Identification of Genetic Sources of Broomrape (Orobanche crenata) Tolerance in Mediterranean Lentil (Lens culinaris Medik.) Population

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Abstract

Lentil (*Lens culinaris* Medik.), an essential food legume for global food security and agricultural sustainability, currently confronts several major challenges related to biotic constraints such as Orobanche. This root parasite significantly reduces yields, requiring effective breeding strategies to develop tolerant genotypes. In this study, we evaluated a Mediterranean population of 112 lentil genotypes to identify sources of tolerance to Broomrape (*Orobanche crenata*) in pots under greenhouse conditions. The results of this study revealed significant genetic variability among genotypes, based on key traits such as performance and parasitism indexes, severity and agronomic traits. By integrating these traits, the Multi-Trait Performance Index (MGIDI) was used and the best-performing genotypes were selected in terms of their broomrape tolerance and productivity. These genotypes represent promising candidates for breeding programs aimed at developing Orobanche-tolerant lentil varieties.

Introduction

Lentil is one of the world's most popular and widely grown grain legumes. The high protein content of lentil seeds proves that it is essential for human nutrition. In regions where access to animal protein is fairly limited for economic reasons, lentils would be a good alternative for preventing diseases due to deficiencies in protein and other trace elements (Ganesan and Xu 2017; Kaale et al. 2023). The domestication of lentils dates back to 13,000 BC in the Indian sub-region towards the Fertile Crescent, making lentils one of the first grain legumes to be domesticated (Ladizinsky 1987; Sandhu and Singh 2007). According to recent data, lentil production stands at 6.6 M tonnes worldwide (*FAOSTAT* 2024). In Morocco, although it is grown in the “bour system”, lentil is, behind broad bean and chickpea, the third most cultivated legume, with 9978.6 tons in 2022 (*FAOSTAT* 2024). However, lentil production is not stable and is subject to abiotic and biotic constraints.

Consequently, its economic importance, particularly in the creation of value chains, is attracting a great deal of interest and requires researchers to constantly find solutions to the biotic and abiotic constraints that disrupt the stability of lentil yields. Traditionally, the main pathogens ravaging lentil cultivation in Morocco are *Ascochyta rabiei*, and *Fusarium oxysporum* f. sp. In addition to these pathogens, lentil productivity in Morocco has in recent years, as elsewhere in the world, been severely tested by the invasion of many crop fields by broomrape. In these circumstances, broomrape seems to be the major biotic constraint to lentil cultivation in Morocco (En-nahli et al. 2021). The holoparasitic species belonging to the broomrape genus include few species that affect cultivated plants. However, some species, notably *O. crenata* Forsk, *O. foetida* Poiret, *O. cumana* Wallr., *O. ramosa* L. and *O. aegyptiaca* L., have a wide host range on legumes, including peas, broad beans, lentils and chickpeas (Abbes et al. 2007).

The rapid adaptation of broomrape species to new hosts, the tenacity of their seeds in the soil, and their production in large numbers mean that this invasive broomrape species is difficult to control. At one time, lentils seemed to be one of the legumes least affected by broomrape (En-nahli et al. 2021). Populations of broomrape collected from lentil plants have shown significant virulence on other crops. This shows that although lentils are less affected by broomrape, they nevertheless contribute to a spread that remains a danger to other crops. The current trend shows considerable losses for lentil growers (Mbasani-Mansi et al. 2019a). Winter sowing, which seems to be particularly conducive to reducing lentil attacks by certain pathogens (*Ascochyta rabiei*, *Fusarium oxysporum* f. sp.), is proving conducive to the multiplication of broomrape. Yield losses caused by broomrape vary between species and depend on the degree of infestation and the sowing date, but are still enormous.

