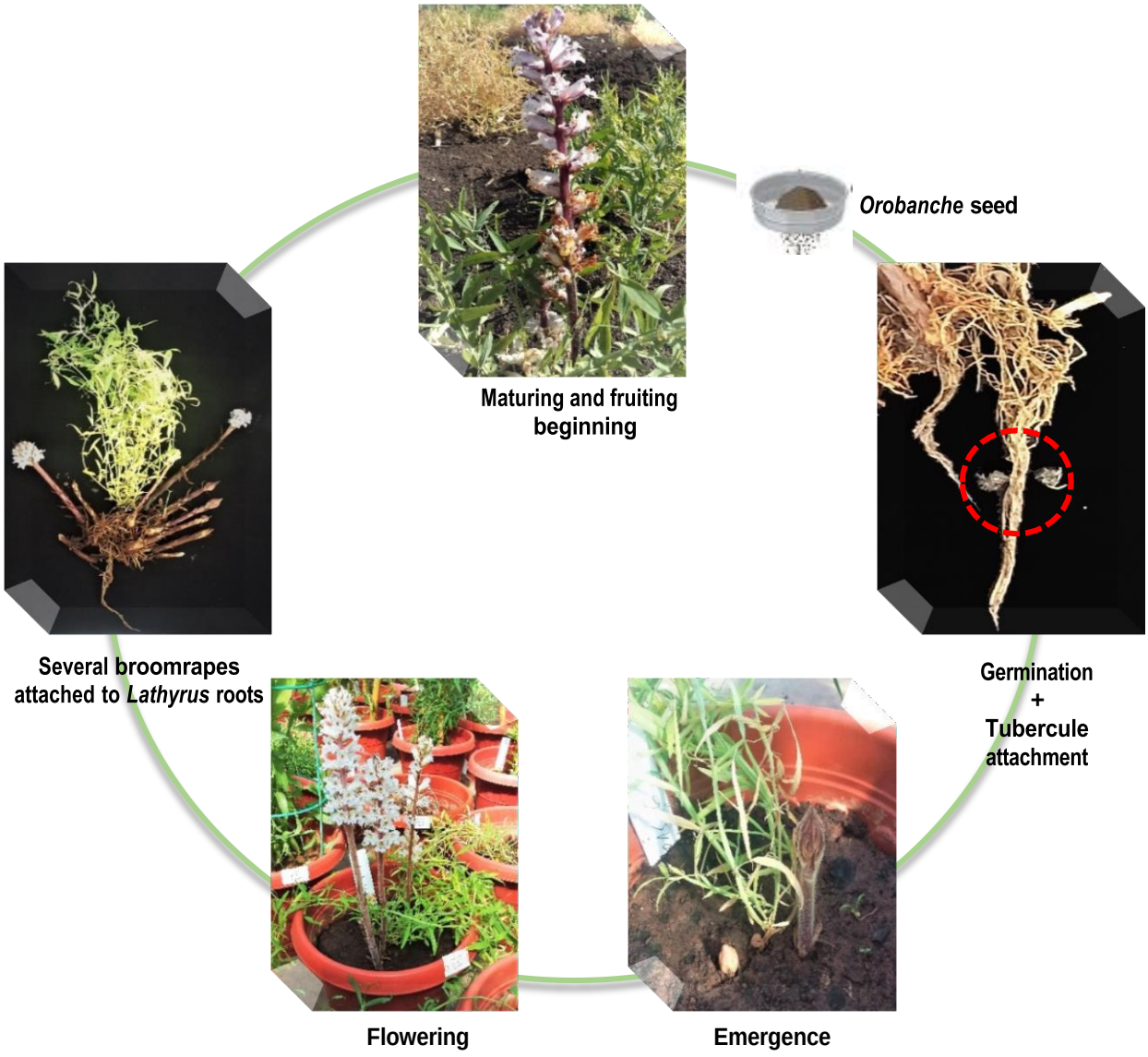


Figure 5: *Orobanche* life cycle in *Lathyrus sativus* infested pots under controlling conditions.

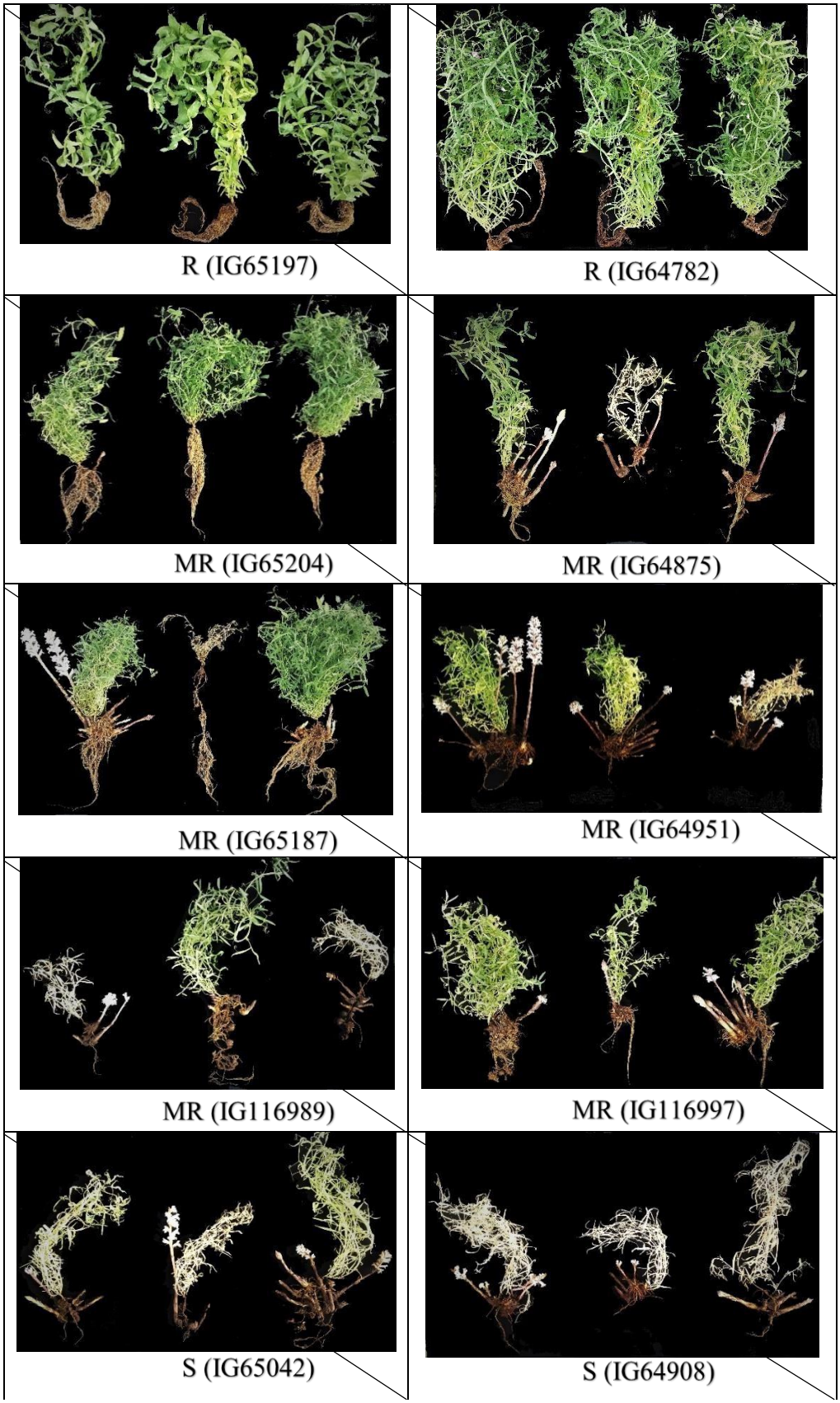


Figure 6: Different reactions of ten *Lathyrus sativus* accessions in *O. crenata* infested pots.

Results showed significant genotypic variation among the tested accessions (table 3). Total *O. crenata* attachment number per plant (TAN) ranged from 0 (IG64782, Ethiopia) to 30 (IG116989, Bangladesh). Averages of 1.8, 1.9, 3.4 and 5 were observed for EODW, UODW, EON and UON, respectively. The maximum EODW, UODW, EON and UON values were recorded for the accessions IG64951 (Cyprus), IG116997 (Bangladesh), IG64875 (Greece) and IG116989 (Bangladesh), respectively, against a minimum observed only in the resistant accession IG64782 originated from Ethiopia.

Table 3: Descriptive statistics and variance registered for five *O. crenata* infestation parameters in selected grass pea accessions in pot experiment during 2018-2019.

	Min.	**Max.**	**Mean**	**F**	**R^2**	**Heritability (h^2)**	**CV(%)**	**Genotypic Variance**	**LSD (95%)**
EODW	0 (**IG64782**)	6.2 (**IG64951**)	1.8	4.9**	0.7	0.8	1.0	4.7	1.2
UODW	0 (**IG64782**)	23 (**IG116997**)	1.9	1.7	0.4	0.6	2.3	11.4	3.9
EON	0 (**IG64782**)	12 (**IG64875**)	3.4	3.6**	0.6	0.9	1.0	14.9	2.6
UON	0 (**IG64782**)	25 (**IG116989**)	5.0	2.2*	0.5	0,9	1.3	33.9	5.6
TAN	0 (**IG64782**)	30 (**IG116989**)	8.43	2.3*	0.5	0,9	0.9	49.0	6.5

TAN: total attachment number per plant; **EON**: emerged *Orobanche* number per plant; **EODW**: emerged *Orobanche* dry weight per plant; **UON**: underground *Orobanche* number per plant; **UODW**: underground *Orobanche* dry weight per plant.

Table 4 (A) represents shoot dry weight (SDW), root dry weight (RDW) and pod number (PDN) per plant of grass pea plants under infested and non-infested conditions. Comparing to non-infested pots, PDN and SDW were decreased significantly, which represents 77.7 % and 50%, respectively. The moderately resistant accession (IG65204) showed the highest PDN in both infested and non-infested pots, as well as a good shoot and root development system (figure 6). As shown in table 4A and figure 6, the resistant accession IG64782 had the highest SDW values, followed by the second resistant one, IG65197. While, IG116989 (MR accession) revealed the lowest SDW in

infested pots (figure 6). Nonetheless, RDW was increased by 300 % in regards to other traits in infested experiments (table 4A). The highest RDW (24) was recorded in IG116997 (MR) (figure 6). Significant genotypic variance and LSD (95%) dissimilarities were revealed for PDN, SDW and RDW (table 4B). The highest genotypic variance was observed for PDN (105.5%) followed by SDW (72.3%) and RDW (16%). The lowest LSD was recorded for RDW.

Table 4: Descriptive statistics and variance registered for three *O. crenata* infestation parameters in selected grass pea accessions in infested and non-infested pot experiment during 2018-2019.

(A)	**Min.**		**Max.**		**Mean**		**% Decrease**
	Infested	**Non-Infested**	**Infested**	**Non-Infested**	**Infested**	**Non-Infested**	
PDN	0 (**IG65187**)	0 (**IG65197**)	24 (**IG65204**)	25 (**IG65204**)	2.8	12.6	77.7
SDW	0.4 (**IG116989**)	3 **(IG116989)**	23.6 (**IG64782**)	21.5 (**IG64782**)	5.0	10.0	50.0
RDW	0.3 (**IG65187**)	0.3 (**IG64951**)	24 (**IG116997**)	2.3 (**IG65197**)	4.0	1.0	-300

(B)	**F**	**R^2**	**Heritability (h^2)**	**CV(%)**		**Genotypic Variance**	**LDS (95%)**
				Infested	**Non Infested**		
PDN	4.5**	0.7	0.7	1.9	0.6	105.5	10.2
SDW	12.2**	0.8	0.6	1.3	0.4	72.3	5.9
RDW	2.8**	0.6	0.5	1.2	0.4	16.0	5.1

2.2.2. Multivariate analysis

Normal frequency distribution was recorded for all the measured parameters (figure 7). The performance for the studied accessions and their correlations with *O. crenata* under controlled conditions were confirmed by PCA and cluster analysis. The first two principal component PC1 (42.1%) and PC2 (22.8%) explained 65.4% of the total genotypic variation (figure 8A). PC1 was positively and strongly correlated with UODW (r = 0.92), RDW (r = 0.88), UON (r = 0.8) and TAN (r = 0.69), but negatively correlated with SDW (r = -0.23), whereas PC2 was positively and highly correlated with EON (r = 0.87) and EODW (r = 0.86) and negatively correlated with SDW (r = -0.7) and PDN (r = -0.42). The biplot (PCA) grouped the *L. sativus* accessions into two different groups. The first cluster contained seven accessions mainly from *L. sativus* with high TAN, UON and RDW values. The second cluster comprised of three *L. sativus* accessions IG64782, IG65204 and IG65187 with lower parameters values in comparison to cluster 1. A

moderate value was observed for TAN and SDW in this cluster. Heat map and hierarchical clustering (figure 8B) were performed to generate and confirm different resistance traits of the ten *L sativus* accessions in the glasshouse, which were selected based on their resistance in the field. Two accessions IG64908 and IG64875 grouped in cluster 1 exhibited high levels of specific resistance traits (EODW and EON). In addition, this cluster showed moderate levels in PDN (IG65187), UODW (IG116997) and UON (IG116989). In the second cluster, seven accessions were distinguished by extremely high values of PDN in IG65204 from Ethiopia and SDW in the most resistant accessions IG64782 and IG65197 originated from Ethiopia. These three accessions revealed a very low TAN, EON and EODW values in this cluster.

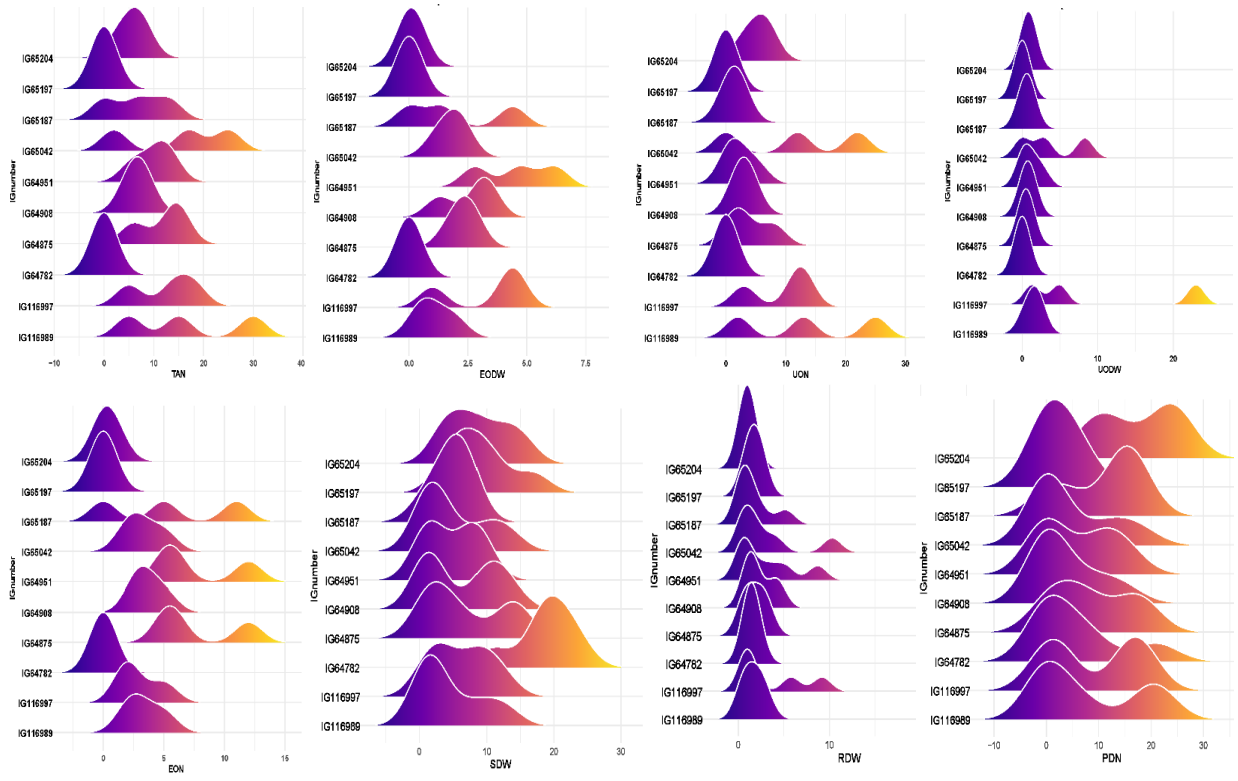


Figure 7: Frequency distribution of morphological and infested traits by *Orobanche crenata* recorded in 10 grass pea accessions in glasshouse under controlling conditions during 2018-2019.

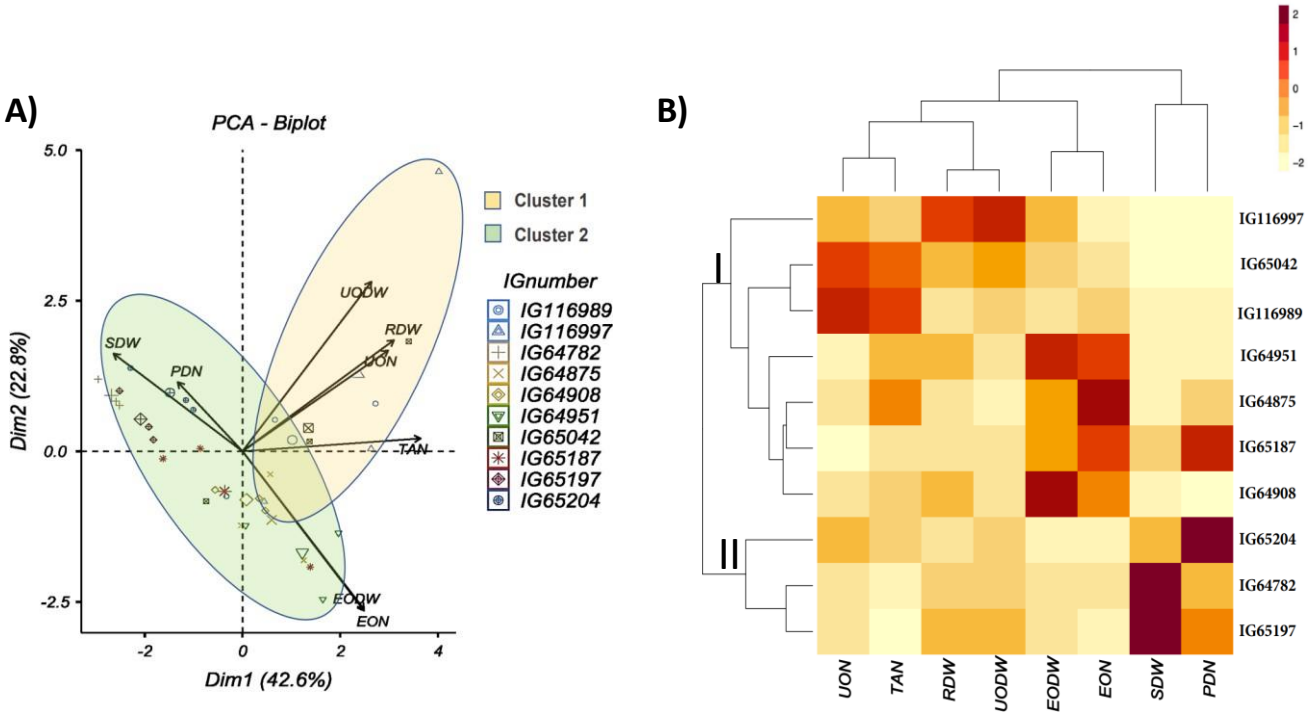
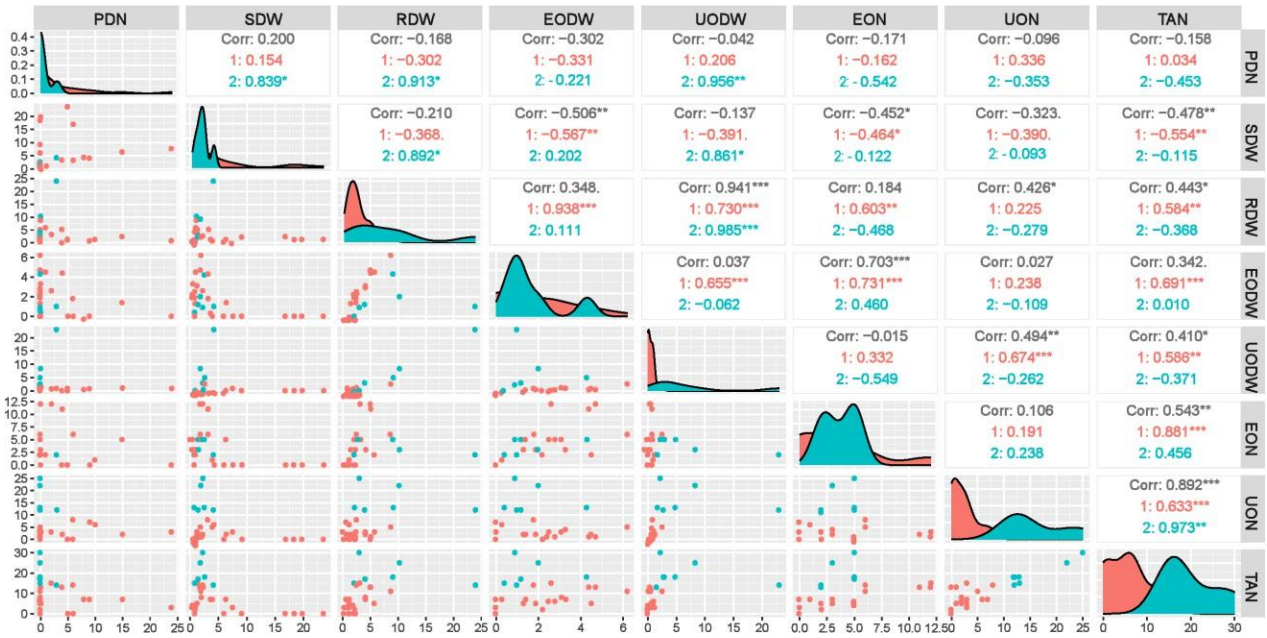


Figure 8: A) Principal component analysis (PCA) and heat map (B) of different infested parameters by *Orobanche crenata* evaluated in 10 grass pea accessions in pot assay under controlling conditions during 2018-2019.