Several practices and strategies have been proposed to mitigate the damage caused by broomrape, including the use of pesticides, biological control and staggered sowing dates (Rubiales et al. 2003, 2006). The effectiveness of certain control strategies against broomrape is certainly approved, but they sometimes show less than total crop protection and present difficulties for their implementation. Beyond the debates surrounding the regulation and use of certain pesticides, this means of control shows its limits, as lentil in particular seems to be very sensitive to certain herbicides, even though they have been proposed for the control of broomrape. Moreover, the application of a herbicide at the broomrape attachment stage did not have a significant effect on yield in certain legumes (Soliman et al. 2012). Some of the more virulent broomrape species are even more successful in breaking the broomrape resistance identified in certain crops. So the race to find broomrape-resistant planting material seems to be the path favored by improvers to limit chickpea yield losses. Although genetic resistance is strongly influenced by the environment, it remains the most economical and environmentally friendly strategy.

However, the best-explored plant defense mechanisms appear to be complex, with low heritability in leguminous plants, which makes it difficult to select for broomrape resistance. This complexity maybe is attributed to the high variability of broomrape populations and the dependence of the infestation on the climatic and environmental conditions. To date, little research has succeeded in identifying total resistance, although in recent years research institutions have been able to propose cultivars that remain less susceptible. At present, sources of resistance to broomrape have been identified in other crops (González-Verdejo et al. 2020; Joel et al. 2007; Rubiales et al. 2006), and in certain legumes such as faba bean (Abbes et al. 2007, 2011) and chickpea, notably with line ILC 3279 (Linke et al. 1992); Rubiales et al. 2004). In lentils, no total resistance has been identified (Muehlbauer et al. 2006). However, using a collection of 234 lentil accessions, (Fernández-Aparicio et al. 2008) were able to identify partial but effective resistance under field conditions. Genetic resistance could in some cases, be combined with other control measures.

In the Mediterranean basin region, where useful agricultural areas reserved for legumes are fairly widespread, broomrape remains a serious threat to legume crops in general and lentils in particular. Of all broomrape species, *O. crenata* is the most widespread in Morocco and the most damaging. This species has been threatening crops since ancient times. Field observations show that lentil yield losses can reach 90%. In Morocco, recent advances in genetic resistance research have revealed the existence of a few partially tolerant resistant accessions. The aim of this research is therefore, firstly, to evaluate the behavior of a collection of 110 lentil accessions (including advanced lines, local genotypes and varieties) exposed to *O. crenata* infestation under controlled greenhouse conditions and, secondly, to study the variability of responses under these biotic stress conditions in order to identify sources of genetic tolerance in the material used.

Material and methods

Plant material

The plant material used in this study comprises 110 lentil Mediterranean accessions, including 4 genotypes from Turkey, 4 genotypes from Greece, 7 genotypes from Italy and 95 local genotypes (landraces), with 2 check accessions (C1: Zaaria (susceptible variety to broomrape from Morocco), C2: EST (Tolerant variety to broomrape from Canada)).

Growth conditions, experimental design, and treatment

The experiment was carried out in greenhouse under natural day-light conditions at the Laboratory of Food Legumes Breeding, Regional Center of Settat, the National Institute of Agricultural Research (INRA Morocco). The experiments was carried out in plastic pots with 1L capacity, containing a homogeneous mixture with 2:1 (v:v) of soil and peat, respectively. The experimental design adopted is an RCBD (randomized completely blocs design) augmented by three blocks. Each block is composed of forty-one genotypes, including the two controls repeated twice. Five seeds per genotype were carefully chosen by their size caliber and health status (without traces of disease) and sown, while only three plants were maintained until the end of the experiment.

The infested pots were inoculated with *O. crenata* seeds (collected during previous seasons on lentil plants in infested plots) at a density of 10 mg.kg – 1 of soil. During the experiment, plants were irrigated every 4 to 7 days depending on the plants growth stages and the corresponding water consumption. Plants were harvested at physiological maturity.

Evaluations and Measurements

A set of traits was evaluated to quantify the impact of Orobanche parasitism on lentil plants. The number of aborted flowers (**NAF**) was determined by counting the aborted flowers per plant. The number of broomrape plants (**NTBP**) was measured as the total number of emerged Orobanche shoots per pot. Additionally, the number of plants per pot (**NPLP**) was recorded by counting the individual plants within each pot, while the number of pods per plant (**NPP**) was assessed by counting the pods produced by each plant.

Orobanche development was classified into six distinct stages (Fig. 5), from stage 0 (**OS0**) to stage 5 (**OS5**), capturing the progression of parasitism from initial attachment to full emergence and seed production, **OS0**: No Orobanche attachment, **OS1**: Tubercle size < 2 mm, **OS2**: Tubercle size > 1cm, **OS3**: Shoots size > 2cm but still below the surface, **OS4**: Emerged shoots in flowering stage, **OS5**: Orobanche seeds formation.

The emerged Orobanche number per plant (**OEN**) was recorded by counting the number of emerged Orobanche shoots per plant.

The parasitism index (**PI**) was calculated using the formula developed by (Abbes et al. 2007):

PI= (OIN×OSV)/100

Where Orobanche incidence (**OIN**) represents the percentage of lentil plants with at least one emerged Orobanche shoot per pot, and Orobanche severity (**OSV**) refers to the degree of damage inflicted on lentil growth and seed production, rated on a 1–9 scale. The seed weight (**SW**) was measured as the total seed mass per plant, using a balance, while both the total biomass of Orobanche (**TBO**) and the total biomass of lentil plants (**TBP**) were determined by weighing all corresponding plant parts in the pot using a balance.

A performance index (**IP)** was developed to integrate the severity of parasitism with lentil productivity. The IP was calculated using the following formula:

IP = 0.6×(normalized PI) + 0.2×(normalized NPP)

This index provides a comprehensive measure of lentil plant performance under Orobanche parasitism by combining the normalized parasitism index and the normalized number of pods per plant.

Statistical analysis

The statistical analysis were performed using R v4.2.2 software. The ANOVA analysis was performed using agricolae package (Mendiburu 2019) according to lentil origins and their biological status. In addition, other analysis of variance according to genotypes, blocs and checks were performed using augmented-RCBD package (Sankar 2021), the descriptive statistics was performed using the same package. Correlation analysis were performed using the metan package (Olivoto and Lúcio 2020) to identify the association between the measured traits. Using the standardized means of each genotype across all traits, principal component analysis (PCA) was performed with the factoextra package (Kassambara and Mundt 2017) to get an idea of the variables that contribute to the high variability of responses to Orobanche. To understand how data points (genotypes) are grouped together, a hierarchical cluster analysis was performed. This analysis was carried out using several statistical r packages, including cluster (Maechler et al. 2013) and fpc (Hennig and Imports 2015). The Multi-Trait Genotype-Ideotype Distance Index (MGIDI) analysis was carried out using metan r package (Olivoto and Lúcio 2020) in order to select accessions with high performance index. This analysis was based on moderate selection intensity with a rate of 15%. The density plots were performed using the ggplot2 package to visualize the distribution of the performance index in the studied Mediterranean lentil population.

Results

1. Response and effect of genotype, origin and biological status according to Orobanche infestation

The analysis of variance (ANOVA) revealed significant variations according to genotype for many measured parameters (Table 1), highlighting the presence of the genetic variability in the tested population. The NPLP, NTBP, OIN, OSV, NAF, OEN, TBO, NPP, SW, PI, IP, TBP revealed significant variations ($P \leq 0.01$), indicating the influence of the genotype on these traits. The tested checks showed significant variations ($P \leq 0.01$), confirming their role as references comparing to genotypes. No significant differences ($P > 0.05$) were showed between the blocks suggesting that the variability between genotypes according to blocks is negligible. Concerning the Orobanche stages OS0 to OS5, the significant differences ($P \leq 0.01$) between genotypes is showed only for OS3, OS4 and OS5,while the OS0, OS1 and OS2 revealed no significant differences ($P > 0.05$). A notable variability ($P \leq 0.01$) according to origin of genotypes was showed only for OIN, OS2, OS3, OS4 and OS5, and according to Biological status was revealed only for SW (Table 2).

Table 1
Means squares and significance levels of the effects of genotype, check and block on the studied traits using analysis of variance (ANOVA).