3. Correlations

Correlogram (figure 9) was accomplished on ten grass pea accessions in pot experiment infested by *O. crenata*. A positive and significant correlation was revealed between UON and TAN (r = 0.892***), EODW and EON (r = 0.703***), RDW and UODW (r = 0.941***). EON and TAN with UON and UODW were positively and significantly correlated ($p \leq 0.01$) with r values of 0.543** and 0.494**. Furthermore, significant but negative correlations ($p \leq 0.01$) of SDW were observed with TAN (r = -0.478**) and EODW (r = -0.506**). Other positive and significant correlations were recorded at $p \leq 0.05$ for three combinations namely, TAN with UODW (r = 0.41*), RDW with UON (r = 0.426*) and RDW with TAN (r = 0.443*). However, SDW of *O. crenata* was significantly and negatively correlated with EON (r = -0.452*) at $p \leq 0.05$.



*** significant $p \leq 0.001$ ** significant $p \leq 0.01$ * significant $p \leq 0.05$.

Figure 9: Correlogram of different parameters infested by *O. crenata* under controlling conditions during 2018-2019 at ICARDA, Morocco.

Discussion

Grass pea is an important legume crop whose cultivation is severely constrained by the parasitic weedy broomrape (*O. crenata* Forsk. and *O. foetida* Poir.) in the Mediterranean basin, North Africa and West Asia. Past screening of grass pea germplasm showed complete, moderate and incomplete resistance in the crop wild relatives (CWR) of *Lathyrus* (Abdallah *et al*., 2020). This germplasm collection was analyzed in this study to evaluate agronomic-physiological performance of 13 *Lathyrus* wild species against *O. crenata* and *O. foetida* under open field conditions in Morocco and Tunisia. The results revealed high genotypic variation and heritability for all traits, in the tested germplasm accession in field and pot conditions. Our study confirmed the significant negative impact of *O. crenata* and *O. foetida* (broomrape) parasitism on grass pea development and fertility. It highlights a clear negative correlation between seed yield (SY) and parasitism index (PI). This correlation suggests that the presence of broomrape is a limiting factor of grass pea production. Furthermore, previous studies reported similar negative effects of broomrape parasitism on other leguminous crops such as faba bean (Messa-Garcia and Garcia-torres, 1984; Grenz *et al*., 2005; Abbes *et al*., 2011; Trabelsi *et al*., 2016; Amri *et al*., 2021), lentil (Kumar *et*

al., 2015; Ennami *et al*., 2017; Amri *et al*., 2019; Ennami *et al*., 2020), chickpea (Nefzi *et al*., 2016) and grass pea (Abdallah *et al*., 2020). In summary, the results of the present study underscored the detrimental impact of broomrape parasitism on grass pea, emphasizing the need for effective management strategies to mitigate its effects and ensure sustainable production.

In our study, we used multiple linear regression (MLR) model to detect the relationships between a dependent variable (SY) and multiple independent variables (PI, OIN, EODW, EON, OSV, D2OE and BY). MLR-based models are widely employed in various fields including agriculture to predict crop characteristics or outcomes based on a set of input variables (Parimala and Mathur, 2006; Huang *et al*., 2013; Baldinger *et al*., 2014; Abdipour *et al*., 2015; Abdipour *et al*., 2016; Ghosh and Khan 2018). Our analysis revealed that 73.3% of SY variation was associated with OIN, PI and BY. Similar results were reported by Abdipour *et al*., (2019) on grass pea, a positive linear relationship between SY and BY. Therefore, an increase in the above traits would lead to an increase in seed yield (Boukecha *et al*., 2018). Our finding based on MLR, revealed also that SY is not significantly associated with others infested traits such as_ EON, EODW, OSV and D2OE. Overall, these relationships indicated negative impact of *Orobanche* incidence and parasitism index on grass pea productivity, both in terms of biomass yield and seed yield. According to the results, SY, BY, PI and OIN might be employed as selection criteria in field trials. Türk *et al*., (2007) reported that the biologic yield and 1000-seed weight could be used as selection criteria in early generations.

Correlations were generally higher in field affected by *O. foetida* in Tunisia compared to those affected by *O. crenata* in Morocco. Parasitism index (PI) showed high correlations with OIN and OSV (r = 1). Additionally, there was a strong positive correlation (r = 1) between OSV and OIN. In contrast, no significant correlation was recorded between OSV and OIN under *O. crenata* infestation. EODW was positively correlated with EON with moderate correlation coefficient (r=0.47) under *O. crenata* infested fields. However, this correlation was non-significant under *O. foetida* infestation. On the other hand, Linke *et al*., (1993) reported a strong correlation between these traits in faba bean infested by *O. crenata*. EODW was positively correlated with D2OE with a high r = 0.77. In a field infested with *Orobanche crenata*, a negative correlation was revealed between seed yield (SY) and the emerged *Orobanche* number (EON). Similar results have been

recorded by En-nahli *et al*., (2023) on lentil. Conversely, under infestation by *Orobanche foetida*, a positive correlation was observed between SY and EON. Seed yield and biological yield showed a strong correlation in both infested fields (r = 0.8). Although, negative correlation was revealed between SY and OSV under two open infested fields. Mesa-Garcia and Garcia-Torres (1984) predicted that four *O. crenata* per plant was the average infection severity responsible for the reduction of faba bean seed yield by half, like our predictive system for grass pea seed reduction. It has been previously suggested that seed yield losses induced by *O. crenata* can reach up to 100% (Sauerborn, 1991). In grass pea, seed yield was negligible at severities higher than four parasites per plant. In contrast, at the highest infection severity (11 broomrapes per plant) faba bean and field pea sustained a low but significant seed production (Fernández-Aparicio *et al*., 2016).

In field infested by *O. foetida*, D2F and D2M recorded a very high correlation (r = 0.98). D2F was also positively correlated with other parameters such as D2OE, BY, SY, EON and EODW. These relationships demonstrated that D2F was related with later stages of *Orobanche* emergence and growth, as well as with crop yield traits. The correlation with D2F indirectly affected PI, OSV and OIN. However, in field infested by *O. crenata*, D2F showed significant correlation with fewer parameters, specifically, it was correlated with OIN, SY, BY, D2M and EODW. This indicates that in fields infested by *Orobanche crenata*, the timing of flowering might have a more direct relationship with *Orobanche* infestation severity and crop yield. Kumar and Abbo (2001) and Farida-Traore *et al*., (2022) reported a positive correlation between GY and D2F in chickpea while other studies recorded an indirectly correlation among these latter traits on faba bean (Alghamdi, 2007) and lentil (Vandemark *et al*., 2018). These correlations provide valuable insights into the relationships between various agronomic and phenotypic traits under different *Orobanche* infestations, suggesting potential differences in host plant responses to two *Orobanche* species.

Reduced infestation levels are often the result of a combination of different escape and resistance mechanisms. These mechanisms act at different phases of the infection process and collectively contribute to minimizing *Orobanche* infestation. This approach is common in many crops affected by *Orobanche* (Rubiales, 2004). The use of very early maturing cultivars has been commonly recommended to escape from broomrape damage due to early pod setting. Early flowering has been reported to play a major role in reducing *Orobanche* infection in various crops including faba

bean (Manschadi *et al*., 1997; Grenz *et al*., 2005), lentil (Fernández-Aparicio *et al*., 2008; Trabelsi *et al*., 2015), and *Lathyrus cicera* (Fernández-Aparicio *et al*., 2009a). Fernández-Aparicio *et al*. (2012) mentioned that very late accessions of *Lathyrus sativus* can also escape *Orobanche* infection. These late-flowering accessions develop most of their roots too late for *Orobanche* establishment, as conditions become less favorable for the parasite.

Based on the combined assessment of PI and SY, this study selected the best-performing accessions that demonstrated resistance or tolerance to *O. crenata*. Their resistance was reconfirmed in the pot experiment under controlled conditions. Number of emerged broomrape per host plant is considered to be the resistance index to broomrape (Gil *et al*., 1987; Cubero, 1991; Qasem and Kasrawi, 1995; Rubiales *et al*., 2005; Abbes *et al*., 2007; Román *et al*., 2011; Trabelsi *et al*., 2016; Briache *et al*., 2019; Cuccurullo *et al*., 2022). Abdallah *et al*., (2020) suggested underground shoot trait as a criterion for resistance assessment against *Orobanche* in the pot experiment. The present study recorded significant genotypic variation with high heritability both under infested and non-infested pots. The coefficient of variation (CV%) was higher in infested pots compared to non-infested ones. This variability could stem from differences in *Orobanche* attachment, nutrient uptake, and other factors that influence plant growth and development. Emerged (EON) and underground (UON) *Orobanche* numbers were positively correlated with total attachment number (TAN) and emerged *Orobanche* dry weight (EODW). These results agree with previous reports on faba bean for resistance to *O. crenata* and *O. foetida* (Trabelsi *et al*., 2016). Two accessions of *L. sativus* (IG64782 and IG651970) originating from Ethiopia showed complete resistance under both field conditions and glasshouse experiments. Resistant and tolerant accessions are mostly originated from Asia (Middle east, Bangladesh, India), Cyprus and Ethiopia and represented *L. ochrus*, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius* and *L. pseudocicera*. Susceptible accessions belonged mainly to *L. sativus*, *L. cicera*, *L. annuus*, *L. hierosolymitanus,* and *L. tingitanus* derived from Europe and North America. This distribution might be influenced by various factors, including environmental conditions, genetic diversity, and historical agricultural practices. The geographical distribution of resistant and susceptible accessions can could be useful in developing breeding programs and management strategies aimed at developing *Orobanche*-resistant cultivars and mitigating the impact of *Orobanche* infestation on legume crops in different regions.

These resistant accessions of grass pea are being explored by molecular and cytogenetic studies to identify quantitative trait loci (QTLs) associated with resistance to *Orobanche*. Molecular techniques such as marker-assisted selection (MAS), genetic mapping, and genome sequencing may be employed to find genomic regions associated with resistance traits. For cytogenetic techniques such as karyotyping, fluorescence in situ hybridization (FISH), and chromosome banding may be used to analyze the chromosome structure and organization related to resistance traits. These studies will provide valuable information for grass pea pre-breeding and breeding programs. Identification of resistance genes/QTLs and understanding their genetic basis will enable breeders to develop improved grass pea varieties with enhanced resistance to *Orobanche*, contributing to sustainable agriculture production.

Conclusion

The parasitic weeds *O. crenata* and *O. foetida* are considered to be one of the most important constraints limiting grass pea cultivation in the Mediterranean region. Breeding for resistance remain the most successful strategy to control these broomrapes. The field and controlled conditions evaluation of the large germplasm collection revealed new potential sources for resistance in *L. sativus* (IG64782 and IG65197) and wild species; *L. ochrus* (IG64823, IG62140, IG64731, IG64801, IG64804, IG64807, IG64808, IG64809, IG64810, IG64812, IG64813, IG64814, 64816,), *L. aphaca* (IG65053), *L. articulatus* (IG64733, IG64735, IG64786, IG64787, IG64795, IG64798, IG64800), *L. blepharicarpus* (IG64865), *L. cassius* (IG65277) and *L. pseudocicera* (IG65065, IG65543) originated mostly from Asia, Cyprus and Ethiopia. These sources can be used in future breeding activities for future breeding activities aimed at developing *Orobanche*-resistant cultivars. BY, PI and OIN were the strongest driver traits that influenced the SY. Root dry weight should always be considered a good selection criterion for identification of *Orobanche* resistance.

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III. Conclusion:

According to the findings of the current study, grass pea exhibits genetic variability, with high heritability and coefficient of variation, as well as a good understanding of the genotype*parasite interaction. The evaluation of a large germplasm collection under both field and controlled conditions has revealed new potential sources of resistance in *Lathyrus sativus* (accessions IG64782 and IG65197) and various wild species, including: *L. ochrus* (IG64823, IG62140, IG64731, IG64801, IG64804, IG64807, IG64808, IG64809, IG64810, IG64812, IG64813, IG64814, 64816,), *L. aphaca* (IG65053), *L. articulatus* (IG64733, IG64735, IG64786, IG64787, IG64795, IG64798, IG64800), *L. blepharicarpus* (IG64865), *L. cassius* (IG65277) and *L. pseudocicera* (IG65065, IG65543). These accessions predominantly originated from Asia, Cyprus, and Ethiopia and can be utilized in future breeding activities aimed at developing *Orobanche*-resistant cultivars.

The Multiple Linear Regression, PCA, Dendrogram and correlogram of field analysis were showed different relationships between all studies traits. Seed yield (SY) was significantly and strongly correlated with biological yield (BY). However, a negative correlation was observed between SY and OSV (*Orobanche* severity). Results concluded that OSV, BY and PI were the strongest influential traits in SY.

The resistance validation was conducted under controlled conditions in the glasshouse. Significant variability, heritability and interactions were observed fo all studied parameters. In fact, strong and positive correlations were observed between UON and TAN ($r = 0.892^{***}$), EODW and EON ($r = 0.703^{***}$), RDW and UODW ($r = 0.941^{***}$). We also reported that an increase in root dry weight in the presence of *Orobanche* in pots could be an indicator of resistance or tolerance. Plants that can maintain or enhance root growth may be better equipped to withstand the negative effects of parasitism. This parameter could be a valuable trait to consider in breeding programs aimed at developing *Lathyrus* cultivars with improved resistance to *Orobanche*.

Chapter IV: Investigating genetic diversity and correlations between mineral concentrations and neurotoxin (β -ODAP) content in Lathyrus genus.

I. Introduction:

Grass pea is an important legume grown in South Asia, sub-Saharan Africa, and to a limited extent in other regions such as Southern Europe and South America (Bishoi and Khetarpaul., 2013; Ramya *et al*., 2022). Its global cultivated area has reduced significantly over the years, from 1.5 million hectares and 1.2 million tons of production to 0.7 million hectares and 0.79 million tons (Kumar *et al*., 2011). Despite this, it remains an essential crop for fragile agro-ecosystems due to its resilience to extreme climatic conditions like drought, waterlogging, soil salinity, and high temperatures (Aarti, 2016). There is great potential for the expansion in the utilization of grass pea in dry areas and zones which are becoming more drought-prone as a result of climate change (Lambein *et al*., 2009).

It also contributes to soil fertility by fixing atmospheric nitrogen. In addition, grass pea seeds are rich in protein, fiber, and essential micronutrients like magnesium, phosphorus, calcium, iron, zinc, manganese, and copper. This makes it a valuable source of nutrition, particularly in regions where other crops may be less productive or harder to grow (Vaz Patto *et al*., 2018; Vaz patto 2016). The most important issue holding back breeding efforts that would enable more widespread utilisation of grass pea as a food and fodder crop is the fact that it contains *β-L*-ODAP, a toxin believed to cause neurolathyrism in humans (Girma and Korbu, 2012; Yang and Zhang, 2005).

The goal of this study is to identify the best performing grass pea accessions with low *β*-ODAP content and high mineral concentrations, to understand the correlations between the levels of *β*-ODAP and other nutritional traits and to explore the genetic variation in wild relatives of grass pea in terms of both nutritional quality and *β*-ODAP content.

The graphical abstract and the supplementary material of the following paper are presented in the Appendix 4a and 4b.

II. Scientific contribution:

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Investigating Genetic Diversity and Correlations Between Mineral Concentration and Neurotoxin (β-ODAP) Content in the *Lathyrus* Genus

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MDPI

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Investigating Genetic Diversity and Correlations Between Mineral Concentration and Neurotoxin (β-ODAP) Content in the *Lathyrus* Genus

Fadoua Abdallah [1,2], Zakaria Kehel [1,*], Mohamed Amine El Kalchi [3], Ahmed Amri [1], Adil el Baouchi [4], Zine El Abidine Triqui [2], Moez Amri [4,*] and Shiv Kumar [1,*]

[1] International Center for Agricultural Research in the Dry Areas (ICARDA), Rabat 10112, Morocco; fadoua.abdallah.fadoua@gmail.com (F.A.)
[2] Faculty of Sciences, University Mohammed V, Rabat 10106, Morocco
[3] Agence National des Eaux et Forêts (ANEF), Agro Paris Tech, 75007 Paris, France
[4] AgroBioSciences Program (AgBS), College of Agriculture and Environmental Science (CAES), University Mohammed VI Polytechnic (UM6P), Ben Guerir 43150, Morocco
* Correspondence: z.kehel@cgiar.org (Z.K.); moez.amri@um6p.ma (M.A.); sk.agrawal@cgiar.org (S.K.)

Abstract: Grass pea (*Lathyrus sativus* L.) is a nutritious legume crop well-adapted to fragile agro-ecosystems that can survive under challenging climatic conditions. The cultivation of grass pea faces stigma primarily due to the presence of β-*N*-Oxalyl-*L*-α, β-diaminopropionic acid (β-ODAP), which is associated with a risk of inducing neurolathyrism upon prolonged consumption of its grains as a staple diet. The grass pea improvement program of the International Center for Agricultural Research in the Dry Areas (ICARDA) aims to reduce β-ODAP content to a safe level along with improving yield potential and nutritional quality of grass pea. In this study, 183 germplasm accessions representing 13 different *Lathyrus* species and 11 *L. sativus* breeding lines were evaluated for β-ODAP content based on Rao protocol and mineral concentration using ICP-OES. Significant variability was observed among the accessions for the studied traits. The results showed low β-ODAP content and high mineral concentration in 25 accessions of crop wild relatives, which included *L. cicera*, *L. ochrus*, and *L. cassius*, with one accession IG65277 of *L. cassius*, in addition to two lines, IG117034 and ACC1335, of *L. sativus* having very low β-ODAP content. Furthermore, some accessions of *L. pseudocicera*, *L. aphaca*, *L. cicera*, *L. marmoratus*, *L. gorgoni*, and *L. tingitanus* also showed low β-ODAP content. The results showed significant positive correlations among different trait combinations, viz., K and P (r = 0.193 ***), K and Fe (r = 0.177 ***), Mn and Fe (r = 0.210 ***), Mn and Se (r = 0.137 ***), β-ODAP and Mg (r = 0.158 **), and β-ODAP and Ca (r = 0.140 **). *L. cicera*, *L. ochrus*, and *L. cassius* were identified as a great source for improving the mineral concentration and reducing β-ODAP content in the cultivated grass pea.

Keywords: *Lathyrus* spp.; grass pea; β-ODAP content; neurotoxins; mineral concentration; wild crop relatives; genetic variability; correlation

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1. Introduction

Grass pea (*Lathyrus sativus* L.) is a nutritious legume popularly known as chickling pea, khesari in India and Bangladesh, pois carré or gesse in France, Cicerchia coltivata in Italy, guaya in Ethiopia, and san li dow in China. It is an annual cool-season crop of economic and ecological significance in South Asia and sub-Saharan Africa, and to a limited extent in Central and West Asia and North Africa (CWANA), Southern Europe, and South America [1]. Globally, grass pea cultivated area at 0.70 million ha with 0.79 million tons' production has observed drastic reduction from the last global estimate of production (1.2 million tons) and area (1.5 million ha) [2]. Grass pea is one of the most resilient crops due to its ability to grow under extreme climatic conditions such as drought, waterlogging,

soil salinity, and high temperature, but remains the most underutilized crop for adaptation to fragile agro-ecosystems [1].