Source of Variation	Df	Mean.Sq											
		NPLP		NTBP		OIN		OSV		NAF		OEN	
Genotype	111	0.75	**	26.90	*	887.96	**	6.03	**	306.96	**	1.56	**
Check	1	2.08	**	420.08	**	18148.15	**	120.33	**	5355.19	**	27.00	**
Block	2	0.08	ns	7.58	ns	75.23	ns	0.33	ns	3.56	ns	0.25	ns
Source of Variation	Df	TBO		NPP		SW		PI		IP		TBP	
Genotype	111	2.09	**	917.59	**	1.94	**	7.36	**	0.02	**	5.01	**
Check	1	23.80	**	11102.08	**	29.57	**	126.75	**	0.33	**	23.73	**
Block	2	0.01	ns	5.58	ns	0.06	ns	1.45	ns	0.01	ns	0.07	ns
Source of Variation	Df	OS0		OS1		OS2		OS3		OS4		OS5	
Genotype	111	0.04	ns	0.04	ns	0.06	ns	0.07	**	0.07	**	0.07	**
Check	1	0.75	*	0.75	*	2.08	**	3.00	**	3.00	**	3.00	**
Block	2	0.25	ns	0.25	ns	0.08	ns	0.00	ns	0.00	ns	0.00	ns

Df: Degree of freedom. NAF: Number of aborted flowers. NTBP: Number of broomrape plants. NPLP: Number of plants per pot. NPP: Number of pods per plant. OS0 to OS5: Orobanche stages, from stage 0 to stage 5. PI: Parasitism index. OIN: Orobanche incidence. OSV: Orobanche severity. SW: Seed weight. TBO: Total biomass of Orobanche. TBP: Total biomass of lentil plants. IP: Performance index. OEN: Emerged Orobanche number per plant. (ns) $P > 0.05$; (*) $P <= 0.05$; (**) $P <= 0.01$.

Table 2
Means squares and significance levels of the effects of origin and biological status on the studied traits using analysis of variance (ANOVA).

Source of Variation	Df	Mean.Sq											
		NTBP		OEN		NAF		OSV		PI		TBP	
Origin	4	25.41	ns	2.5718	ns	584.1	ns	10.647	ns	14.780	ns	1.272	ns
Biological Status	2	26.20	ns	0.9363	ns	493.8	Ns	3.460	ns	2.670	ns	3.390	ns
Source of Variation	**Df**	**NPLP**		**OIN**		**IP**		**TBO**		**NPP**		**SW**	
Origin	4	1.0499	ns	2064.1	*	0.03647	ns	2.9025	ns	1862	ns	3.853	*
Biological Status	2	1.2869	ns	354.8	ns	0.00071	ns	0.9811	ns	4024	**	9.349	**
Source of Variation	**Df**	**OS0**		**OS1**		**OS2**		**OS3**		**OS4**		**OS5**	
Origin	4	0.05859	ns	0.05859	ns	0.16425	***	0.23772	***	0.23772	***	0.23772	***
Biological Status	2	0.00101	ns	0.00101	ns	0.00101	ns	0.00101	ns	0.00101	ns	0.00101	ns

Df: Degree of freedom. NAF: Number of aborted flowers. NTBP: Number of broomrape plants. NPLP: Number of plants per pot. NPP: Number of pods per plant. OS0 to OS5: Orobanche stages, from stage 0 to stage 5. PI: Parasitism index. OIN: Orobanche incidence. OSV: Orobanche severity. SW: Seed weight. TBO: Total biomass of Orobanche. TBP: Total biomass of lentil plants. IP: Performance index. OEN: Emerged Orobanche number per plant. (ns) P > 0.05; (*) P < = 0.05; (**) P < = 0.01; (***) P < = 0.001.

2. Genotype performance in response to Orobanche infestation: descriptive analysis

The descriptive statistics (Table 3) were made in order to better understand the performance of the tested genotypes across measured traits, and to characterize the impact of Orobanche parasitism on lentil genotypes. The total number of broomrape plants per pot (NTBP) shows substantial variability, with a mean of 8.49 and a standard deviation of 5.62, suggesting heterogeneity in infestation levels, with values ranging from 0 to 25.92. The Orobanche incidence (OIN), representing the percentage of lentil plants with at least one emerged Orobanche shoot, has a high mean of 82.15 and a standard deviation of 26.60, indicating significant infestation levels across genotypes. The number of aborted flowers per plant (NAF) shows also a high variability, with a mean of 32.48 and a standard deviation of 16.43, suggesting significant differences in flowering responses. The number of emerged Orobanche shoots per plant (OEN) averages 3.02 with a standard deviation of 1.20, indicating moderate but varied infestation levels, with some plants free from Orobanche emergence. The parasitism index (PI), combining Orobanche incidence and severity, averages 5.34 with a standard deviation of 2.74, showing moderate variation in parasitism levels among genotypes. The performance index (IP), integrating parasitism index and severity, has a mean of 0.41 and a standard deviation of 0.15, indicating a wide range of performance under Orobanche parasitism conditions. Total biomass of lentil plants (TBP) shows moderate variation with a mean of 5.61 and a standard deviation of 2.20, while total Orobanche biomass (TBO) has a higher degree of variability (maean of 2.79, standard deviation of 1.38), ranging from 0 to 6.13, reflecting the extent of infestation. The seed weight (SW) shows considerable variability, with a mean of 1.24 and a standard deviation of 1.30, probably influenced by the effects of parasitism.

Table 3
Descriptive statistics for Orobanche related traits in lentil genotypes

Trait	Mean	Std.Deviation	Min	Max
NPLP	3.70	0.88	0.92	5.92
NTBP	8.49	5.62	0.00	25.92
OIN	82.15	26.60	0.00	100
OSV	6.17	2.42	0.67	9.17
NAF	32.48	16.43	5.00	100.13
OEN	3.02	1.20	0.00	5.25
PI	5.34	2.74	0.00	9.44
IP	0.41	0.15	0.04	0.68
TBP	5.61	2.20	0.04	12.96
TBO	2.79	1.38	0.00	6.13
NPP	29.86	29.14	0.00	117.67
SW	1.24	1.30	0.00	5.39

NAF: Number of aborted flowers. NTBP: Number of broomrape plants. NPLP: Number of plants per pot. NPP: Number of pods per plant. PI: Parasitism index. OIN: Orobanche incidence. OSV: Orobanche severity. SW: Seed weight. TBO: Total biomass of Orobanche. TBP: Total biomass of lentil plants. IP: Performance index. OEN: Emerged Orobanche number per plant.

3. Classification of lentil genotypes according to their tolerance to Orobanche using performance index (IP)

Based on the Fig. 1, we can see a clear differentiation of the 112 lentil genotypes into four distinct groups, according to their tolerance to Orobanche, measured by the infection parameter (IP). The circular dendrogram (a) reveals a hierarchy where each cluster, colored differently, groups together genotypes with varying levels of tolerance. Cluster 4 (green), with the lowest IP values, groups together the most tolerant genotypes, as also indicated by its density, strongly concentrated towards low values in the distribution graph (b). In contrast, Cluster 2 (blue) shows the highest IP values, representing genotypes more sensitive to Orobanche parasitism as genotype 70 presented in Fig. 6. Clusters 1 (red) and 3 (yellow) occupy intermediate positions: Cluster 1 with moderate tolerance and Cluster 3 with greater variability, as shown by its bimodal density. Boxplot (c) summarizes these differences, confirming the general cluster trend, with a lower dispersion in Cluster 4 for tolerant genotypes and a high dispersion in Cluster 2 for susceptible ones. This stratification of genotypes according to their IP could guide the choice of genotypes for lentil breeding programs aimed at improving tolerance to Orobanche.