The genus *Lathyrus* comprises about 181 annual and perennial species [3]. *L. sativus* is widely cultivated as a food and feed crop [4], mostly in the Indian subcontinent, Ethiopia, and the Mediterranean region [5]. *L. cicera* is grown mainly as stock feed, both as fodder and grain, and its cultivation has been confined to South-western Europe and, to a limited extent, in West Asia and North Africa [6]. Recently, grass pea has received considerable attention from scientific and farming communities, and it is considered an excellent option for building agri-food sustainability under stress conditions such as drought, waterlogging, high temperature, cold, and salinity [7]. It has a very hardy and penetrating root system and, therefore, can be grown on a wide range of soil types, including very poor soil and heavy clays. Its ability to fix atmospheric nitrogen up to 124 kg/ha, especially in dry conditions [8], makes the crop well suited for harsh conditions [9]. In South Asia, grass pea is well-suited to the rice ecosystem as it is typically broadcasted in the standing rice crop before its harvest, thriving on the residual moisture. Therefore, it makes a valuable contribution for the development of a sustainable rice production system [9,10]. Compared with the other legumes, grass pea is relatively resistant to insect pests [11,12] and diseases but highly susceptible to parasitic weeds, *Orobanche crenata* and *O. foetida* [13]. The grass pea seeds are rich in protein, micronutrients, and fiber [14–16]. In addition to that, it is also rich in magnesium, phosphorus, calcium, iron, zinc, manganese, and copper. Despite these advantages, its consumption has declined due to the presence of a neurotoxin known as β-*N*-oxalyl-*L*-α, β-diaminoproprionic acid (β-ODAP) in seeds and vegetative tissues that can lead to neurolathyrism if taken in large quantities continuously for long periods as a staple diet [10,17–20]. Previous studies conducted on *L. sativus* showed significant variability in seeds' β-ODAP content ranging from 0.518 to 1.001 mg/100 g [21]. These levels are 2 to 5 times higher than the level presumed to be safe for human consumption [22]. Various processing methods have been suggested to reduce the β-ODAP content [23,24]. However, none has proven entirely effective in eliminating β-ODAP, including techniques such as soaking and boiling, roasting, extrusion, cooking, fermenting, and autoclaving [25]. Crop improvement efforts are currently underway, employing conventional methods to identify grass pea accessions with lower neurotoxin levels at ICARDA [26]. Exploring the inter- and intra-specific genetic diversity within the *Lathyrus* genus could serve as a valuable strategy for discovering and mining low β-ODAP traits. The other published materials on this topic mainly focus on other aspects of *Lathyrus sativus*. The objectives of this study were as follows: (1) to identify germplasm of *Lathyrus sativus* with low β-ODAP levels but high concentrations of macro- and micronutrients; (2) to evaluate the relationships among nutritional traits and β-ODAP content; and (3) to investigate the genetic diversity in terms of nutrient and β-ODAP concentrations among the wild relatives of *Lathyrus*.

2. Results

2.1. β-ODAP Content and Macro- and Micronutrient Concentrations

The MANOVA (Table 1) showed significant genotypic differences ($p < 0.01$) for macro- and micronutrients and for β-ODAP content among the accessions. The results showed that potassium (K) and zinc (Zn) reached the highest mean values of 2953.8 ppm and 33.4 ppm, respectively. Contrariwise, the lower concentrations have been recorded in magnesium (Mg) (1027.5 ppm) and selenium (Se) (0.1 ppm). Phosphorus (P) content varied from 953.7 (IG65224) to 3788.9 ppm (IG64807). Notably, IG64807 of *L. ochrus* exhibited a maximum value of P (Table 1). Calcium (Ca) content ranged from 688.4 ppm (IG64834) to 1678.4 ppm (IG116889). The highest Ca and K concentrations were observed in *L. sativus* (IG116889) and (IG117365), respectively; both originated from Bangladesh (Table S1). The maximum Mg concentration was observed in IG65074 (*L. cicera*). Two accessions, namely, IG117012 and ACC1916, of *L. sativus* showed the highest values of iron (Fe) (57.1 ppm) and manganese (Mn) (30.0 ppm) content. ACC1348 revealed the highest Zn (48.7 ppm) content among all the accessions. The highest concentration of Se was recorded in IG62145 (*L. odoratus*)

(Table 1). Low β-ODAP content was found in IG65277 (0.01%), while IG65340 showed a high β-ODAP content of 0.3%. Indeed, this *L. cassius* accession, IG65277, originated from Syria. High heritability was recorded for Mn, Fe, Se, and β-ODAP contents; moderate heritability was recorded for P, K, Ca, and Mg contents; and low heritability was recorded for Zn content. Significant genotypic variation was observed for all the parameters. The coefficients of variance (CV%) and LSD (95%) showed variation in nutrient content and β-ODAP. The highest value of CV was recorded for Mn (122.51%). Low genotypic variance (0.02) and LSD (0.1) were observed for Se.

Table 1. Range, mean, F, R^2, heritability (h^2), coefficient of variance (CV%), genotypic variance, and LSD of nutrients and β-ODAP for 183 grass pea accessions belonging to 13 species.

Traits	Min.	Max.	Mean	F	R^2	Heritability (h^2)	CV (%)	Genotypic Variance	LSD (95%)
P	953.7 (IG65224)	3788.9 (IG64807)	2405.8	63.9 **	0.9	0.5	31.6	308,479.4	177.9
K	1478.9 (ACC1916)	4189.1 (IG117365)	2953.8	40.9 **	0.9	0.4	19.0	157,105.1	169.2
Ca	688.4 (IG64834)	1678.4 (IG116889)	1080.1	5.7 **	0.8	0.5	18.9	23,025.8	74.6
Mg	601.1 (IG64858)	1765.4 (IG65074)	1027.5	10.4 **	0.9	0.5	24.3	33,138.6	69.6
Mn	0.1 (IG117018)	30.0 (ACC1916)	6.3	21.5 **	0.9	0.6	122.5	37.5	9.6
Fe	14.4 (ACC1330)	57.1 (IG117012)	30.1	75.3 **	0.9	0.6	27.0	43.6	6.0
Zn	20.3 (IG65147)	48.7 (ACC1348)	33.4	48.4 **	0.9	0.2	18.0	8.3	8.39
Se	0.01 (IG65053)	0.6 (IG62145)	0.1	66.3 **	0.9	0.7	77.2	0.02	0.1
β-ODAP%	0.01 (IG65277)	0.3 (IG65340)	0.1	2.5 **	0.7	0.5	66.0	38.6	5.0

** singnificant at ($p < 0.01$).

2.2. Principal Component Analysis of Macronutrients

Normal distribution frequency was observed for all macronutrients (Figure 1). Principal component analysis (PCA) was used to investigate the correlations among different study traits in the examined *Lathyrus* accessions. The first two principal components explained together 59.4% of the total variance for macronutrient contents (Figure 2A). The first component PC1 accounted for 31.5%, and the second component PC2 explained an additional 27.9% of the observed variation.

Table 2 revealed that PC1 was positively correlated with Mg (r = 0.599). PC2 was positively correlated with P (r = 0.630) and K (r = 0.753), but negatively correlated with Ca and Mg. PC3 recorded a positive correlation with Ca (r = 0.678), a low positive correlation with Mg (r = 0.187), and a negative correlation with P (r = −0.014) and K (r = −0.033). The results showed that PC4 was positively correlated with P (r = 0.335), Ca (r = 0.177), and Mg (r = 0.524) but negatively correlated with K (r = −0.068).

The principal component analysis biplot (Figure 2A) grouped these accessions into two clusters. The first cluster comprised 72 accessions, mainly from breeding lines *L. cicera*, *L. gorgoni*, *L. ochrus*, and *L. sativus* (Figure 2C), with high P and K and moderate Mg and Ca contents. The second cluster, consisting of 111 accessions, exhibited comparable Mg and Ca values to those in cluster 1, along with lower K and P contents than cluster 1. Cluster 2 included accessions belonging to *L. annuus*, *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius*, *L. marmoratus*, *L. odoratus*, *L. pseudocicera*, and *L. tingitanus* (Figure 2B,C).

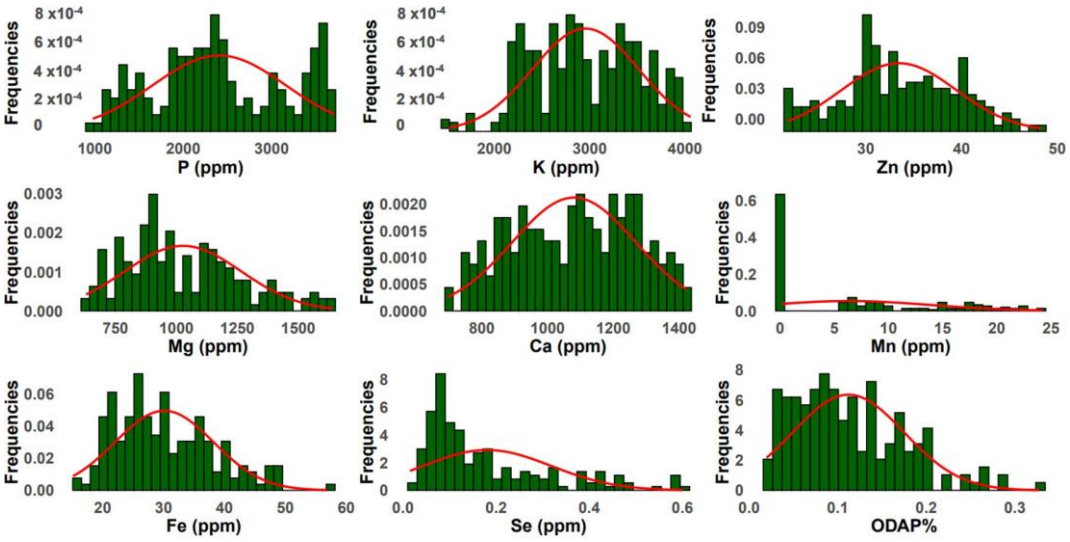

Figure 1. Frequency distribution of macro- and micronutrients and β-ODAP concentrations in grass pea species.

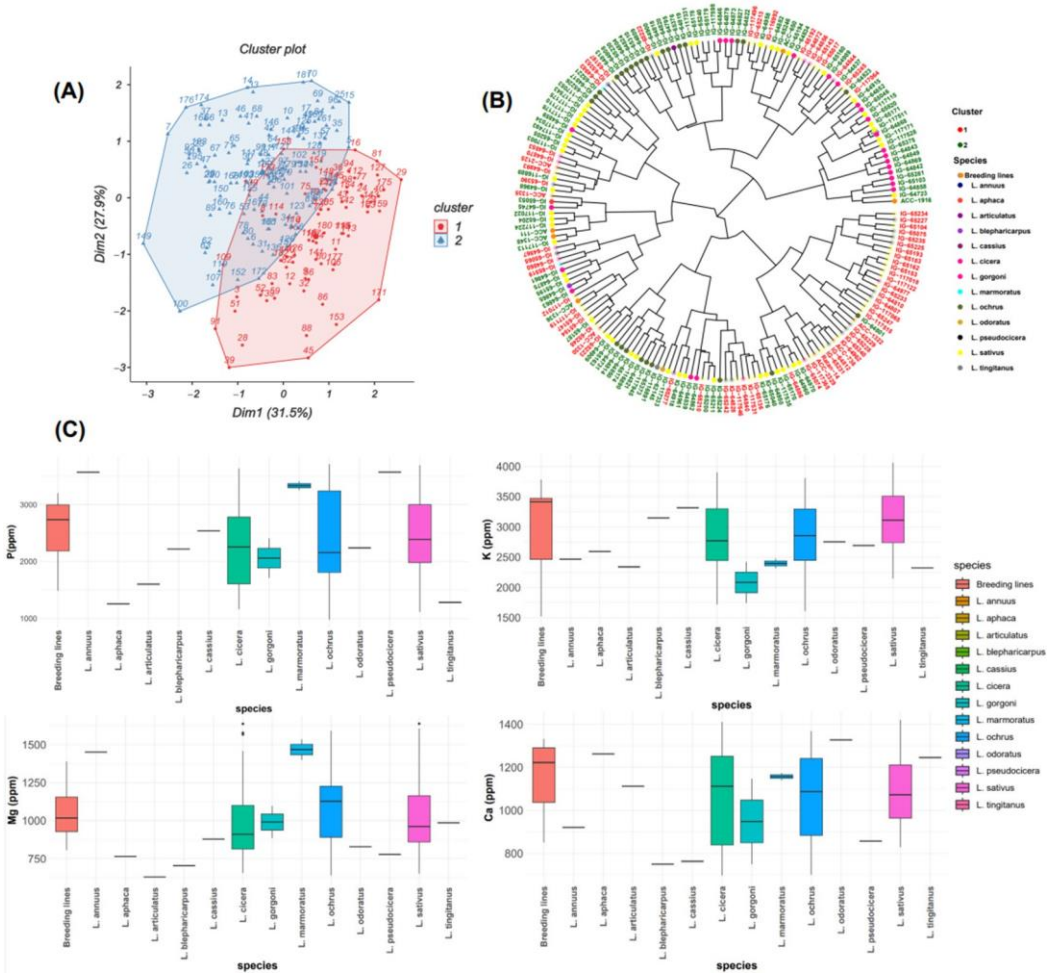

Figure 2. (**A**) Biplot of the first two dimensions of PCA based on the macronutrient contents for all tested grass pea germplasms. (**B**) Dendrogram showing the level of macronutrients in the total examined accessions. (**C**) Box plots showing a comparison of different macronutrient concentrations among the whole analyzed species.

Table 2. The first four principal components of principal component analyses (PCAs) for 183 grass pea accessions based on their neurotoxins (β-ODAP%) and macro- and micronutrient concentrations.

	PC1	PC2	PC3	PC4
P	0.044	**0.630**	−0.014	0.335
K	0.052	**0.753**	−0.033	−0.068
Ca	0.055	−0.250	**0.678**	0.177
Mg	**0.599**	−0.191	0.187	0.524
Mn	**0.734**	0.003	0.178	−0.141
Fe	**0.646**	0.252	−0.246	−0.134
Zn	−0.019	−0.156	0.373	**−0.416**
Se	0.038	0.391	**0.717**	−0.078
β-ODAP%	−0.242	0.075	0.104	**0.706**

Bold values for each column correspond to the parameters highly correlated (positive or negative) with each of the four PCs.

2.3. Principal Component Analysis of Micronutrients

The frequency distribution of the three micronutrients Zn, Fe, and Se followed a normal distribution among the tested grass pea germplasm (Figure 1). Principal component analysis for all micronutrients (Figure 3A) revealed that the first two principal components explained 56.5% of the total variation. PC1 (31%) was highly associated with Mn (r = 0.734) and Fe (0.646) and revealed positive correlation with Se (r = 0.38) and negative correlation with Zn (r = −0.19) (Table 2). A positive correlation was observed between PC2 (25.4%) and all micronutrients (Mn, Fe, and Se) except Zn (r = −0.156). A positive correlation was recorded between PC3 and Se (r = 0.717), Mn (r = 0.178), and Zn (r = 0.373), while negative correlation was revealed with Fe (r = −0.246). PC4 had negative correlations with Se (r = −0.078), Fe (r = −0.134), Mn (r = −0.141), and Zn (r = −0.416). PCA grouped all 183 accessions into two clusters comprising 114 and 69 accessions (Figure 3A). Cluster 1 grouped the accessions with high Fe and Zn concentrations. Box plots (Figure 3C) showed that cluster 1 contained breeding lines and accessions of *L. cassius*, *L. cicera*, *L. gorgoni*, *L. marmoratus*, *L. ochrus*, and *L. sativus*. The second cluster included accessions with a moderate value of Mn and Se and almost the same Zn and Fe contents (Figure 3A,B). This group embraced other species, namely, *L. pseudocicera*, *L. blepharicarpus*, *L. articulatus*, *L. annuus*, *L. tingitanus*, *L. odoratus*, and *L. aphaca* (Figure 3B,C).

2.4. Principal Component Analysis of β-ODAP Content

Principal component analysis indicated that the first two principal components, PC1 (16.4%) and PC2 (15%) explained together 31.4% of the total variability of β-ODAP content (Figure 4A). β-ODAP content was positively correlated with PC4 (r = 0.706) and moderately associated with PC2 (r = 0.075) and PC3 (r = 0.104). A frequency distribution representing the normal distribution of β-ODAP content across the tested accessions is illustrated in Figure 1. A negative correlation was observed between β-ODAP content and PC1 (r = −0.242) (Table 2).

Further, Biplot and ggtree (Figure 4A,B) showed 72 accessions in cluster 1 and 111 accessions in cluster 2. Clusters 1 and 2 were characterized by a high content of Zn and Fe, with moderate concentrations in Mg and Ca. On the other hand, P and K content were higher in cluster 1 than in cluster 2. On the other side, the β-ODAP value was 0.11% in cluster 1 and 0.10% in cluster 2. The dendrogram was used to identify and select the germplasm with good performance for nutrient and β-ODAP contents (Figure 4B). Three species, *L. sativus*, *L. ochrus*, and *L. cicera*, and breeding lines belonging to *Lathyrus sativus* were grouped into two clusters. Accessions belonging to *L. annuus*, *L. blepharicarpus*, *L. marmoratus*, and *L. pseudocicera* were included in cluster 1, and *L. articulatus*, *L. odoratus*, *L. cassius*, *L. aphaca*, *L. gorgoni*, and *L. tingitanus* were included in cluster 2 (Figure 4B). The box plot analysis revealed species with low and high β-ODAP content (Figure 4C). Accession with the lowest β-ODAP content belonged to *L. cassius* (0.01%, IG65277) (Table 1, Figure 4B), followed by accessions of *L. pseudocicera*, *L. aphaca*, *L. cicera*, *L. marmoratus*, *L. gorgoni*, and

L. tingitanus. Species that revealed high β-ODAP concentrations (≥0.10%) were *L. ochrus* (0.3%, IG65340), *L. articulatus*, *L. odoratus*, *L. annuus*, *L. sativus*, and *L. blepharicarpus*. In general, results revealed that *L. cicera* showed the lowest β-ODAP content (0.02% to 0.1%), followed by *L. sativus* (0.03% to 0.2%) and *L. ochrus* (0.1% to 0.3%) (Figure 4C).

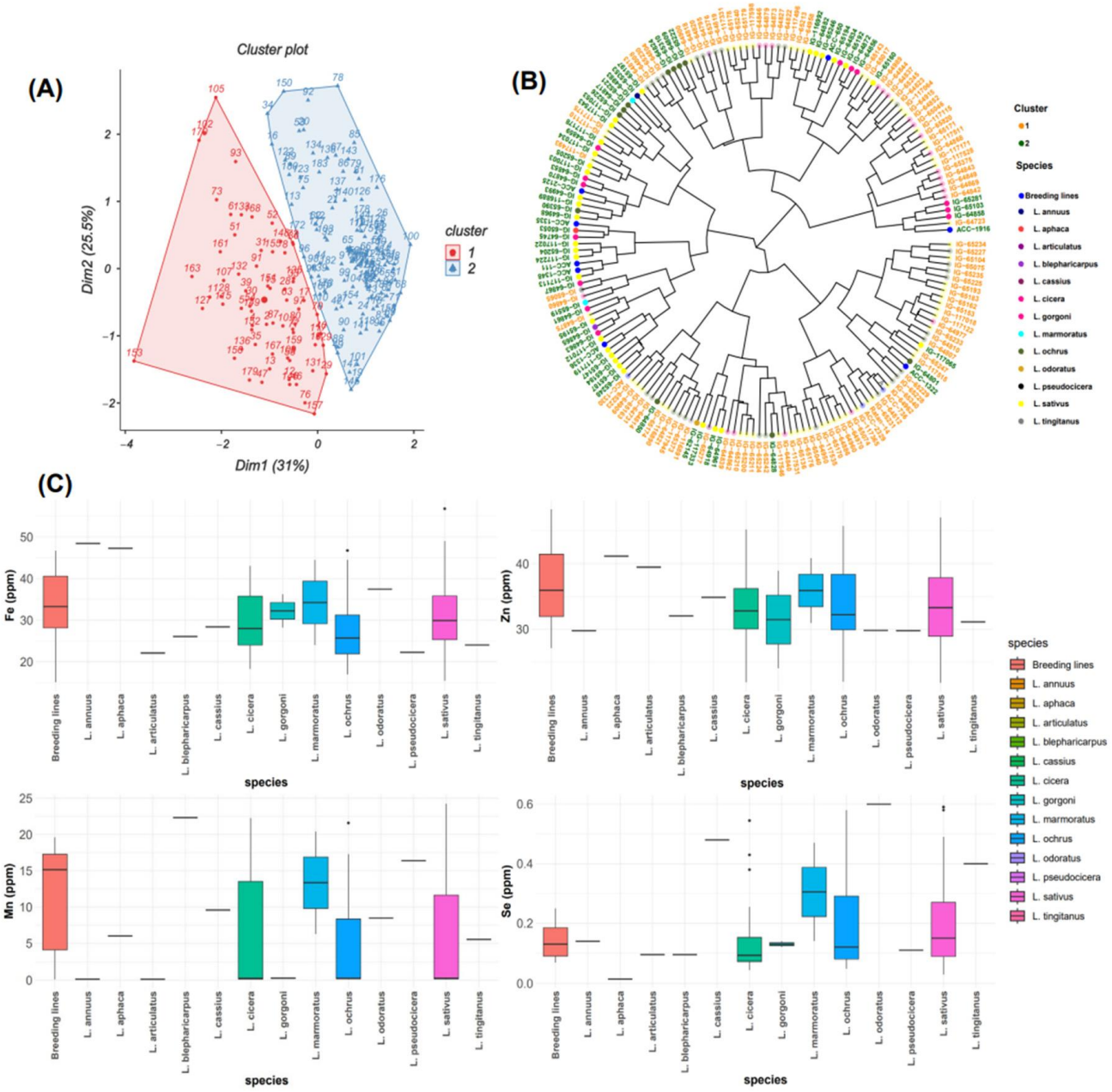

Figure 3. (**A**) Biplot of the first two dimensions of PCA based on the micronutrient contents for grass pea accessions. (**B**) Dendrogram showing the level of micronutrients in the total examined accessions. (**C**) Box plots showing a comparison of different micronutrient concentrations among the whole analyzed species.