4. Phenotypic correlations between traits under Orobanche infestation

Pearson correlation analysis was used to determine relationships between the studied variables (Fig. 2). The results reveal varied relationships between traits, significant and non-significant correlations were identified. The high correlation between OSV and PI (0.86) suggests that the severity of parasitism influences the lentil genotypes performance under Orobanche parasitism, as IP includes elements related to lentil productivity (NPP). The highly significant correlation between PI and IP (0.97) confirms that the performance index of lentil genotypes under Orobanche parasitism is strongly correlated with the level of parasitism in general, indicating that IP integrates well the effects of parasitism in its measure of performance. The significant correlations between OIN and IP (0.70) and between OIN and PI (0.72) indicate that Orobanche incidence is related to parasitism severity (PI) and lentil performance (IP). A high incidence of Orobanche is therefore associated with higher parasitism and reduced lentil performance. The correlation of 0.74 between OIN and OEN shows that the presence of Orobanche increases proportionally with the number of emerged Orobanche shoots per lentil plant. This is logical, since a higher incidence means that more plants are affected, with an increased number of emerged Orobanche shoots. The high correlation between SW and NPP (0.96) indicates that the number of pods is a key factor in seed production. Indeed, the more pods a plant produces, the higher its total seed weight, reflecting higher productivity under parasitism conditions. Similarly, the positive correlations between SW and TBP (0.78), and TBP and NPP (0.76) show that total lentil biomass (TBP) is positively associated with pod number and, eventually, seed weight. This indicates that lentil plants with higher biomass tend to produce more pods and higher seed weight, which is an indicator of tolerance to parasitism.

5. Principal Component Analysis of Lentil tolerance and Productivity Traits under Orobanche Parasitism

Principal Component Analysis (PCA) is performed to evaluate Orobanche parasitism tolerance traits in lentils, the first two dimensions together explain 70.53% of the total variance, reflecting strong information extraction (Fig. 3). The first dimension (58.12% of variance) is mainly associated with indicators of parasitism severity, notably the parasitism index (PI), performance index (PI), and Orobanche severity (OSV). These variables were highly correlated with this dimension (PI: 0.96, IP: 0.88, OSV: 0.86), indicating that this component represents the total negative effect of Orobanche on lentil performance. Furthermore, negative correlations with seed weight (SW: -0.80) and number of pods per plant (NPP: -0.83) suggest that higher levels of parasitism are associated with reduced plant productivity. The second dimension (12.41% of variance) represents a complementary axis, integrating variables related to biomass and number of infested plants. This component is influenced mainly by the total biomass of lentil plants (TBP: 0.52) and the total number of Orobanche shoots per pot (NTBP: 0.48), underlining the importance of plant biomass in the plants' response to parasitism. These associations suggest that, despite parasitism having a direct negative effect on biomass production, some plants show a capacity for growth despite the presence of Orobanche. This could indicate a compensatory response whereby some plants maintain a higher biomass, even under conditions of parasitism, potentially via mechanisms of tolerance or resource allocation.

6. Selection of performant genotypes in response to Orobanche tolerance using MGIDI

MGIDI analysis was carried out to select the performant genotypes according to Orobanche tolerance. This analysis focused on two pertinent traits, which related to Orobanche paratitisme (IP) and yield (NPP). In addition, a selection intensity of 15% was applied, facilitating the selection of genotypes characterized by low performance index and high number of pods per plant. The MGIDI analysis resulted in the selection of 17 performant genotypes (Fig. 4a and b), including the genotype (check 2) as tolerant control. Furthermore, among the selected genotypes, 15 originate from Morocco including 4 advanced lines, 10 landraces and 1 cultivar, and 1 landraces genotype from Italy. It should be noted that all genotypes selected by MGIDI based on parasitism index and number of pods per plant (Fig. 4), are identified and selected also using performance index only in the cluster 4 (Fig. 1).

Discussion

The present investigation aimed to explore the performance of diverse lentil genotypes from a Mediterranean germplasm collection under Orobanche infestation. Significant variations were revealed within the tested germplasm collection. The Orobanche parasitism affect significantly lentil growth and productivity, this is well showed in our results by high parasitism index, severity and performance index, and by low number of pods and decreased seed weight. In contrast to our results, (En-nahli et al. 2023) although using a large lentil germplasm collection under high *O. crenata*-infested field conditions, but they have reported that no significant variation according to parasitism index, severity, incidence and seed yield has been showed. However, this can be attributed to limited genetic differences within their germplasm collection, or to experimental conditions. In fact, their study was conducted in a naturally infested field, which may have resulted in a less homogeneous infestation and a less pronounced response from accessions. In contrast, in our study, we used controlled greenhouse conditions by precisely introducing Orobanche seeds into each pot, thus creating a more uniform and severe infestation. This approach enables us to better isolate the genetic effect and detect more significant genetic variation among accessions, which explains why we observed significant genetic differences for these variables.