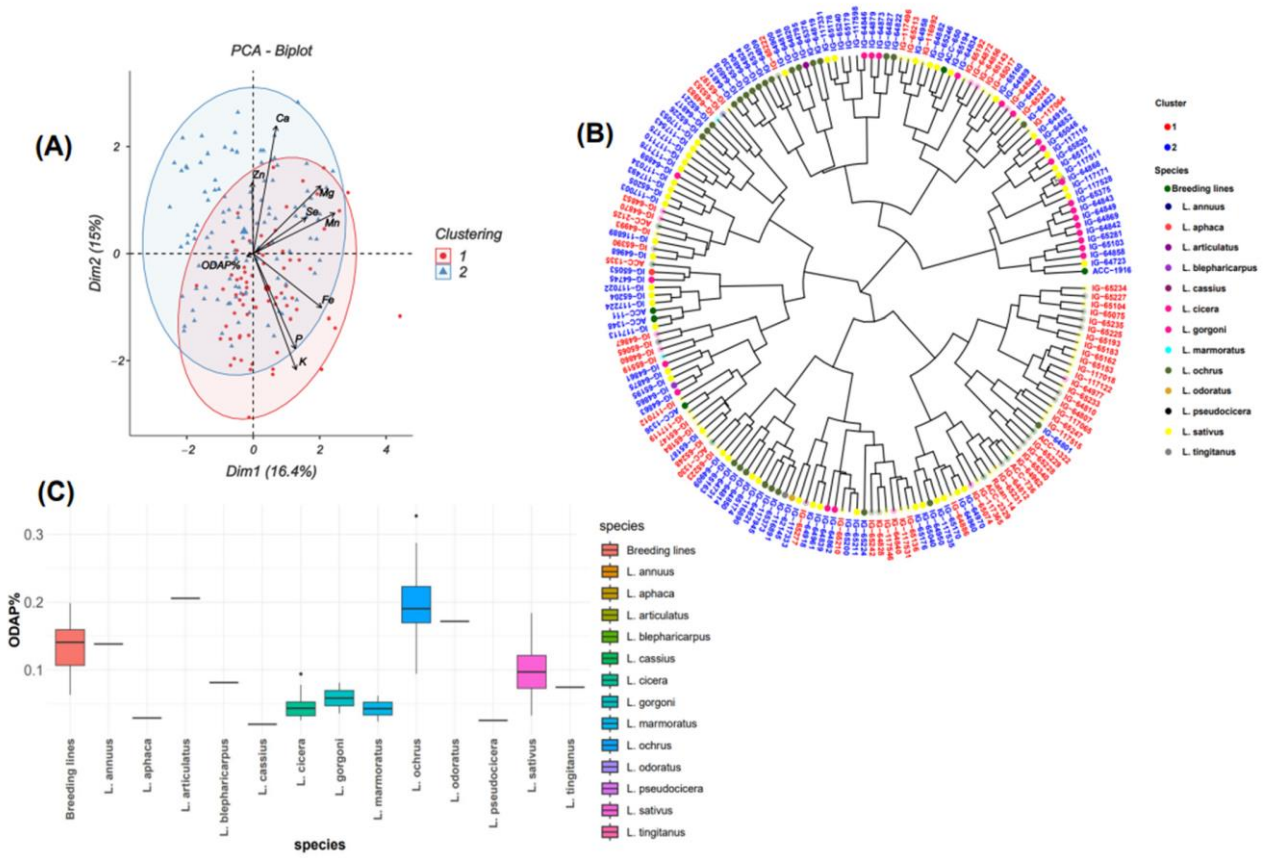

Figure 4. (**A**) Biplot of the first two dimensions of PCA based on β-ODAP content and nutrient concentration for grass pea accessions. (**B**) Dendrogram revealing the neurotoxins and nutrient levels of different tested accessions. (**C**) Box plot showing a comparison of different β-ODAP% concentrations among the whole analyzed accessions.

2.5. Correlations Among Nutrients and β-ODAP Content

Pairwise correlation (Figure 5) was carried out on all 183 analyzed accessions in this study. Correlation analysis indicated significantly positive associations of Mg with Mn (r = 0.215 ***) and Ca (r = 0.211 ***) (Figure 5). Significant positive correlations of K were observed with P (r = 0.193 ***) and Fe (r = 0.177 ***), and for Mn with Fe (r = 0.210 ***) and Se (r = 0.137 ***). However, low significant positive correlations were recorded for three combinations, namely, Se with P (r = 0.103 *), Zn with Ca (r = 0.103 *), and Mg with Fe (r = 0.130 *). β-ODAP revealed a significant positive correlation with Mg (r = 0.158 **) and Ca (r = 0.140 **). Furthermore, β-ODAP showed a positive correlation with P (r = 0.088), K (r = 0.085), Zn (r = 0.024), and Se (r = 0.035). The results also revealed that β-ODAP content was negatively correlated with Mn (r = −0.084) and Fe (r = −0.047).

2.6. Best-Performing Accessions

Twenty-five accessions were selected based on their high nutrient composition and low β-ODAP concentration (Figure 6). Most of these accessions represented *L. sativus*, followed by *L. cicera* and *L. marmoratus*. The first cluster contained 11 accessions with high P and Fe concentrations, such as ACC 650 (*L. sativus*), IG64834, IG64856, and IG64872 (*L. cicera*, Greece), and IG65184 and IG65192 (*L. sativus*, Ethiopia) (Figure 6). Moderate concentration was found for K in IG64863 (*L. cicera*, Greece) and for Mg in IG117034 (*L. sativus*, Bangladesh). Low β-ODAP content was revealed in IG64872 (*L. cicera*) from Greece and in IG117034 (*L. sativus*) from Bangladesh. In the second cluster, 14 accessions were characterized by high concentrations of Mg, Ca, and Zn. This included IG117022, IG65204, and IG65074 from *L. sativus*, IG64858 from *L. cicera*, and IG64983 from *L. marmoratus*.

Furthermore, moderate content was obtained for K and Fe in *L. sativus* (IG64886) originated from Greece and in ACC1335. Two accessions revealed low β-ODAP in this cluster, which are IG64862 (*L. cicera* from Greece) and ACC1335.

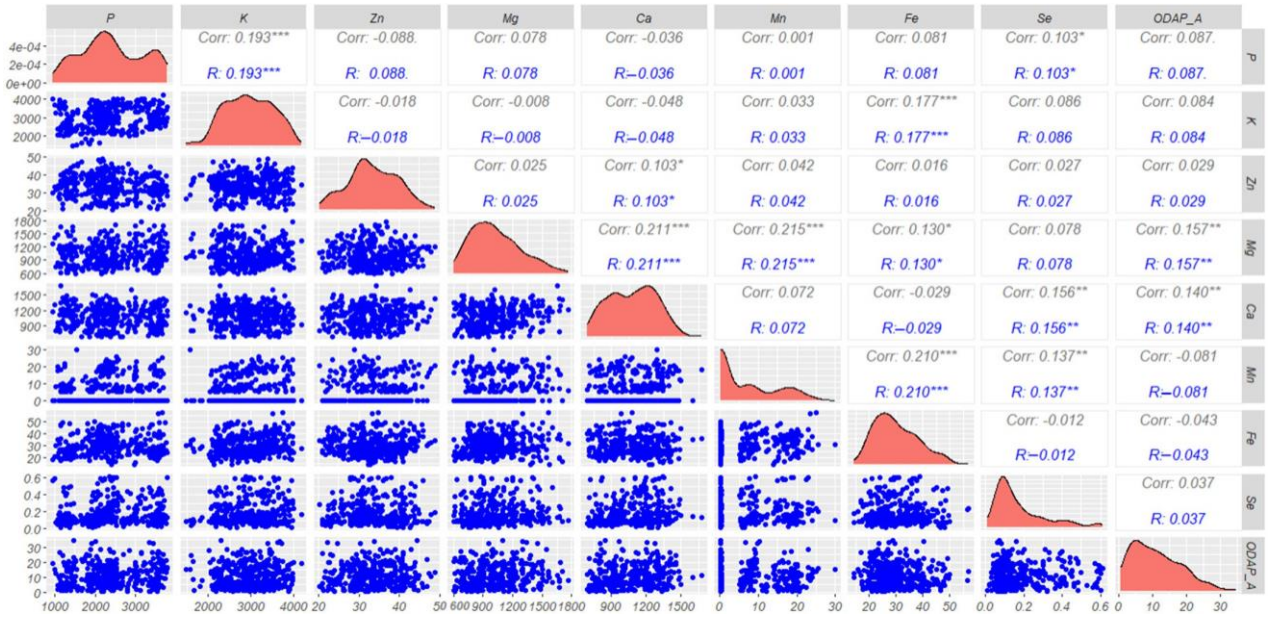

Figure 5. Correlogram with ggpairs among different parameters (macro- and micronutrient concentrations and percentage of β-ODAP) recorded for all grass pea accessions *** significant at ($p < 0.001$); ** significant at ($p < 0.01$); * significant at ($p < 0.05$).

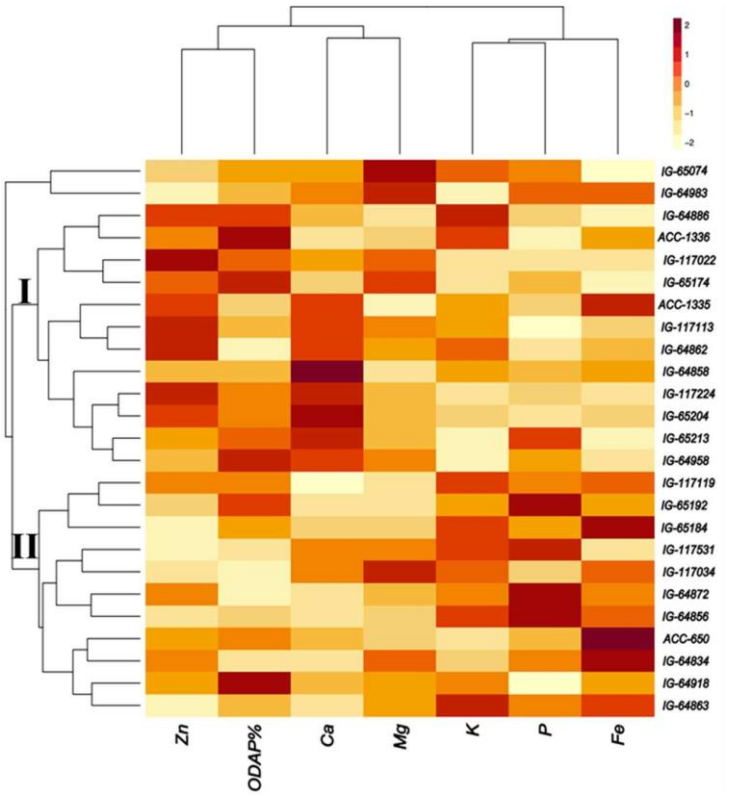

Figure 6. Heat map and hierarchical clustering of 25 selected grass pea accessions for the best performance parameters (high nutrient level and low β-ODAP content).

3. Discussion

Traditional grass pea varieties and landraces, especially from South Asia and Sub-Saharan Africa, contain higher levels of the neurotoxin β-ODAP content, which can cause neurolathyrism if consumed in large quantities for prolonged periods by the undernourished population. Despite its β-ODAP content, grass pea is also recognized as a good source of protein, carbohydrates, and homoarginine that can sustain life during periods of famine when other food is unavailable [27]. The mineral composition of grass pea is comparable to other grain legume crops [28], although this will likely vary with soil mineral content. Global and regional efforts are underway to develop low β-ODAP varieties, utilizing the genetic variability available within the cultivated genepool and in the crop wild relatives. Past studies have indicated the presence of low β-ODAP content in the related wild species. Keeping that in mind, we analyzed 183 germplasm accessions representing 13 *Lathyrus* species originated from different continents for nutrient and β-ODAP contents. Results revealed significant genetic variation and high heritability for most of the traits studied. A large variation was observed for macro- and micronutrient concentrations and β-ODAP content.

For macronutrients, the highest values were recorded for K, followed by P, Ca, and Mg. This agrees with the findings of White et al. [28] in *L. cicera* [K (0.91%), P (0.33%), Ca (0.25%), and Mg (0.13%)] as well as in *L. sativus* [K (0.64%), P (0.42%), Ca (0.16%), and Mg (0.11%)]. In this study, the maximum values for K and Ca were observed in *L. sativus* (IG117365, IG116889, Bangladesh) and for P in *L. ochrus* (IG64807, Greece). Özyazıcı and Açıkbas¸ [29] reported the highest K ratio in Sel706 (2.44%), Sel1837 (2.44%), ETH-24 (2.44%), and ETH WIR-70 (2.46%) accessions. Among the studied nutrients, Zn emerged as the most abundant micronutrient, particularly in ACC1348, followed by Fe in IG117012 (*L. sativus*, Bangladesh), Mn in ACC1916, and Se in IG62145 (*L. odoratus*, Italy). Similar results were reported in *L. sativus* and *L. cicera* [28,30], in lentil [31,32], and in chickpea [33]. Lisiewska et al. [34] have reported relatively low concentrations of Mg (473–642 ppm) and Fe (20–36.7 ppm) in *L. sativus* seeds compared to what was observed in our study with Mg (1027.5 ppm) and Fe (30.1 ppm). On average, Chavan et al. [35] announced that the trace element contents for Mg and Fe of *Lathyrus maritimus* were 1800 mg/kg and 94 mg/kg, respectively, and of *L. sativus* were 1500 mg/kg and 82 mg/kg, respectively. A large number of grass pea accessions have been evaluated for nutritional value: K (8.33-11.05 ppm) [36,37], Mg (0.86–1.61 ppm), Mn (7.86–42.5 ppm) [37,38], Fe (41–73 ppm), and Zn (19–54 ppm) [16,21,36].

In the present research, lower neurotoxin concentration (β-ODAP) was detected in *L. cassius* with 0.01% in IG65277 (from Syria). Urga et al. [21] also reported significant variability in β-ODAP levels among *Lathyrus* species, with some accessions having low β-ODAP content considered safe for human consumption [22]. *L. cicera* cultivar "Chalus" was selected based on high yield and low β-ODAP (0.09%) in comparison with many other *L. cicera* accessions tested by Hanbury et al. [5]. Our results identified accessions of *L. pseudocicera*, *L. aphaca*, *L. cicera*, *L. marmoratus*, *L. gorgoni*, and *L. tingitanus* with low β-ODAP content. Our findings indicated that in *L. ochrus*, the β-ODAP content varied significantly, with IG65340 having the highest β-ODAP content (0.33%). Identical results were also reported by Aletor et al. [39]. In contrast to our results, Urga et al. [40] reported the highest β-ODAP content in *L. sativus* (IG46075) from Ethiopia. Four lines of *L. sativus*, viz., IFLLS 522 (Syria), IFFLS 588 (Cyprus), IFLLS 516 (Turkey), and IFLLS 563 (Turkey), showed low β-ODAP content ranging from 0.02 to 0.07%. The level presumed safe for human consumption is <0.2% [41]. As stated in our results, β-ODAP content ranged from 0.01% to 0.3% with an average of 0.1% for all studied species. According to Kumar et al. [2], the β-ODAP content of a large collection of *L. sativus* (1128 accessions) ranged from 0.150 to 0.952%, with only IG118563 (0.150%) and IG64888 (0.198%) having low content. Pandey et al. [42] have also stated that the β-ODAP content for 1963 entries of grass pea was between 0.067 and 0.712% and recorded that the percentage of β-ODAP in *Lathyrus sativus* (IPLY9, Prateek, AKL 19, BioL202, BioL203, Ratan, No. 2203, and No. 2208.) is lower than 0.10%. The range of β-ODAP content varied across studies. Other results have included ranges of 0.2–2%

among 1000 accessions [43]; 0.02–0.74% among 81 accessions [44]; and 0.149–0.916% in a set of 150 accessions [45]. Moreover, our analysis revealed the lowest β-ODAP content was in *L. cicera* (0.05%), followed by *L. sativus* (0.1%) and *L. ochrus* (0.2%), which is consistent with the results of Hanbury et al. [5], Abd-El-Moneim et al. [26], and Aletor et al. [39]. Likewise, the classification was found to be identical by Eichinger et al. [46] while using capillary electrophoresis. The evaluation of 142 accessions of *L. cicera* at ICARDA during 2009 showed a range of 0.073–0.513% for β-ODAP content, which is much lower than the cultivated species. Therefore, *L. cicera* accessions hold promise as a source of low β-ODAP content in grass pea breeding programs [1]. Low β-ODAP was detected mainly in *L. sativus*, *L. cicera*, *L. clymenum*, *L. ochrus*, *L. hirsutus*, and *L. sylvestris*, and also with lesser content in *L. aphaca*, *L. sphaericus*, and *L. gorgoni* [47–50].

We selected the 25 best germplasm accessions with high mineral concentration and low β-ODAP content. The results recorded high levels of P and Fe in ACC650, IG64834, IG64856, and IG64872 (*L. cicera*) from Greece, and IG65184 and IG65192 (*L. sativus*) from Ethiopia. Furthermore, we found high levels of Mg, Ca, and Zn in certain accessions, which include *L. sativus* (IG117022, IG65204, and IG65074) from Bangladesh, Ethiopia, and Maldova, respectively; *L. cicera* (IG64858) from Greece; and *L. marmoratus* (IG64983) from Iraq. Low β-ODAP content was reported in *L. cicera* from Greece (IG64872, IG64862), *L. sativus* derived from Bangladesh (IG117034), and in a breeding line (ACC1335). We notice that the selected accessions differed in their origin, covering different continents (Africa, Asia, Europe, Australia). As proved by Bandana et al. [51], Prateek, Ratan, and Mahateora showed a significantly low amount of β-ODAP content; instead, Local Khesari and Boga Khesari revealed a very high nutritional characteristic. According to our results, Europe is the continent that contains most of the best-performing accessions, followed by Asia, Ethiopia, and then Australia. Abd-El-Moneim et al. [26] also reported that grass pea germplasm from Ethiopia and the Indian subcontinent is generally higher in β-ODAP (0.7–2.4%) compared to germplasm from the Near East (0.02–1.2%). This is interpreted to mean that the species' origin influences the β-ODAP content in *Lathyrus*. The β-ODAP content in grass pea may be influenced by environment and agronomic practices [43] within the same species. Hanbury et al. [5] found for 407 *L. sativus* and 96 *L. cicera* lines collected from three geographic origins (Ethiopian, Mediterranean, European), that genotype was the most important determinant of β-ODAP concentration and that environment had less influence. However, Wuletaw [52] concluded for *L. sativus* that genotype and its interaction with the environment are the most significant determinants of β-ODAP levels.

Our research revealed that Mn was significantly and positively correlated with Fe (r = 0.210 ***) and Se (r = 0.137 ***). Zn showed moderate associations with all other micronutrients. Arslan [53] revealed a significant correlation (at 5%) between Se and Mg and a highly significant relation (at 1%) among all the other minerals. Significant and positive correlation were found in this study between Mg and Ca (r = 0.211***), while Se showed moderate relationships with P (r = 0.103*), K (r = 0.086) and Zn (r = 0.027). This study suggest that β-ODAP was negatively correlated with Mn (r = −0.081) and Fe (r = −0.043). Nonetheless, Basaran et al. [54] also reported that β-ODAP was highly correlated with Zn (r = 0.732 **) and Bore (B) (r = −0.507 *) and poorly correlated with P, K, Mg, and Fe. These latter authors indicated that interactions between β-ODAP and minerals are complex and vary depending on species. So, any observations regarding β-ODAP x mineral interaction in certain species cannot be generalized for the whole genus.

Taking into account our results, we identified grass pea germplasm with a high content of minerals and low β-ODAP content, which could be useful for integrating them into a genetic improvement program. Breeding for low β-ODAP was achieved through somaclonal mutation, which has allowed the release of several viable diploid mutants with marked alterations in plant characters [55]. The accessions of grass pea with valuable traits can also be used by the breeding programs. However, the mobilization of the genes for low β-ODAP and for high macro- and microelements from other *Lathyrus* species will require a lot of pre-breeding efforts. Interspecific crosses of grass pea with more than

11 *Lathyrus* species were attempted at ICARDA and allowed to develop germplasm from crosses with *L. cicera*, *L. ochrus*, *L. inconspectus*, *L. marmoratus*, and *L. hierosolymitanus* (Amri A., unpublished data). Further crosses using the identified accessions of *Lathyrus* species with low β-ODAP and high Zn, Fe, and Se will allow the development of high-yielding varieties with low β-ODAP content and biofortified for microelements.

4. Materials and Methods

4.1. Plant Material

A set of 183 germplasm accessions belonging to 13 wild *Lathyrus* species originated from different continents (Asia, Africa, Europe) and 11 *L. sativus* breeding lines were provided by the ICARDA gene bank (Table S1). During the crop seasons of 2016 and 2017, these accessions were grown under net cages to avoid outcrossing and ensure seed purification. Two samples from each accession were subjected to β-ODAP% and nutritional quality analysis at the ICARDA, Morocco, quality laboratory.

4.2. β-ODAP Protocol

Seeds harvested from a representative single plant of each accession were collected and grinded separately. A total of 5 g of powdered sample of each accession was diluted in 60% ethanol and mixed in a volumetric flask for 45 min at 200 rpm. All the tubes were centrifuged at 4500 rpm for 15 min, followed by the collection of 2 mL of supernatant in centrifuge tubes. Afterwards, 4 mL of 3 N potassium hydroxide (KOH) was added to each tube, vortexed, and placed in a boiling water bath at 100 °C for 30 min. The sample tubes were once again centrifuged at 4500 rpm for 15 min, and 250 µL of the hydrolyzed supernatant was pipetted. These samples were then combined with 750 µL of H_2O and 2000 µL of O-phthalaldehyde (OPT) solution in tetraborate. The procedure included three blanks: 0.25 mL non-hydrolyzed extract + 0.75 mL distilled water + 2 mL of the OPA reagent (OPA blank); 0.25 mL non-hydrolyzed extract + 0.75 mL distilled water + 2 mL OPT (sample blank); and 0.25 mL hydrolyzed extract + 0.75 mL distilled water + 2 mL OPT (buffer blank). Afterward, the tubes were vortexed and incubated for 2 h at 40 °C. The spectrophotometric measurement was recorded at 425 nm with the absorbance at blank set as zero [56]. The final absorbance was given by

$$A = (A\ (\text{sample}) - A\ (\text{buffer blank}) - 1/3\ (A\ (\text{OPA blank}) - A\ (\text{sample blank})), \quad (1)$$

A is a calibration curve made using DL-2,3-diaminopropionic acid mono hydrochloride as standard and a conversion factor to β-ODAP of 1.69 [39]. The general equation for calculating the concentration of β-ODAP is as follows:

$$C = ((A_3 – A_4) - 1/3\ (A_1 – A_2) - \text{y intercept})/\text{Slope}, \quad (2)$$

where C is the concentration of β-ODAP in the amino acid extract; the y intercept and the slope were extrapolated from a regression line calculated from absorbance readings of D*L*-α-β-diamino propionic acid (DAP) as standard [56]. The DAP solutions were prepared and reacted with OPT precisely in the same way as described for the amino acid extracts. A1 to A4 are absorbance values for amino acid solutions.

4.3. Mineral Analysis

The seeds were grounded using a Cyclone mill (Twister, 10 mm–250 µm, Retsch, Éragny, France), and mineral concentration was measured using a modified HNO_3 and H_2O_2 method [57]. For each accession, 500 mg of sample was placed in digestion tubes with 6 mL nitric acid (HNO_3) at 90 °C for 60 min in the digestion block (QBlock series, Horiba, Burlington, Canada). Then, 3 mL of 30% hydrogen peroxide (H_2O_2) was added to each tube, followed by the second digestion when the samples were heated again at 90 °C for 15 min. Next, 3 mL of 6 M hydrochloric acid (HCl) was added to each digestion tube. Finally, all samples were cooled, filtered (Whatman No. 1 filter papers), and then diluted

with distilled water to 10 mL. The mineral concentrations were carried out by inductively coupled plasma–optical emission spectroscopy (ICP-OES) (iCAP-7000 Duo, Thermo Fisher Scientific, Waltham, MA, USA). The results were based on lab references and NIST standard references using a specific calibration for the micronutrients with a dilution from 0.1 to 10 mg/L.

4.4. Statistical Analysis

The data were analyzed using two software tools, SPSS version 25 (IBM Corporation, New York, NY, USA) and R Core Team (2023). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/ (accessed on 1 October 2023). Multivariate Analysis of Variance (MANOVA) for ODAP and nutrient contents was performed. Mean, max, min, R^2, heritability, genotypic variance, coefficient of variation CV (%), and least significant difference (LSD) were determined using SPSS. All statistical descriptive parameters were tested for significance at $p < 0.05$. Principal component analysis (PCA) was performed to explore the relationships among accessions and to determine the number of clusters when accessions are associated with major nutrient concentration and low ODAP contents using ggplot, factoextra, and factoMineR in Rstudio. Hierarchical clustering analysis (HCA) was employed to form different groups of accessions based on their performance in nutrient and β-ODAP values, using Ward's method based on Euclidean distance. Tree diagrams resulting from hierarchical clustering analysis were represented using the ggplot2 and ggtree packages in Rstudio. A correlogram was used to study the relationships among all studied traits with the ggpairs function. Boxplots and the frequency histograms were established with the ggplot2 package in Rstudio to provide a quick summary of the values' variabilities.

5. Conclusions

This study indicates the potential of crop wild relatives as a source of novel variation for mainstreaming in the grass pea improvement program to develop improved varieties with high mineral concentration and low β-ODAP content. Some of the species, such as *L. cicera* (IG64872, IG64862), *L. sativus* (ACC 650, IG65184, IG65192, IG117022, IG65204, IG65074, IG117034), and *L. cassius* (IG65277), present an excellent source of desirable traits useful for enhancing the mineral concentration and reducing β-ODAP content in the cultivated species. *L. cassius* (IG65277 from Syria) showed the lowest β-ODAP content. Some of the species, namely *L. pseudocicera*, *L. aphaca*, *L. marmoratus*, *L. gorgoni*, and *L. tingitanus*, showed crossing barriers with cultivated species and require special breeding tools and techniques to overcome the bottleneck to integrating them in the grass pea improvement program.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants13223202/s1, Table S1: *Lathyrus* accessions, their origin, and IG number included in the study for β-ODAP and nutrient content.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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III. Conclusion:

In conclusion, while grass pea is a resilient and nutritious crop, its high toxicity remains a barrier to its widespread use. Ongoing research into breeding and genetic diversity is critical for making grass pea a safer and more sustainable crop for agricultural systems in resource-limited regions.

Recent reviews (Sarkar *et al*., 2019, Lambein *et al*., 2019, Rathi *et al*., 2021, Das *et al*., 2021) highlighted grass pea as a stress-resilient crop with high nutritional value, contributing to a better health state and capable to withstand climate change impacts. Additionally, the progress of its improvement by conventional breeding until the more recent genomics techniques was also highlighted in those works.

If the diversity observed for other phenotypes also exists for *β-L*-ODAP concentration in the seed, then low- or zero-toxin accessions may already occur within the existing germplasm of grass pea. Screening germplasm collections for such accessions therefore could provide the basis for the development of new, safer varieties. Assessment of ODAP in different species of *Lathyrus* indicated that none of the species are free from ODAP (Aletor *et al*., 1994; Hanbury *et al*., 1999).

In collaboration with the International Center for Agriculture Research in the Dry Areas in Morocco, our research highlights the significant potential of crop wild relatives (CWRs) as a source of valuable genetic variation for improving the grass pea (*Lathyrus sativus*) breeding program. Notably, *L. cassius* accession IG65277, originating from Syria, exhibited the lowest *β*-ODAP content, suggesting its potential as a valuable parent for breeding programs aimed at reducing the toxin in grass pea such as somaclonal variation. Low-toxin varieties have also been released by the Bangladesh Agricultural Research Institute (BARI) under the names BARI Khesari 1, 2 and 3 (Akter *et al*., 2015). One low-toxin variety, Ceora, was developed by the Centre for Legumes in Mediterranean Agriculture (CLIMA) in Australia (Siddique *et al*., 2006). Twenty-five accessions were evaluated in this study for their potential (high nutrients level and low ODAP content) and will be integrated to the improvement program.

General Discussion

Legumes are the second most important family of cultivated plants after cereals. They not only play a key role in agri-food systems as sources of food and feed but also provide ecosystem services by improving soil fertility, biodiversity and environmental sustainability (Ditzler *et al*., 2021; Ianetta *et al*., 2021). The increasing demand for environmental-friendly agricultural practices and food security establishes a favourable context for new cropping systems that include grain and forage legumes (Duc *et al*., 2015; Rubiales *et al*., 2021). Grain legumes, also known as pulses, are major foodstuffs and important sources of protein in most countries (Arnoldi *et al*., 2015). They are also environmentally friendly sustainable sources of many other nutritional and health-beneficial components (Vaz Patto, 2016; Vaz Patto *et al*., 2018).

Grass pea (*Lathyrus sativus* L.) is an annual cool-season grain legume crop, that due to its relatively low input requirements compared to major crops, is considered a model crop for sustainable agriculture and an interesting alternative for cropping systems diversification in marginal lands (Almeida *et al*., 2014; Vaz Patto *et al*., 2006). It is characterized by a wide adaptation to different soils and climates, to low temperatures, showing flood and drought tolerance, insect and disease resistance, and high protein content for human and animal feed (Campbell *et al*., 1994; Hanbury *et al*., 2000). Moreover, it is superior in yield, nitrogen fixation, and salinity tolerance, when compared to other legume crops (Vaz Patto *et al*., 2006). These traits make it an outstanding crop for ensuring nutritional security, especially in the face of impending climate challenges (Sarkar *et al*., 2019).

Cultivation of annual grain and forage temperate legumes is strongly hampered in the farming systems of the Mediterranean and Middle East by the widespread occurrence of broomrapes, which cause important yield losses (Fernández-Aparicio *et al*., 2016). The most widespread and damaging broomrape is crenate broomrape (*Orobanche crenata* Forsk.), but minor broomrape (*O. minor* Sm), foetida broomrape (*O. foetida* Poir), and Egyptian broomrape (*Pelipanche aegyptiaca* (Pers.) Pomel) can be of importance locally (Parker, 2012; Das *et al*., 2020; Rubiales, 2020). *O. crenata* is not a new problem in legume farming, having been described by authors from ancient Rome. Little progress has been made in its management since then, and unfortunately, the real situation is that instead of being controlled, it is a problem that is spreading to new areas that were considered free of infestation, even outside the Mediterranean Basin, to the north in Europe, to the south in Africa, and to the east in Asia, representing a situation that could worsen with climate change (Grenz and Sauerborn, 2007; Rubiales, 2020; Negewo *et al*., 2022). *O.*

foetida seems to be a relatively recent problem. Natural non-weedy populations of *O. foetida* are widespread in the western Mediterranean, infecting wild legume species only, not legume crops. However, only a few decades ago, weedy populations of *O. foetida* on faba bean were reported in the Beja region of Tunisia that became established in that area (Kharrat *et al*., 1992).

The first and the second parts of this study have focused on identification of resistance for 285 accessions of 13 *Lathyrus sativus* L. species against *O. crenata* and *O. foetida* in two different locations, the correlations between all tested traits and the most influential parametres which could be a criterion of selection for *Orobanche* infestation. Results of ANOVA revealed that field trials indicated that *Orobanche crenata* in Morocco was more parasitic on *Lathyrus* species than *O. foetida* in Tunisia, consistent with findings in other legumes like faba bean (Trabelsi *et al*., 2015) and chickpea (Abbes *et al*., 2008). Six wild *Lathyru*s species; *L. aphaca*, *L. articulatus*, *L. blepharicarpus*, *L. cassius*, *L. pseudocicera*, *L. ochrus* have indicated a complete resistance to *O. crenata* and *O. foetida* in field experiments during the 2017-2018 cropping season. High resistance was recorded in all wild and cultivated *Lathyrus* species to *O. foetida*. These wild species present opportunities for introgression breeding to enhance resistance in cultivated varieties. Still, valuable sources of resistance have been identified in germplasm of most legume crops, including faba bean (Maalouf *et al*., 2011; Fernández-Aparicio *et al*., 2012; Rubiales *et al*., 2014; Briache *et al*., 2019), pea (Pérez-De-Luque *et al*., 2005; Rubiales *et al*., 2005; Pavan *et al*., 2016 ; Abd El-Fatah and Nassef, 2020), lentil (Pérez-De-Luque *et al*., 2005; Fernández-Aparicio *et al*., 2008b; En-nahli *et al*., 2023), vetches (Nadal *et al*., 2007; Rubio and Rubiales, 2021), chickpea (Rubiales *et al*., 2003c; Brahmi *et al*., 2016), grass pea and related *Lathyrus* species (Sillero *et al*., 2005; Fernández-Aparicio *et al*., 2009; Fernández-Aparicio and Rubiales, 2010 ; Fernández-Aparicio *et al*., 2011).

Rubiales *et al*., 2003b have reported that field pea germplasm has little resistance to *O. crenata*, but wild relatives within the genus *Pisum* have been identified as potential sources of resistance (Rubiales *et al*., 2005), which have successfully been hybridized with cultivated pea (Pérez-de-Luque *et al.*, 2005).

The collection described above revealed in chapter III a significant genetic variability among all tested *Lathyrus* species for various agro-physiological and infestation traits. High heritability, high CV % and low LSD were observed in field tested parameters. Figure 3B showed that Asia (Middle East, Bangladesh, India), Cyprus, and Ethiopia are the main origins of resistant and tolerant

accessions, which are made up of *L. ochrus* (IG64823, IG62140, IG64731, IG64801, IG64804, IG64807, IG64808, IG64809, IG64810, IG64812, IG64813, IG64814, IG64816), *L. articulatus* (IG64733, IG64735, IG64786, IG64787, IG64795, IG64798, IG64800), *L. blepharicarpus* (IG64865), *L. Cassius* (IG65277), and *L. pseudocicera* (IG65065, IG65543). Also, we have identified the susceptible accessions belonging to *Lathyrus sativus*, *Lathyrus cicera*, *Lathyrus annuus*, *Lathyrus hierosolymitanus*, and *Lathyrus tingitanus* from Europe and North America. These regions could be influenced by environmental or agricultural practices that favor the establishment and proliferation of *Orobanche*. This distribution could be a result of historical agricultural practices that did not prioritize the selection of resistant varieties. In the case of legumes, progress in broomrape resistance breeding has been slow, as they are rather minor crops in which relatively little has been invested in the last half century (Cusworth *et al*., 2021; Rubiales, 2023; Rubiales *et al*., 2023). Most studies on broomrape resistance in legumes have concluded that there is low heritability and that inheritance is complex, highly influenced by the environment (Román *et al*., 2002).

The low establishment of broomrape is attributed to the low induction of germination of broomrape seeds in the presence of resistant host plants (Sillero *et al*., 2005). It is suggested that resistant accessions may produce root exudates or other signals that prevent *Orobanche* seeds from germinating. This mechanism is significant because it demonstrates a proactive form of resistance, in which the host plant can inhibit the start of parasite's life cycle. The mechanical mechanisms of resistance to Broomrape are primarily investigated in certain cultivars. Resistant cultivars showed its effects through cell necrosis in faba bean (Zaitoun and Borg, 1994) or high osmotic pressure (Wegmann *et al*., 1991). Nassib *et al*., (1982) attribute the resistance of the Giza 402 of *Vicia faba* L. cultivar to the morphology of its root system and Khalaf and El-Bastawesy (1989) explain it by a fairly reduced root biomass. Other authors have concluded that there is no effect of biomass on the resistance and sensitivity (Ter Borg and Van Ast, 1991; Kharrat *et al*., 1994). In addition, the secretion of exudates which stimulate seed germination broomrape does not appear to influence the host response (Khalaf and El-Bastawesy, 1989; Van Woerden *et al*., 1994).

Multiple Linear Regression was reported in this research that SY was significantly associated to PI, OIN and BY. However, SY was not significantly associated to others infested parameters. We then concluded that PI, OIN, BY were the strongest infulantial traits on SY in field trials. In similar

study, En-nahli *et al*., 2023, were reported that the D2F, NEO, D2OE, SEV and parasitism index (PI) were the strongest driver traits that influenced the seed yield (SY).

Correlations between all tested traits were observed in field trials in chapter III of this research. The higher correlations in field affected by *O. foetida* may indicate a more consistent or predictable relationship between the traits measured (such as SY, EON, and EODW) in response to *Orobanche*. In contrast, the lower correlations under *O. crenata* infested field may reflect greater variability in how this parasite interacts with its host, possibly due to differences in environmental conditions, host plant responses, or the biological characteristics of the parasite itself. Negative correlation was observed between SY and EON. Similar results were recorded by Fernández-Aparicio *et al*., 2009 on *L. cicera* in infested filed by *O. crenata*. However, positive association between host plant biomass and level of infection would be expected, as the more vigorous the host plants the higher the root biomass to be infected and the higher nutrient supply for the parasite. This association was earlier suggested in faba bean (Aalders and Pieters, 1987) and in pea (Fondevilla *et al*., 2009a) showing that low poor vegetative host growth might result in reduced infection, being a confounding factor in resistance screenings. The attachment of many *Orobanche* tubercles/shoots to the host root system results in increasing the parasitism impact and severity (SEV) through increased uptake of water and nutrients from the host (Trabelsi *et al*., 2016). The limited number of emerged broomrape shoots was previously used as an indicator of resistance to broomrape (Trabelsi *et al*., 2017; Van Bruggen *et al*., 2018; Amri *et al*., 2021). In the other study, En-nahli *et al*., 2023 were recorded that SY was negatively and significantly correlated with SEV and D2F. Conversly, under *O. foetida* infested field, SY was highly correlated with EON. D2F was also positively correlated with other parameters such as D2OE, BY, SY, EON and EODW. As proved in chickpea by Kumar and Abbo, 2001 and Farida-Traore *et al*., 2022 that a positive correlation between GY and D2F was revealed. Another studies were recorded negative relationships between D2F and GY (grain yield) in faba bean (Alghamdi, 2007) and lentil (Vandemark *et al*., 2018). However, negative correlations were observed between D2F and PI, OSV and OIN. This suggests that early flowering is advantageous in reducing *O. crenata* infestation but is less efficient against *O. foetida*. Breeding programs might prioritize early-flowering types in areas where *O. crenata* is prevalent

The resistance confirmation for ten best-performing accessions selected in previous collection for their response to both *Orobanche* infestations, was achieved in pot experiment. Results validated

a complete resistance for two *L. sativus* accessions IG64782 and IG65197 from Ethiopia against *O. crenata* in the glasshouse. However, only low levels of resistance to *O. crenata* were available in faba bean, until the appearance of the Egyptian line F402 (Nassib *et al*., 1982). Similarly, resistance against *O. foetida* Poir. has also been identified in faba bean germplasm. Some lines (674/154/85 L3-4 and 402/29/84) selected against *O. crenata* have also shown a high level of resistance to *O. foetida*, with a high yield potential (Abbes *et al*., 2008). En-nahli *et al*., 2021 reported on lentil, two genotypes ILL6415 and ILL7723, which have a good resistance level Furthermore, this investigation demonstrated that all MR accessions in field experiments maintained the same reaction under controlled conditions, with the exception of IG116989. This accession showed a susceptible reaction with the highest number of emerged *Orobanche* due to to the underground development of *Orobanche* shoots at the host plant root level. Briache *et al*., 2019 indicated that it makes a false selection of resistant genotypes in field screening. When underground *Orobanche* sprouts are not accounted for test entries may be incorrectly judged as resistant (Rubiales *et al*., 2006; Ennami *et al*., 2019). This misclassification can have significant consequences for breeding programs, as it may lead to the selection of genotypes that are not truly resistant to *Orobanche* infestation. For this reason, the underground number of *Orobanche* shoots is a good selection criterion for evaluation of resistance in grass pea accessions. In chapter III we also revealed that root dry weight could be used for evaluation resistance *Orobanche* in grass pea.

Grass pea is also characterized by a low content of the essential amino acid methionine (Lambein *et al*., 2001) and by the presence of high levels of anti nutritional factors (ANFs) (Wang *et al*., 1998), particularly of the neurotoxic *β*-ODAP (*β-N*-oxalyl-*L*-α, *β*-diaminopropionic acid). By 1964, *β*-ODAP was considered the cause of neurolathyrism (Murti *et al*., 1964; Rao *et al*., 1964), a neurodegenerative disease often occurring in epidemics after famines in the Indian subcontinent or in Ethiopia, when grass pea seed has been the exclusive or main nutrient for several months (Getahun *et al*.,1999). Grass pea seeds may represent a potential source of several important nutrients for human and animal nutrition. *Lathyrus* is also an important source of minerals which is used as a forage crop for livestock in Mediterranean type environments (Skiba *et al*., 2007; Polignano *et al*., 2009). Therefore, we studied in the last part of this thesis the *L. sativus* germplasm with low *β*-ODAP content and high nutrient concentrations. The current study showed that the *Lathyrus* species had a high variation for all studied traits, which had a coefficient of variation greater than 15%. The highest variation among the investigated species was observed in Mn (CV

= 122.5%) that was varied from 0.1 ppm (*L. sativus)* to 30 ppm (breeding lines) and averaged with 6.3 ppm. In contrast, Basaran *et al*., 2019 observed that ODAP content contained the highest coefficient of variation compared to all tested mineral concentrations. Similarly, high coefficient of variation for ODAP (CV = 99%) was reported among 18 species of *Lathyrus* (Sommour *et al*., 2007a). This variation was explained in many earlier studies on the basis of morphological, agronomic, biochemical traits and genetic (Jackson and Yunus, 1984; Sammour *et al*., 2007a; Acar and Basaran, 2007; Emre *et al*., 2015; Wang *et al*., 2015). Among the major minerals, our analysis showed that K and P were the most abundant among all species followed by Ca and Mg. For micronutrients, Zn and Fe exhibited higher values compared to Se and Mn. Similar ranking for *L. sativus*, *L. cicera* and *L. ochrus* was reported earlier by Aletor *et al*., 2011; Basaran *et al*., 2016, for chickpea by Farida-traore *et al*., 2022 and for lentil by Choukri *et al*., 2022. Legumes generally have high mineral content (Sandberg, 2002). Additionally, our results were in agreement with Urga *et al*., 2005, who stuwandied mineral contents in 450 Ethiopian *Lathyrus sativus* samples.