The high parasitism index, performance index and severity observed in many accessions largely explain why these accessions have a low number of pods and reduced seed weight. This, in turn, has a negative impact on plant biomass, slowing growth and eventually reducing the productivity. These results are in agreement with those observed on lentil (En-nahli et al. 2021, 2023; Mbasani-Mansi et al. 2019b; Yolcu et al. 2020), and on others food legumes crops as chickpea (*Cicer arietinum* L.) (Nefzi et al. 2016; Rubiales et al. 2003), faba bean (*Vicia faba* L.) (Negewo et al. 2022; Pérez-de-Luque et al. 2010; Rubiales et al. 2014), pea (*Pisum sativum*) (Fernández-Aparicio et al. 2016; Fernández-Aparicio and Rubiales 2012; Rubiales et al. 2020), and grass pea (*Lathyrus sativus* L.) (Abdallah et al. 2021).

The genetic diversity according to Orobanche tolerance observed in this study, namely the high significant variation for the parasitism index, performance index, severity, pods number and seeds weight, highlight the crucial role of the genetic resources in the Orobanche tolerance. These results reveal that some accessions have specific mechanisms of tolerance or escape to Orobanche, via limiting the synthesis of chemical stimuli including strigolactones (SLs) which are the most active stimulants inducing Orobanche seeds germination (Trabelsi et al. 2017; Xie et al. 2010). This genetic variability is an important opportunity for the lentil breeding programs enabling to select and integrate the traits of Orobanche tolerance in the commercialized lentil varieties. The structuration of the distinct group of genotypes identified by hierarchical cluster analysis, reveal a significant genetic variability, highlighting the response of the genotypes to Orobanche. (Pérez De La Vega et al. 2022) found that the tolerance of biotic stresses is associated with specific genetic groups for legumes. In particular, the cluster 4 genotypes, which demonstrate a certain homogeneity in their tolerance to Orobanche, seem to share some common genetic traits favoring this ability. This perhaps related to specific tolerance loci or converging physiological mechanisms.

The Orobanche development stages for the tested lentil genotypes (Fig. 5), from Orobanche seed germination and tubercle formation (OS1) to Orobanche shoot emergence and seeds formation (OS5), reveal a contrasted response, highlighting a significant variability within the tested lentil germplasm. This heterogeneity suggests the existence of distinct tolerance or inhibition mechanisms. For example, some lentil accessions show a remarkable inhibition of Orobanche seeds germination (OSO) (Fig. 6 (b-d)), probably due to the low production of strigolactones. Other genotypes, while promoting initial Orobanche seed attachment, germination and tuber formation (OS1 – OS2), they limited shoot formation and emergence, which could indicate post-attachment resistance mechanisms, such as structural or biochemical barriers at the plant-host boundary. Similar results were observed for pea (*Pisum sativum*) (Pérez-De-Luque et al. 2005), for (*Vicia faba* L.) (Sillero et al. 2005), and for lentil (*Lens culinaris* Medik) (Fernández-Aparicio et al. 2008) Plants showing high parasitism (Fig. 6 (a-c)), severity and performance index, despite reaching the OS5 stage (Orobanche seed maturation), appear to have low intrinsic resistance. This may be due to the high production of strigolactones that promote Orobanche germination, the absence of inhibitory biochemical mechanisms, or an easily colonized root system. These observations confirm the idea that tolerance to Orobanche is not a single phenomenon, but relies on a combination of physiological and complex genetic processes.