No species was found *β*-ODAP free, although very low *β*-ODAP content with 0.01% in IG65277 (*L. cassius* from Syria) was observed in the present study. Our analysis investigated some accessions among all studied species with low *β*-ODAP content, which are- *L. pseudocicera, L. aphaca, L. cicera, L. marmoratus, L. gorgoni, and L. tingitanus.* Furthermore, our findings showed the highest value of *β*-ODAP in L. *ochrus* (IG65340) with 0.33%. Basaran *et al*., 2016 were also recorded that besides the cultivar species (*L. clymenum* and *L. sativus*), *L. ochrus* and *L. cicera* had higher ODAP content and also higher seed weight. *L. ochrus* and *L. cicera* investigated are wild but have culture forms in Turkey and other parts of the world (Kendir, 1999). Especielly, *L. cicera* has been cultivated since ancient times. Moreover, it is the most grown species of the genus after *L. sativus* in worldwide (Kislev, 1989).

For safe human consumption ODAP should be lower than 2 mg g^{-1} (Abd-El-Moneim *et al*., 1999). In this respect, it can be said that cultivars exhibited higher ODAP content than critical value (2 mg g^{-1}) while literally wild species had lower values. On the other hand, perennial species (*L. laxiflorus* and *L. tuberosus*) had lower ODAP content than that of annuals. In addition, Hanbury *et al*., 2000 were reported that the *β*-ODAP concentrations in *L. sativus* were generally higher (0.04–0.76%) compared to *L. cicera* (0.08–0.34%). As per our findings, the *β*-ODAP content varied from 0.01% to 0.3%, with an average of 0.1% for all tested species. The range of *β*-ODAP content differed among studies. Robertson and Abd-El-Moneim 1997, have shown a wide range

of variation within the existing germplasm, ranging from 0.02% to 2.59% (ICARDA). Hanbury *et al*., 1999 also reported a range of 0.04-0.76% for ODAP content in a set of 503 accessions procured from ICARDA. Pandey *et al*., 1997 reported a range of 0.128-0.872% for ODAP content among 1187 accessions. Some of the accessions having <0.1% ODAP are IPLY9, Prateek, AKL 19, BioL202, BioL203, Ratan, No. 2203 and No. 2208. Kumar *et al*., 2011 also screened 1128 accessions of *L. sativus* and found a wide range (0.150-0.952%) for ODAP content. Only two accessions, IG118563 (0.150%) and IG64888 (0.198%), had low ODAP content. Furthermore, our research indicated that *L. cicera* had the lowest *β*-ODAP content (0.05%), followed by *L. sativus* (0.1%) and *L. ochrus* (0.2%), is a significant finding that aligns with previous research. Hanbury *et al*., 1999 observed the lowest ODAP in *L. cicera* (0.18%) followed by *L. sativus* (0.39%) and *L. ochrus* (1.01%). Aletor *et al*., 1994 reported four to five times lower ODAP content in *L. cicera* (0.13%) than in *L. ochrus* (0.56%) and *L. sativus* (0.49%). Similarly, Abd-El-Moneim *et al*., 2000 described ranges of 0.02-2.40% in *L. sativus*, 0.03-0.22% in *L. cicera* and 0.46-2.50% in *L. ochrus*. Eichinger *et al*., 2000 screened *Lathyrus* germplasm using capillary electrophoresis and found that *L. cicera* is consistently low in ODAP as compared to *L. sativus* and *L. ochrus*. Evaluation of 142 accessions of *L. cicera* at ICARDA during 2009 showed a range of 0.073-0.513% for ODAP content, which is much lower than the cultivated species. Therefore, *L. cicera* accessions hold promise as a source of low ODAP content in grass pea breeding programmes (Kumar *et al*., 2013).

Heatmap showed the best 25 best-performing selected grass pea accessions with high mineral concentrations and low *β*-ODAP content. Results revealed high level in P, Fe, Zn, Mg and Ca in ACC650, IG64834, IG64856, and IG64872 (*L. cicera*) from Greece, in IG65184 and IG65192 (*L. sativus*) from Ethiopia, in IG117022, IG65204, and IG65074 (*L. sativus*) from Bangladesh, Ethiopia, and Maldova, respectively, in IG64858 (*L. cicera*) from Greece and *L. marmoratus* (IG64983) from Iraq. In addition, we found that in *L. cicera* from Greece (IG64872, IG64862), as well as, *L. sativus* derived from Bangladesh (IG117034) and breeding line (ACC1335), exihibit low *β*-ODAP content. In previous study, Prateek, Ratan, and Mahateora showed low ODAP content and Local Khesari and Boga Khesari revealed high nutrient concentrations (Bandana *et al*., 2022). Our findings investigate that Europe has the highest number of best-performing accessions of *Lathyrus sativus*, followed by Asia, Ethiopia, and Australia. Grass pea germplasm from Ethiopia and the Indian subcontinent is generally high in ODAP (0.7-2.4%) as compared to 0.02-1.2% in germplasm from the Near East (Abd-El-Moneim *et al*., 2000). Another study by

Gutiérrez-Marcos *et al*., 2006, on 2987 individuals belonging to 110 different global samples revealed considerable genetic diversity in grass pea collections throughout the world.

General conclusion and perspectives

Numerous known or underappreciated species face endangerment, in parts of the world. The excessive focus on a handful of crops such as rice, corn and wheat has marginalized varieties resulting in a loss of genetic diversity in agriculture.

The proliferation of high yield crop varieties often pushes ones to remote or marginalized areas. This shift has led to a decrease in the cultivation of these varieties, impacting diversity negatively.

Research on crop diversity indicates that although traditional varieties may be less productive they exhibit variability compared to modern breeds. This genetic variation plays a role in enhancing crop resilience against diseases, climate fluctuations and other environmental pressures.

The study offered insights into the *Lathyrus* genus particularly focusing on aspects such as resistance to broomrape infestation, genetic variability, agro-physiological characteristics, mineral concentration levels and the presence of ODAP (*β-N*-oxalyl-*L-α, β*-diaminopropionic acid) content.

An initial examination in the field, identified a group of *Lathyrus* varieties originating from different regions that showed resistance to *O. Crenata* in Morocco and *O. Foetida* in Tunisia. This group contains eight wild species namely- *L. aphaca* (IG65053), *L. gorgoni* (IG65375), *L. cicera* (IG64844, IG64867, IG64873, and IG64864), *L. articulatus* (IG64733, IG64735, IG64786, IG64787, IG64795, IG64798, IG64800), *L. blepharicarpus* (IG64865), *L. cassius* (IG65277), *L. pseudocicera* (IG65065, IG65543), *L. ochrus* (IG62140, IG64731, IG64801, IG64804, IG64807, IG64808, IG64809, IG64810, IG64812, IG64813, IG64814, IG64816, IG64823) and ten accessions of cultivated species *L. sativus* (IG64782,IG64875, IG65187, IG65197, IG116989, IG65204, IG64951, IG116997, IG117544, and IG65149) with PI <1 against broomrape, originating from Asia, Cyprus and Ethiopia.

Furthermore, the moderate resistance (MR) response observed in fifty accessions of *L. cicera* and four of *L. gorgoni* species, with PI scores ranging from 1 to 2. In contrast, the susceptibility as indicated by PI scores ranging between 4 and 5 was observed in 151 accessions of cultivated species *L. sativus*, four *L. inconspicuous* accessions, the remainder of *L. cicera*, *L. annuus*, *L. hierosolymitanus* and *L. tingitanus* originating mostly from Ethiopia and North America. In field

conditions, the wild species in Tunisia exhibited a higher level of resistance against *O. foetida* compared to *O. crenata* in Morocco.

Agro-morpho-physiological analysis constitutes a first approach to evaluating genetic diversity of *Lathyrus* species, which showed a significant genetic variability for most studied traits in response to brommrape (*O. crenata* and *O. foetida*). Both the correlation and multiple linear regression analyses showed that parasitism index (PI), *Orobanche* incidence (OIN), biological yield (BY), days to flowering (D2F), days to *Orobanche* emergence (D2OE) and *Orobanche* severity (OSV) were the strongest predictors or driver's traits that influenced the seed yield (SY).

The ten best-performing *Lathyrus sativus* accessions were selected based on their reactions (resistant (R), moderately resistant (MR), and susceptible (S)) during field screening at Marchouch station. According to parasitism index and seed yield, their resistance was confirmed in pot experiment under controlled conditions.

Two specific accessions of *L. sativus*, IG64782 and IG651970, originating from Ethiopia, demonstrated and confirmed complete resistance to *O. crenata*. Four accessions, specifically IG65204, IG64875, IG64951, and IG65187, displayed a moderately resistant (MR) reaction during pot experiment.

However, the two remaining accessions, IG116989 and IG116997, which exhibited low infestation levels during the field experiment, revealed high susceptibility in the glasshouse under controlled conditions. In this case, two traits; emerged *Orobanche* number (EON) and underground *Orobanche* number (UON) could be considered to be a criterion for selection and identification the resistance of the best grass pea entries. As such, RDW serves as a reliable selection parameter in breeding programs aimed at developing *Orobanche*-resistant cultivars, contributing to more sustainable grass pea cultivation.

This present sudy revealed significant genotypic variation, high heritability, high coefficient of variance, R^2 and LSD for all tested traits under infested and non-infested pots in the glasshouse. Total attachment number (TAN) was highly and positively correlated with both underground *Orobanche* number (UON) ($r = 0.892^{***}$) and emerged *Orobanche* number (EON) ($r = 0.543^{**}$) under controlled conditions.

As for the nutritional value of 183 germplasm accessions implicating to 13 wild *Lathyrus* species and 11 *Lathyrus sativus* of breeding lines was determined. The results showed that the highest values were recorded for K in *L. sativus* (IG117365, Bangladesh) followed by P in *L. ochrus* (IG64807, Greece), Ca in *L. sativus* (IG116889, Bangladesh) and Mg in *L. cicera* (IG65074, Australia). Zn was found to be the most abundant micronutrient in ACC1348, followed by Fe in IG117012 (*L. sativus* from Bangladesh), Mn in ACC1916, and Se in IG62145 (*L. odoratus* from Italy). Significant genetic variation was observed for all the traits within the entire collection.

ODAP analysis revealed significant variability among 13 *Lathyrus* species, with having low ODAP content, originated from different regions. *L. cassius* accession (IG65277, Syria) has relatively a lower level of ODAP compared to other species with a concentration of 0.01%. Our results indicate that some accessions of *L. pseudocicera*, *L. aphaca*, *L. cicera*, *L. marmoratus*, *L. gorgoni*, and *L. tingitanus*, exhibit low levels of ODAP content.

Principal component analysis and hierarchical classification revealed two groups containing 25 best performing grass pea accessions based on their high nutrient composition and low *β*-ODAP concentration. The first group included 11 accessions with high concentrations of P and Fe. These accessions were ACC650 (*L. sativus*), IG64834, IG64856, and IG64872 (*L. cicera* from Greece), as well as IG65184 and IG65192 (*L. sativus* from Ethiopia). Moderate concentrations were found for K in IG64863 (*L. cicera* from Greece) and for Mg in IG117034 (*L. sativus* from Bangladesh). Low levels of the neurotoxin ODAP were detected in IG64872 (*L. cicera* from Greece) and IG117034 (*L. sativus* from Bangladesh) within this group.

The second group consisted of 14 accessions characterized by high concentrations of Mg, Ca, and Zn. Accessions comprised IG117022, IG65204, and IG65074 from *L. sativus*, IG64858 from *L. cicera*, and IG64983 from *L. marmoratus*. Moderate levels of K and Fe were observed in *L. sativus* (IG64886) from Greece and in ACC1335. Two accessions within this group, IG64862 (*L. cicera* from Greece) and ACC1335, exhibited low *β*-ODAP content.

Moreover, our study revealed significant positive correlations between different parameters namely_ K and P ($r = 0.193^{***}$), K and Fe ($r = 0.177^{***}$), Mn and Fe ($r = 0.210^{***}$), Mn and Se ($r = 0.137^{***}$), ODAP and Mg ($r = 0.158^{**}$), and ODAP and Ca ($r = 0.140^{**}$).

All the results allowed a good understanding of the structure and distribution of the diversity of the different species of various origins studied and will make it possible to define the strategies of future collection missions for better conservation and use of selection programs, valorization and promotion of grass:

- Genetic study using molecular markers must complete these observations and provide a more detailed analysis which will help to undertake a conservation program by establishing a core collection. Diverse molecular markers like Random Amplified Polymorphic DNA (RAPD); Restriction Fragment Length Polymorphism (RFLP); Amplified Fragment Length Polymorphism (AFLP); Inter Simple Sequence Repeat (ISSR); Expressed Sequence Tag-Simple Sequence Repeats (EST-SSR); Sequence-Related Amplification Polymorphism (SRAP); and Sequence Tagged Site (STS) could be used in grass pea breeding programs, aiding in deciphering genetic diversity, population structure and phylogenetic relationships among different species within the *Lathyrus* genus.

- Overall, by combining traditional field observations with advanced molecular and cytogenetic techniques, researchers can identify and utilize genetic traits associated with *Orobanche* resistance in grass pea, ultimately leading to the development of improved varieties with enhanced resistance to *Orobanche* infestation. This contributes to sustainable agriculture production by reducing crop losses and improving food security for farmer's in regions affected by *Orobanche*.

- By employing special tools and techniques (wide hybridation "embryo rescue or tissue culture" and genomic tools "CRISPR-Cas9"), we can work towards overcoming crossing barriers and harnessing the genetic diversity present in wild or less cultivated *Lathyrus* species like *L. pseudocicera*, *L. aphaca*, *L. marmoratus*, *L. gorgoni*, and *L. tingitanus* to improve the cultivated grass pea for desirable traits such as yield, nutritional quality, and stress tolerance.

- Best-performing accessions of wild or cultivated grass pea (resistant lines, low ODAP content, high mineral concentrations) will be integrated in the grass pea improvement

programme. Many approachs will be utilized for selecting these varieties like in vitro methods to identify a somaclonal variants for developing low ODAP accessions, comparative genomics identified valuable genes and QTLs is thus required for deployment of MAS in breeding strategies of grass pea. Linkage maps, gene cloning, and MAS are expected to hasten the introgression of novel genes for low ODAP.

By integrating these approaches and leveraging advancements in genomics, breeding, and biotechnology, the goal is to develop improved varieties of grass pea with reduced ODAP content, enhanced nutritional value and increased resilience to *Orobanche* resistance.

For these reasons, International Cooperation and Investment in research at national, regional, and international (ICARDA) levels are crucial to transform grass pea into a mainstream pulse legume globally.

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Appendices

Appendix 1a: Augmented design used under open field conditions at Marchouch station, Morocco. ⟶ continuation

1001	Faba bean	1042	Faba bean	1043	Faba bean	1084	Faba bean	1085	Faba bean	1126	Faba bean	1127	Faba bean	1168	Faba bean	1169	Faba bean	1210	Faba bean
1002	GP90174	1041	IG147655GP	1044	GP90174	1083	IG147655GP	1086	GP90174	1125	IG147655GP	1128	GP90174	1167	IG147655GP	1170	GP90174	1209	IG147655GP
1003	65065	1040	64836	1045	64837	1082	64843	1087	64734	1124	64813	1129	64814	1166	65373	1171	65376	1208	65179
1004	65543	1039	64833	1046	64838	1081	65823	1088	64841	1123	64812	1130	64816	1165	65340	1172	65390	1207	65040
1005	65053	1038	64832	1047	64839	1080	65281	1089	64745	1122	64810	1131	64817	1164	65310	1173	117945	1206	117496
1006	64865	1037	64831	1048	64842	1079	65103	1090	64830	1121	64809	1132	64818	1163	65242	1174	65235	1205	116889
1007	65277	1036	64800	1049	64844	1078	64878	1091	64834	1120	64808	1133	64819	1162	65237	1175	64782	1204	65186
1008	65330	1035	64798	1050	64845	1077	64876	1092	64840	1119	64807	1134	64820	1161	65230	1176	64875	1203	64935
1009	65375	1034	64795	1051	64846	1076	64873	1093	64853	1118	64804	1135	64821	1160	65229	1177	64882	1202	116890
1010	65750	1033	IG147607GP	1052	64848	1075	IG147607GP	1094	65046	1117	IG147607GP	1136	64822	1159	IG147607GP	1178	64886	1201	IG147607GP
1011	Faba bean	1032	Faba bean	1053	Faba bean	1074	Faba bean	1095	Faba bean	1116	Faba bean	1137	Faba bean	1158	Faba bean	1179	Faba bean	1200	Faba bean
1012	IG147607GP	1031	64787	1054	IG147607GP	1073	64872	1096	IG147607GP	1115	64801	1138	IG147607GP	1157	65228	1180	IG147607GP	1199	65223
1013	63034	1030	64786	1055	64849	1072	64870	1097	64879	1114	64731	1139	64823	1156	65227	1181	64900	1198	65213
1014	64989	1029	64735	1056	64851	1071	64869	1098	64977	1113	62140	1140	64824	1155	65226	1182	64908	1197	65212
1015	65735	1028	64733	1057	64855	1070	64868	1099	64852	1112	65949	1141	64825	1154	65225	1183	64909	1196	65211
1016	65494	1027	65463	1058	64857	1069	64867	1100	64856	1111	65074	1142	64827	1153	65224	1184	64915	1195	65210
1017	65279	1026	65353	1059	64858	1068	64866	1101	64864	1110	64847	1143	64828	1152	65222	1185	64918	1194	65197
1018	65519	1025	116891	1060	64859	1067	64863	1102	64854	1109	65820	1144	64850	1151	65221	1186	65136	1193	65195
1019	64983	1024	65500	1061	64861	1066	64862	1103	64860	1108	65252	1145	64950	1150	65075	1187	65184	1192	65187
1020	IG147655GP	1023	GP90174	1062	IG147655GP	1065	GP90174	1104	IG147655GP	1107	GP90174	1146	IG147655GP	1149	GP90174	1188	IG147655GP	1191	GP90174
1021	Faba bean	1022	Faba bean	1063	Faba bean	1064	Faba bean	1105	Faba bean	1106	Faba bean	1147	Faba bean	1148	Faba bean	1189	Faba bean	1190	Faba bean

1211	Faba bean	1252	Faba bean	1253	Faba bean	1294	Faba bean	1295	Faba bean	1336	Faba bean	1337	Faba bean	1378	Faba bean	1379	Faba bean
1212	GP90174	1251	IG147655GP	1254	GP90174	1293	IG147655GP	1296	GP90174	1335	IG147655GP	1338	GP90174	1377	IG147655GP	1380	GP90174
1213	117178	1250	117504	1255	117528	1292	117171	1297	117224	1334	65233	1339	65234	1376	117544	1381	117604
1214	65178	1249	117113	1256	117022	1291	116913	1298	117511	1333	65205	1340	65236	1375	117533	1382	65149
1215	65183	1248	64993	1257	65176	1290	65244	1299	117531	1332	65193	1341	65245	1374	117530	1383	117118
1216	65189	1247	64975	1258	65232	1289	65231	1300	117543	1331	65173	1342	65246	1373	117509	1384	117197
1217	65194	1246	117553	1259	65240	1288	65104	1301	64970	1330	65171	1343	65247	1372	117507	1385	117220
1218	65200	1245	117551	1260	65248	1287	64723	1302	65157	1329	65170	1344	116818	1371	117506	1386	117223
1219	116893	1244	117548	1261	116992	1286	65204	1303	117352	1328	65163	1345	116997	1370	117373	1387	117279
1220	117117	1243	IG147607GP	1262	117012	1285	IG147607GP	1304	64961	1327	IG147607GP	1346	117004	1369	IG147607GP	1388	117336
1221	Faba bean	1242	Faba bean	1263	Faba bean	1284	Faba bean	1305	Faba bean	1326	Faba bean	1347	Faba bean	1368	Faba bean	1389	Faba bean
1222	IG147607GP	1241	117369	1264	IG147607GP	1283	117598	1306	IG147607GP	1325	65160	1348	IG147607GP	1367	117368	1390	IG147607GP
1223	65039	1240	117065	1265	117018	1282	117365	1307	117515	1324	65156	1349	117060	1366	117358	1391	117540
1224	117003	1239	65201	1266	117034	1281	65162	1308	117535	1323	65154	1350	117095	1365	117334	1392	64964
1225	117333	1238	65108	1267	117047	1280	65175	1309	117546	1322	65153	1351	117182	1364	117332	1393	116949
1226	65017	1237	116989	1268	117053	1279	65174	1310	65147	1321	65042	1352	117196	1363	117328	1394	117204
1227	65112	1236	117493	1269	117064	1278	65143	1311	64951	1320	64968	1353	117222	1362	117283	1395	117211
1228	65192	1235	117331	1270	117110	1277	117175	1312	64958	1319	64963	1354	117225	1361	117277	1396	117510
1229	117121	1234	117122	1271	117115	1276	117119	1313	64960	1318	64962	1355	117229	1360	117255	1397	117526
1230	IG147655GP	1233	GP90174	1272	IG147655GP	1275	GP90174	1314	IG147655GP	1317	GP90174	1356	IG147655GP	1359	GP90174	1398	IG147655GP
1231	Faba bean	1232	Faba bean	1273	Faba bean	1274	Faba bean	1315	Faba bean	1316	Faba bean	1357	Faba bean	1358	Faba bean	1399	Faba bean

Appendix 1b: Germplasm information in field experiment.

Plot Number	TAXNAME	Bloc	EN2017	Blk2017	IG Number
1001		1			Faba Bean
1002		1			GP90174
1003	*L. pseudocicera*	1	115	3	65065
1004	*L. pseudocicera*	1	291	7	65543
1005	*L. aphaca*	1	113	3	65053
1006	*L. blepharicarpus*	1	72	2	64865
1007	*L. cassius*	1	206	5	65277
1008	*L. gorgoni*	1	227	6	65330
1009	*L. gorgoni*	1	242	6	65375
1010	*L. gorgoni*	1	328	8	65750
1011		1			Faba Bean
1012		1			IG147607GP
1013	*L. gorgoni*	1	3	1	63034
1014	*L. gorgoni*	1	106	3	64989
1015	*L. hierosolymitanus*	1	322	8	65735
1016	*L. hierosolymitanus*	1	281	7	65494
1017	*L. inconspicuous*	1	207	5	65279
1018	*L. inconspicuous*	1	287	7	65519
1019	*L. inconspicuous*	1	104	3	64983
1020		1			IG147655GP
1021		1			Faba Bean
1022		2			Faba Bean
1023		2			GP90174
1024	*L. inconspicuous*	2	283	7	65500
1025	*L. tingitanus*	2	363	9	116891
1026	*L. annuus*	2	234	6	65353
1027	*L. annuus*	2	275	7	65463
1028	*L. articulatus*	2	6	1	64733
1029	*L. articulatus*	2	8	1	64735
1030	*L. articulatus*	2	12	1	64786
1031	*L. articulatus*	2	13	1	64787
1032		2			Faba Bean
1033		2			IG147607GP
1034	*L. articulatus*	2	14	1	64795
1035	*L. articulatus*	2	15	1	64798
1036	*L. articulatus*	2	16	1	64800
1037	*L. cicera*	2	39	1	64831
1038	*L. cicera*	2	40	1	64832
1039	*L. cicera*	2	41	1	64833
1040	*L. cicera*	2	43	1	64836
1041		2			IG147655GP

1042		2			Faba Bean
1043		3			Faba Bean
1044		3			GP90174
1045	*L. cicera*	3	44	1	64837
1046	*L. cicera*	3	45	1	64838
1047	*L. cicera*	3	46	2	64839
1048	*L. cicera*	3	49	2	64842
1049	*L. cicera*	3	51	2	64844
1050	*L. cicera*	3	52	2	64845
1051	*L. cicera*	3	53	2	64846
1052	*L. cicera*	3	55	2	64848
1053		3			Faba Bean
1054		3			IG147607GP
1055	*L. cicera*	3	56	2	64849
1056	*L. cicera*	3	58	2	64851
1057	*L. cicera*	3	62	2	64855
1058	*L. cicera*	3	64	2	64857
1059	*L. cicera*	3	65	2	64858
1060	*L. cicera*	3	66	2	64859
1061	*L. cicera*	3	68	2	64861
1062		3			IG147655GP
1063		3			Faba Bean
1064		4			Faba Bean
1065		4			GP90174
1066	*L. cicera*	4	69	2	64862
1067	*L. cicera*	4	70	2	64863
1068	*L. cicera*	4	73	2	64866
1069	*L. cicera*	4	74	2	64867
1070	*L. cicera*	4	75	2	64868
1071	*L. cicera*	4	76	2	64869
1072	*L. cicera*	4	77	2	64870
1073	*L. cicera*	4	78	2	64872
1074		4			Faba Bean
1075		4			IG147607GP
1076	*L. cicera*	4	79	2	64873
1077	*L. cicera*	4	81	2	64876
1078	*L. cicera*	4	82	2	64878
1079	*L. cicera*	4	121	3	65103
1080	*L. cicera*	4	208	5	65281
1081	*L. cicera*	4	337	8	65823
1082	*L. cicera*	4	50	2	64843
1083		4			IG147655GP
1084		4			Faba Bean
1085		5			Faba Bean

1086		5			GP90174
1087	*L. cicera*	5	7	1	64734
1088	*L. cicera*	5	48	2	64841
1089	*L. cicera*	5	9	1	64745
1090	*L. cicera*	5	38	1	64830
1091	*L. cicera*	5	42	1	64834
1092	*L. cicera*	5	47	2	64840
1093	*L. cicera*	5	60	2	64853
1094	*L. cicera*	5	112	3	65046
1095		5			Faba Bean
1096		5			IG147607GP
1097	*L. cicera*	5	83	2	64879
1098	*L. cicera*	5	103	3	64977
1099	*L. cicera*	5	59	2	64852
1100	*L. cicera*	5	63	2	64856
1101	*L. cicera*	5	71	2	64864
1102	*L. cicera*	5	61	2	64854
1103	*L. cicera*	5	67	2	64860
1104		5			IG147655GP
1105		5			Faba Bean
1106		6			Faba Bean
1107		6			GP90174
1108	*L. cicera*	6	200	5	65252
1109	*L. cicera*	6	336	8	65820
1110	*L. cicera*	6	54	2	64847
1111	*L. cicera*	6	116	3	65074
1112	*L. cicera*	6	342	8	65949
1113	*L. ochrus*	6	1	1	62140
1114	*L. ochrus*	6	5	1	64731
1115	*L. ochrus*	6	17	1	64801
1116		6			Faba Bean
1117		6			IG147607GP
1118	*L. ochrus*	6	18	1	64804
1119	*L. ochrus*	6	19	1	64807
1120	*L. ochrus*	6	20	1	64808
1121	*L. ochrus*	6	21	1	64809
1122	*L. ochrus*	6	22	1	64810
1123	*L. ochrus*	6	23	1	64812
1124	*L. ochrus*	6	24	1	64813
1125		6			IG147655GP
1126		6			Faba Bean
1127		7			Faba Bean
1128		7			GP90174
1129	*L. ochrus*	7	25	1	64814

1130	*L. ochrus*	7	26	1	64816
1131	*L. ochrus*	7	27	1	64817
1132	*L. ochrus*	7	28	1	64818
1133	*L. ochrus*	7	29	1	64819
1134	*L. ochrus*	7	30	1	64820
1135	*L. ochrus*	7	31	1	64821
1136	*L. ochrus*	7	32	1	64822
1137		7			Faba Bean
1138		7			IG147607GP
1139	*L. ochrus*	7	33	1	64823
1140	*L. ochrus*	7	34	1	64824
1141	*L. ochrus*	7	35	1	64825
1142	*L. ochrus*	7	36	1	64827
1143	*L. ochrus*	7	37	1	64828
1144	*L. ochrus*	7	57	2	64850
1145	*L. ochrus*	7	92	3	64950
1146		7			IG147655GP
1147		7			Faba Bean
1148		8			Faba Bean
1149		8			GP90174
1150	*L. ochrus*	8	117	3	65075
1151	*L. ochrus*	8	176	4	65221
1152	*L. ochrus*	8	177	4	65222
1153	*L. ochrus*	8	179	4	65224
1154	*L. ochrus*	8	180	4	65225
1155	*L. ochrus*	8	181	5	65226
1156	*L. ochrus*	8	182	5	65227
1157	*L. ochrus*	8	183	5	65228
1158		8			Faba Bean
1159		8			IG147607GP
1160	*L. ochrus*	8	184	5	65229
1161	*L. ochrus*	8	185	5	65230
1162	*L. ochrus*	8	192	5	65237
1163	*L. ochrus*	8	194	5	65242
1164	*L. ochrus*	8	219	5	65310
1165	*L. ochrus*	8	229	6	65340
1166	*L. ochrus*	8	241	6	65373
1167		8			IG147655GP
1168		8			Faba Bean
1169		9			Faba Bean
1170		9			GP90174
1171	*L. ochrus*	9	243	6	65376
1172	*L. ochrus*	9	248	6	65390
1173	*L. ochrus*	9	485	11	117945

1174	*L. ochrus*	9	190	5	65235
1175	*L. sativus*	9	11	1	64782
1176	*L. sativus*	9	80	2	64875
1177	*L. sativus*	9	84	2	64882
1178	*L. sativus*	9	85	2	64886
1179		9			Faba Bean
1180		9			IG147607GP
1181	*L. sativus*	9	86	2	64900
1182	*L. sativus*	9	87	2	64908
1183	*L. sativus*	9	88	2	64909
1184	*L. sativus*	9	89	2	64915
1185	*L. sativus*	9	90	2	64918
1186	*L. sativus*	9	135	3	65136
1187	*L. sativus*	9	155	4	65184
1188		9			IG147655GP
1189		9			Faba Bean
1190		10			Faba Bean
1191		10			GP90174
1192	*L. sativus*	10	157	4	65187
1193	*L. sativus*	10	162	4	65195
1194	*L. sativus*	10	163	4	65197
1195	*L. sativus*	10	172	4	65210
1196	*L. sativus*	10	173	4	65211
1197	*L. sativus*	10	174	4	65212
1198	*L. sativus*	10	175	4	65213
1199	*L. sativus*	10	178	4	65223
1200		10			Faba Bean
1201		10			IG147607GP
1202	*L. sativus*	10	362	9	116890
1203	*L. sativus*	10	91	3	64935
1204	*L. sativus*	10	156	4	65186
1205	*L. sativus*	10	361	9	116889
1206	*L. sativus*	10	452	11	117496
1207	*L. sativus*	10	110	3	65040
1208	*L. sativus*	10	153	4	65179
1209		10			IG147655GP
1210		10			Faba Bean
1211		11			Faba Bean
1212		11			GP90174
1213	*L. sativus*	11	408	10	117178
1214	*L. sativus*	11	152	4	65178
1215	*L. sativus*	11	154	4	65183
1216	*L. sativus*	11	158	4	65189
1217	*L. sativus*	11	161	4	65194

1218	*L. sativus*	11	164	4	65200
1219	*L. sativus*	11	364	9	116893
1220	*L. sativus*	11	388	9	117117
1221		11			Faba Bean
1222		11			IG147607GP
1223	*L. sativus*	11	109	3	65039
1224	*L. sativus*	11	371	9	117003
1225	*L. sativus*	11	427	10	117333
1226	*L. sativus*	11	108	3	65017
1227	*L. sativus*	11	127	3	65112
1228	*L. sativus*	11	159	4	65192
1229	*L. sativus*	11	391	9	117121
1230		11			IG147655GP
1231		11			Faba Bean
1232		12			Faba Bean
1233		12			GP90174
1234	*L. sativus*	12	392	9	117122
1235	*L. sativus*	12	425	10	117331
1236	*L. sativus*	12	451	11	117493
1237	*L. sativus*	12	367	9	116989
1238	*L. sativus*	12	124	3	65108
1239	*L. sativus*	12	165	4	65201
1240	*L. sativus*	12	382	9	117065
1241	*L. sativus*	12	435	10	117369
1242		12			Faba Bean
1243		12			IG147607GP
1244	*L. sativus*	12	470	11	117548
1245	*L. sativus*	12	471	11	117551
1246	*L. sativus*	12	472	11	117553
1247	*L. sativus*	12	102	3	64975
1248	*L. sativus*	12	107	3	64993
1249	*L. sativus*	12	386	9	117113
1250	*L. sativus*	12	453	11	117504
1251		12			IG147655GP
1252		12			Faba Bean
1253		13			Faba Bean
1254		13			GP90174
1255	*L. sativus*	13	461	11	117528
1256	*L. sativus*	13	375	9	117022
1257	*L. sativus*	13	151	4	65176
1258	*L. sativus*	13	187	5	65232
1259	*L. sativus*	13	193	5	65240
1260	*L. sativus*	13	199	5	65248
1261	*L. sativus*	13	368	9	116992

1262	*L. sativus*	13	373	9	117012
1263		13			Faba Bean
1264		13			IG147607GP
1265	*L. sativus*	13	374	9	117018
1266	*L. sativus*	13	376	9	117034
1267	*L. sativus*	13	377	9	117047
1268	*L. sativus*	13	378	9	117053
1269	*L. sativus*	13	381	9	117064
1270	*L. sativus*	13	385	9	117110
1271	*L. sativus*	13	387	9	117115
1272		13			IG147655GP
1273		13			Faba Bean
1274		14			Faba Bean
1275		14			GP90174
1276	*L. sativus*	14	390	9	117119
1277	*L. sativus*	14	407	10	117175
1278	*L. sativus*	14	136	4	65143
1279	*L. sativus*	14	149	4	65174
1280	*L. sativus*	14	150	4	65175
1281	*L. sativus*	14	144	4	65162
1282	*L. sativus*	14	433	10	117365
1283	*L. sativus*	14	479	11	117598
1284		14			Faba Bean
1285		14			IG147607GP
1286	*L. sativus*	14	166	4	65204
1287	*L. sativus*	14	4	1	64723
1288	*L. sativus*	14	122	3	65104
1289	*L. sativus*	14	186	5	65231
1290	*L. sativus*	14	195	5	65244
1291	*L. sativus*	14	365	9	116913
1292	*L. sativus*	14	406	10	117171
1293		14			IG147655GP
1294		14			Faba Bean
1295		15			Faba Bean
1296		15			GP90174
1297	*L. sativus*	15	417	10	117224
1298	*L. sativus*	15	458	11	117511
1299	*L. sativus*	15	463	11	117531
1300	*L. sativus*	15	467	11	117543
1301	*L. sativus*	15	101	3	64970
1302	*L. sativus*	15	142	4	65157
1303	*L. sativus*	15	430	10	117352
1304	*L. sativus*	15	96	3	64961
1305		15			Faba Bean

1306		15			IG147607GP
1307	*L. sativus*	15	459	11	117515
1308	*L. sativus*	15	465	11	117535
1309	*L. sativus*	15	469	11	117546
1310	*L. sativus*	15	137	4	65147
1311	*L. sativus*	15	93	3	64951
1312	*L. sativus*	15	94	3	64958
1313	*L. sativus*	15	95	3	64960
1314		15			IG147655GP
1315		15			Faba Bean
1316		16			Faba Bean
1317		16			GP90174
1318	*L. sativus*	16	97	3	64962
1319	*L. sativus*	16	98	3	64963
1320	*L. sativus*	16	100	3	64968
1321	*L. sativus*	16	111	3	65042
1322	*L. sativus*	16	139	4	65153
1323	*L. sativus*	16	140	4	65154
1324	*L. sativus*	16	141	4	65156
1325	*L. sativus*	16	143	4	65160
1326		16			Faba Bean
1327		16			IG147607GP
1328	*L. sativus*	16	145	4	65163
1329	*L. sativus*	16	146	4	65170
1330	*L. sativus*	16	147	4	65171
1331	*L. sativus*	16	148		65173
1332	*L. sativus*	16	160	4	65193
1333	*L. sativus*	16	167	4	65205
1334	*L. sativus*	16	188	5	65233
1335		16			IG147655GP
1336		16			Faba Bean
1337		17			Faba Bean
1338		17			GP90174
1339	*L. sativus*	17	189	5	65234
1340	*L. sativus*	17	191	5	65236
1341	*L. sativus*	17	196	5	65245
1342	*L. sativus*	17	197	5	65246
1343	*L. sativus*	17	198	5	65247
1344	*L. sativus*	17	346	8	116818
1345	*L. sativus*	17	369	9	116997
1346	*L. sativus*	17	372	9	117004
1347		17			Faba Bean
1348		17			IG147607GP
1349	*L. sativus*	17	380	9	117060

1350	*L. sativus*	17	384	9	117095
1351	*L. sativus*	17	409	10	117182
1352	*L. sativus*	17	410	10	117196
1353	*L. sativus*	17	415	10	117222
1354	*L. sativus*	17	418	10	117225
1355	*L. sativus*	17	419	10	117229
1356		17			IG147655GP
1357		17			Faba Bean
1358		18			Faba Bean
1359		18			GP90174
1360	*L. sativus*	18	420	10	117255
1361	*L. sativus*	18	421	10	117277
1362	*L. sativus*	18	423	10	117283
1363	*L. sativus*	18	424	10	117328
1364	*L. sativus*	18	426	10	117332
1365	*L. sativus*	18	428	10	117334
1366	*L. sativus*	18	432	10	117358
1367	*L. sativus*	18	434	10	117368
1368		18			Faba Bean
1369		18			IG147607GP
1370	*L. sativus*	18	437	10	117373
1371	*L. sativus*	18	454	11	117506
1372	*L. sativus*	18	455	11	117507
1373	*L. sativus*	18	456	11	117509
1374	*L. sativus*	18	462	11	117530
1375	*L. sativus*	18	464	11	117533
1376	*L. sativus*	18	468	11	117544
1377		18			IG147655GP
1378		18			Faba Bean
1379		19			Faba Bean
1380		19			GP90174
1381	*L. sativus*	19	480	11	117604
1382	*L. sativus*	19	138	4	65149
1383	*L. sativus*	19	389	9	117118
1384	*L. sativus*	19	411	10	117197
1385	*L. sativus*	19	414	10	117220
1386	*L. sativus*	19	416	10	117223
1387	*L. sativus*	19	422	10	117279
1388	*L. sativus*	19	429	10	117336
1389		19			Faba Bean
1390		19			IG147607GP
1391	*L. sativus*	19	466	11	117540
1392	*L. sativus*	19	99	3	64964
1393	*L. sativus*	19	366	9	116949

1394	*L. sativus*	19	412	10	117204
1395	*L. sativus*	19	413	10	117211
1396	*L. sativus*	19	457	11	117510
1397	*L. sativus*	19	460	11	117526
1398		19			IG147655GP
1399		19			Faba Bean

Appendix 1c: Multivariate analysis of field experiment.

Rotated Component Matrix[a]

	PC1	PC2	PC3
OIN	**,847**	,393	-,077
D2F	**-,746**	-,006	,044
PI	**,737**	,423	-,377
OSV	**,649**	,509	-,358
D2M	**-,629**	,060	,589
D2OE	**,593**	,545	,274
EODW	,205	**,942**	-,170
EON	,128	**,919**	-,188
SY	-,076	-,210	**,928**
BY	-,093	-,123	**,897**

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 6 iterations.

Clustering Table

Cluster No	Count
1	85
2	20
3	180

Centroids of clusters Table

	D2F	D2OE	D2M	OIN	OSV	PI	BY	SY	EON	EODW
1	77.982	34.441	149.606	21.676	2.724	0.079	468.752	147.737	0.860	0.141
2	87.675	14.575	152.650	7.875	2.200	0.657	926.780	217.436	0.355	0.065
3	64.579	52.783	136.119	27.885	4.258	2.124	154.880	38.575	1.524	0.516

Tables: Linear Regression

Assumption Checks

Collinearity Statistics

	VIF	Tolerance
D2F	1.564	0.639
D2OE	1.846	0.542
D2M	1.845	0.542
OIN	5.480	0.182
OSV	2.609	0.383
PI	6.080	0.164
BY	1.937	0.516
EON	9.742	0.103
EODW	9.555	0.105

Model Fit Measures

Model	R	R^2	RMSE
1	0.859	0.737	38.803

Omnibus ANOVA Test

	Sum of Squares	Df	Mean Square	F	P
D2F	1035.229	1	1035.229	0.663	0.416
D2OE	4257.597	1	4257.597	2.728	0.100
D2M	2078.762	1	2078.762	1.332	0.249
OIN	17288.216	1	17288.216	11.079	< .001
OSV	526.795	1	526.795	0.338	0.562
PI	22954.235	1	22954.235	14.710	< .001
BY	482451.266	1	482451.266	309.178	< .001
EON	4307.520	1	4307.520	2.760	0.098
EODW	4015.665	1	4015.665	2.573	0.110
Residuals	429118.215	275	1560.430		

Note. Type 3 sum of squares

Model Coefficients – SY

Predictor	Estimate	SE	T	P
Intercept	-17.204	29.184	-0.590	0.556
D2F	-0.105	0.129	-0.815	0.416
D2OE	-0.205	0.124	-1.652	0.100
D2M	0.264	0.229	1.154	0.249
OIN	0.894	0.269	3.329	< .001
OSV	1.157	1.992	0.581	0.562
PI	-12.936	3.373	-3.835	< .001
BY	0.231	0.013	17.583	< .001
EON	4.410	2.654	1.661	0.098
EODW	-3.437	2.143	-1.604	0.110

Appendix 2a: RCBD design used in pot experiement.

TRT	Pot N°	N ° ORDER	IG number	Rep
with ORB	1001	7	IG64951	1
with ORB	1002	6	IG65042	1
with ORB	1003	3	IG116997	1
with ORB	1004	2	IG64875	1
with ORB	1005	1	IG65204	1
with ORB	1006	10	IG64908	1
with ORB	1007	8	IG64782	1
with ORB	1008	4	IG65197	1
with ORB	1009	5	IG65187	1
with ORB	1010	9	IG116989	1
with ORB	1011	5	IG65187	2
with ORB	1012	4	IG65197	2
with ORB	1013	7	IG64951	2
with ORB	1014	1	IG65204	2
with ORB	1015	6	IG65042	2
with ORB	1016	8	IG64782	2
with ORB	1017	3	IG116997	2
with ORB	1018	9	IG116989	2
with ORB	1019	2	IG64875	2
with ORB	1020	10	IG64908	2
with ORB	1021	1	IG65204	3
with ORB	1022	9	IG116989	3
with ORB	1023	6	IG65042	3
with ORB	1024	4	IG65197	3
with ORB	1025	7	IG64951	3
with ORB	1026	3	IG116997	3
with ORB	1027	5	IG65187	3
with ORB	1028	8	IG64782	3
with ORB	1029	10	IG64908	3
with ORB	1030	2	IG64875	3
without ORB	2001	2	IG64875	1

without ORB	2002	3	IG116997	1
without ORB	2003	7	IG64951	1
without ORB	2004	5	IG65187	1
without ORB	2005	6	IG65042	1
without ORB	2006	1	IG65204	1
without ORB	2007	9	IG116989	1
without ORB	2008	8	IG64782	1
without ORB	2009	10	IG64908	1
without ORB	2010	4	IG65197	1
without ORB	2011	10	IG64908	2
without ORB	2012	6	IG65042	2
without ORB	2013	2	IG64875	2
without ORB	2014	3	IG116997	2
without ORB	2015	7	IG64951	2
without ORB	2016	4	IG65197	2
without ORB	2017	5	IG65187	2
without ORB	2018	1	IG65204	2
without ORB	2019	9	IG116989	2
without ORB	2020	8	IG64782	2
without ORB	2021	3	IG116997	3
without ORB	2022	4	IG65197	3
without ORB	2023	6	IG65042	3
without ORB	2024	1	IG65204	3
without ORB	2025	9	IG116989	3
without ORB	2026	5	IG65187	3
without ORB	2027	2	IG64875	3
without ORB	2028	8	IG64782	3
without ORB	2029	7	IG64951	3
without ORB	2030	10	IG64908	3

Appendix 2b: Multivariate analysis of pot assay.

Rotated Component Matrix[a]

	PC1	PC2
UODW	**0.915**	-0.100
RDW	**0.848**	0.147
UON	**0.801**	0.160
TAN	**0.694**	0.528
EON	0.039	**0.866**
EODW	0.042	**0.862**
SDW	-0.226	**-0.704**
PDN	-0.061	**-0.415**

Note. 'varimax' rotation was used

Clustering Table

Cluster No	Count
1	7
2	3

Centroids of clusters Table

	PDN	SDW	RDW	EODW	UODW	EON	UON	TAN
1	0.500	2.217	8.817	1.633	7.183	3.667	16.167	19.833
2	3.417	5.729	2.746	1.792	0.538	3.375	2.208	5.583

Appendix 2c: Neurotoxins ODAP Protocol: UV-Spectrophotometer method (Rao, 1978).

1. **Preparation of reagents**

- **Potassium hydroxide (3N)**

  168.33 g KOH per liter distilled water.

- **Potassium borate buffer**

  3.092 g potassium tetra- borate ($K_2B_4O_7 .4H_2O$).

  3.728 g potassium chloride (KCl).

  3 pellets sodium hydroxide (NaOH).

  Make up volume 1 L with distilled water.

  Adjust pH to 9.9 with (1N) sodium hydroxide solution.

- **O-phthalaldehyde reagent (OPT)**

  100 mg (0.1 g) O-phthalaldehyde ($C_8H_6O_2$).

  200 µl (0.2 ml) mercaptoethanol (C_2H_6OS).

  1000 µl (1 ml) ethanol (95%).

  99 ml potassium borate buffer.

2. **Standard Curve**

Diaminopropionic acid (DAP) is hygroscopic and absorbs CO_2 from air. The stable salt, Diaminopropionic acid hydrochloride, which is commercially available, is normally used. DAP stock solution = (400 ug DAP/ml of 60% ethanol).

Weigh 0.054 g DAP hydrochloride ($C_3H_8N_2O_2$.HCl) and dissolve in 100 ml of 60% ethanol. (Conversion factor from DAP to DAP-hydrochloride = 1.35). Make dilutions of the DAP stock, as in Table 1.

Table 1. Standard curve and conversation from DAP to ODAP%.

Tube No.	DAP stock solution (ml)	60% ethanol (ml)	μg DAP in 2 ml solution	μg DAP/g seed	ODAP* %
1	0.3	1.7	120	1200	0.203
2	0.6	1.4	240	2400	0.406
3	0.9	1.1	360	3600	0.609
4	1.2	0.8	480	4800	0.812
5	1.5	0.5	600	6000	1.015
6	1.8	0.2	720	7200	1.218

*Conversion factor from DAP to ODAP = 1.691643

- **Standard curve preparation:**
    - Prepare test tubes with different concentrations of (0.3, 0.6, 0.9, 1.2 ml etc) DAP stock solution and make volume 2 ml with 60% ethanol.
    - Add 4 ml of 3 N KOH to each tube, cap tightly and vortex.
    - Place all tubes in boiling water bath for 30 minutes and then vortex.
    - Centrifuge at 4500 rpm for 15 minutes.
    - 0.25 ml hydrolyzed supernatant + 0.75 ml H_2O + 2 ml OPT and mix
    - Incubate at 40° C for 2 hrs.
    - Take spectrophotometer reading at 425 nm by setting absorbance of blank as zero.
    - Construct a graph of absorbance of standards against BOAA%.

3. Extraction procedure

- Weigh 5mg (or 1g/20ml = 20 x dilution) powdered seed and place into volumetric flask.
- Add 10 ml of 60% ethanol.
- Shake tubes in shaker/mixer for 45 minutes @ 200 rpm.
- Centrifuge at 4500 rpm for 15 minutes.
- Decant ethanol extract (unhydrolyzed fraction) into pre-labeled test tubes.

Figure 1: Isolation of ODAP from grasspea seed.

4. **Sample hydrolysis:** (Conversion from BOAA in the sample to DAP takes place)
    - Pipette 2 ml of ethanol extract into screw top test tube.
    - Prepare a blank tube by 2 ml of 60% ethanol instead of ethanol extract.
    - Add 4 ml of 3N potassium hydroxide (KOH).
    - Cap tightly and vortex and place all tubes in boiling water bath for 30 minutes.
    - Centrifuge at 4500 rpm for 15 minutes.
    - Hydrolyzed sample transfer in pre-labeled test tube
5. **Color Reaction**

Pipette the following into screw-top test tubes for all samples/standard curve tubes/ blank tube

- 250 µl hydrolyzed sample/standard/blank
- 750 µl distilled water
- 2000 µl OPT solution
- Vortex all the tubes
- Incubate for two hours (2hrs) at 40 ^{O}C
- Take spectrophotometric reading at 425 nm by setting absorbance of blank as zero
- Determine the BOAA% in the samples from the standard graph.
- After incubation color development and spectrophotometric reading at 425nm**.**

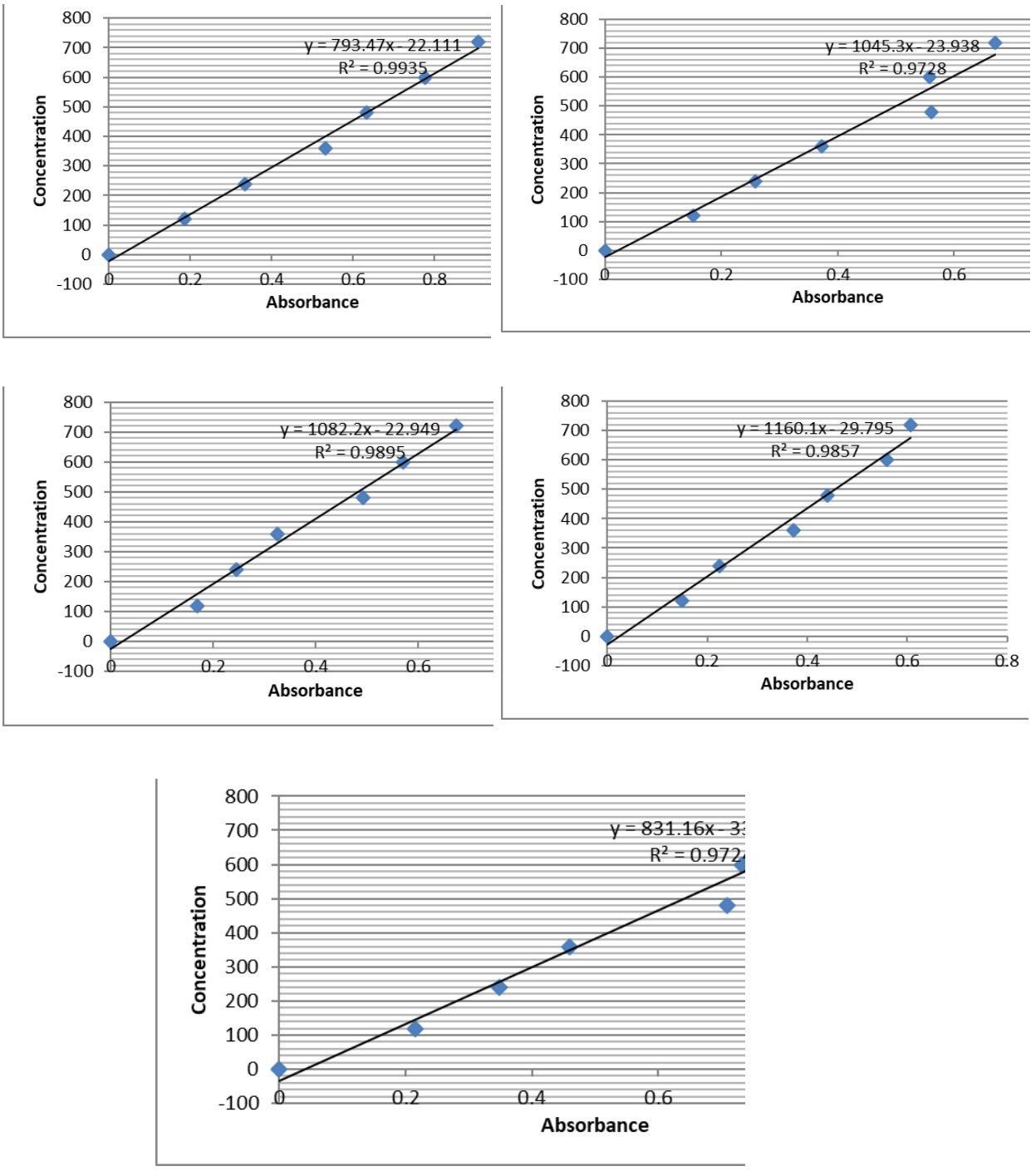


Figure 2: Standard curves for the measurement of *β*-ODAP concentrations of grass pea.

Appendix 3a: Macro and micronutrients: ICP-OES method (ICARDA book).

1. **Sample preparation:**

Mineral (Fe, Zn, Cu, Ca, Mg) concentrations in seeds were determined using a modified HNO^3-H_2O_2 method, (A. pequerul *et al*., Alcok *et al*., 2009).

- Placed sample in new, clean paper bags and then dried in a clean oven at 75 ^oC for 48h to destroy any pathogen spores.
- Whole grain flour samples were obtained by grinding dry seeds in cyclone sample mill, the cyclone should be cleaned adequately after grinding each sample to avoid cross contamination.
- Place finely ground seed samples (500 mg) in individual digestion tubes.
- Add 6- 8 ml of concentrated (70%) nitric acid (HNO^3) to each digestion tube, and leave for overnight.
- The digestion tubes were placed in a 90 ^oC-95 ^oC digestion block for one hour.
- Shake at 15 and 45 minutes.
- Add 3- 4 ml of 30% w/w hydrogen peroxide (H_2O_2) to each tube at 90 ^oC.
- Upon complete digestion (the time required for complete digestion was determined in earlier laboratory experiments, the complete digestion is indicated by the discontinuation of brown smoke coming from the digestion tubes).
- Remove the tubes from the digestion block and let them cool to room temperature.
- Filter the digest (Whatman papers), and then dilute 1:10 with 6M Hcl.

2. **Start up:**

- Open valve on Argon/Nitrogen Tank. Pressure should read 90psi. Allow instrument to purge for 20 minutes.
- After purge, turn on chiller. Temperature should read 18.
- Turn on the extraction.
- Position pump tubing and set clamps on instrument. Set clamp on autosampler rinse tubing. Place sipper into manual rinse container.
- Open Qtegra software. Open operating parameters by clicking on “torch” icon (lower right). Click on “instrument statut”. Set pump rate to 50rpm and set nebulizer to “on”. Click “apply”.

- Confirm that rinse water is pumping through tubing and that drain is properly working.
- Ignite plasma. Allow instrument to stabilize (15 minutes).

3. Software:

- Open “Qtegra” select method from list or create a new method.
- Click on “Analyt” and then select the mineral to analyse, select also the wavelength based on interference and intensity.
- Click on “method mode” and select whitch mode of analysis “Axial (for low concentration) or Radial (for high concentration)”.
- Click on “Standards” and create the list of standards with concentration to be used.
- Click on “Sample liste” and create the order, position and the type of the analysis.
- Record and start the analysis.

4. Shutdown:

- Turn off plasma by clicking on “torch” icon.
- Connect sample pump tubing to manual rinse. Start sample punp to flush tubing.
- Remove sipper from rinse and allow to pump dry.
- Set pump speed to zero. Release tubing from pump.
- Turn off chiller.
- Release tubing from autosampler.
- Allow a few minutes for Nitrogen to purge, and then shut valve on tank. Leave instrument ON.

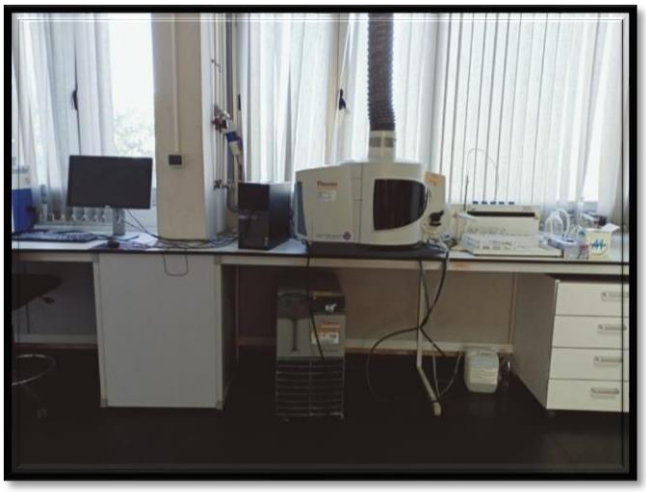


Figure 1: ICP-OES in quality laboratory at ICARDA, Rabat, Morocco.

Appendix 3b: Multivariate analysis of macro and micronutrients analyse.

Centroids of clusters Table (Macro)

	Cluster No	P	K	Ca	Mg
1	1.000	3182.569	3131.858	1065.672	1053.415
2	2.000	1902.662	2841.320	1089.717	1009.841

Clustering Table (Macro)

Cluster No	Count
1	72
2	111

Centroids of clusters Table (Micro)

	Cluster No	Zn	Mn	Fe	Se
1	1.000	33.424	2.627	25.746	0.182
2	2.000	33.520	12.371	37.310	0.178

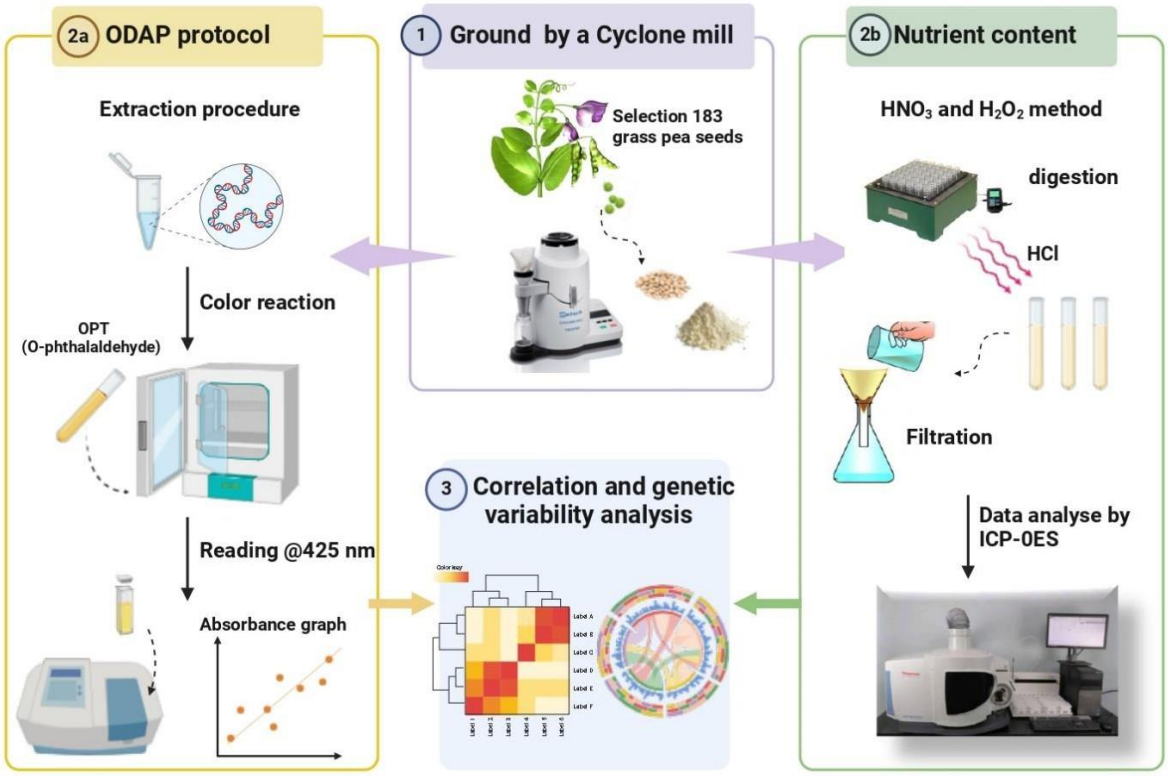
Clustering Table (Micro)

Cluster No	Count
1	114
2	69

Centroids of clusters Table

	Cluster No	P	K	Zn	Ca	Mg	Mn	Fe	Se	%ODAP
1	1.000	3182.569	3131.858	32.317	1065.672	1053.415	5.858	30.230	0.194	0.118
2	2.000	1902.662	2841.320	34.202	1089.717	1009.841	6.589	30.026	0.172	0.108

Appendix 4a: Graphical abstract (Chapter IV).



Appendix 4b: Supplementary material (Chapter IV).

Table S1. *Lathyrus* accessions, their origin and IG number included in the study for *β*-ODAP and nutrient analysis.

Species	Geographic distribution	IG number
L. annuus **(1)**	Syria	65353
L. aphaca **(1)**		65053
L. articulatus **(1)**	Czech Republic	64795
L. blepharicarpus **(1)**		64865
L. cassius **(1)**	Syria	65277
L. cicera **(33)**	India **(1)**	64745
	Turkey **(1)**	65820
	Germany **(1)**	64879
	Syria **(1)**	65103
	Australia **(2)**	65046, 65074
	Greece **(27)**	64834, 64837, 64839, 64840, 64842, 64843, 64844, 64846, 64849, 64852, 64853, 64856, 64858, 64859, 64860, 64861, 64862, 64863, 64865, 64867, 64868, 64869, 64870, 64872, 64873, 64875, 64977

***L. gorgoni* (2)**	Syria **(2)**	65375, 64989
***L. marmoratus* (2)**	Iraq **(1)**	64983
	Syria **(1)**	65519
***L. ochrus* (42)**	India **(1)**	64731
	Czech Republic **(1)**	64827
	Portugal **(1)**	64828
	Russia **(1)**	65075
	Cyprus **(11)**	64950, 65222, 65224, 65225, 65226, 65227, 65228, 65229, 65230, 65235,65242
	Syria **(7)**	65221, 65310, 65340, 65373, 65376, 65390, 117945
	Greece **(20)**	64801, 64804, 64807, 64808, 64809, 64810, 64812, 64813, 64814, 64817, 64818, 64819, 64820, 64821, 64822, 64823, 64824, 64827, 64828, 64850
***L. odoratus* (1)**	Italy **(1)**	62145
***L. pseudocicera* (1)**		65065
***L. sativus* (85)**	Canada **(1)**	64993
	Bulgaria **(1)**	64900
	Iran **(1)**	65040
	Moldova **(1)**	65074
	Germany **(2)**	64915, 64918
	Greece **(2)**	64886, 64909
	Turkey **(2)**	64882, 65017
	Afghanistan **(8)**	64723, 64958, 64960, 64961, 64962, 64968, 64970, 64875
	Cyprus **(9)**	65223, 65231, 65233, 65234, 65240, 65245, 65246, 65247, 65248
	Ethiopia **(28)**	65104, 65136, 65143, 65147, 65153, 65160, 65162, 65163, 65170, 65171, 65174, 65176, 65178, 65179, 65183, 65184, 65187, 65192, 65193, 65194, 65195, 65197, 65200, 65204, 65205, 65210, 65211, 65213
	Bangladesh **(33)**	116889, 116890, 116992, 117003, 117012, 117018, 117022, 117034, 117053, 117064, 117065, 117110, 117113, 117115, 117119, 117122, 117171, 117175, 117178, 117224, 117331, 117333, 117365, 117493, 117496, 117511, 117515, 117528, 117531, 117535, 117543,117546, 117598
	India **(1)**	Ratan
***L. tingitanus* (1)**	Bangladesh **(1)**	116891
Breeding lines (11)	ICARDA **(11)**	ACC111, ACC1322, ACC1330, ACC1335, ACC1336, ACC1348, ACC1916, ACC2125, ACC2329, ACC650, ACC736

***(..)**; Number of accessions of each species.