Conclusion

The results of this study reveal a genetic variability for the Orobanche tolerance, according to parasitism index, performance index, and severity. The performance index, integrating the parasitism index and the number of pods per plant, has proved an effective parameter for selecting genotypes combining a higher tolerance to Orobanche and high yield. The MGIDI analysis enable us to identify 17 genotypes presenting a high Orobanche tolerance, these genotypes can be used in lentil breeding programs. These results open the way for the selection of lentil genotypes more resistant to Orobanche for sustainable management programs against this parasite.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

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Figures

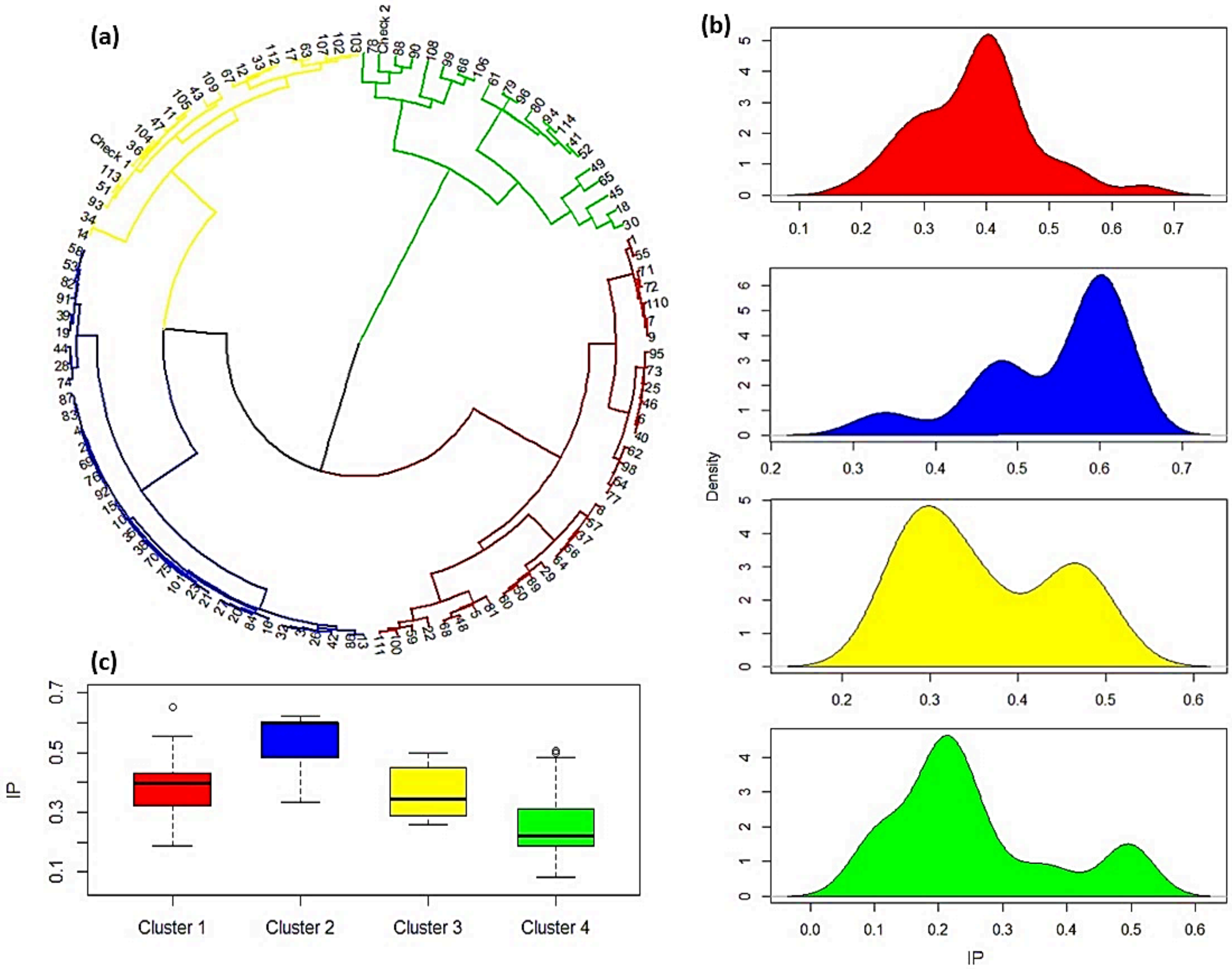

Figure 1

(a) Hierarchical clustering, (b) density plot, and (c) boxplots based on performance index (IP) for lentil-tested genotypes

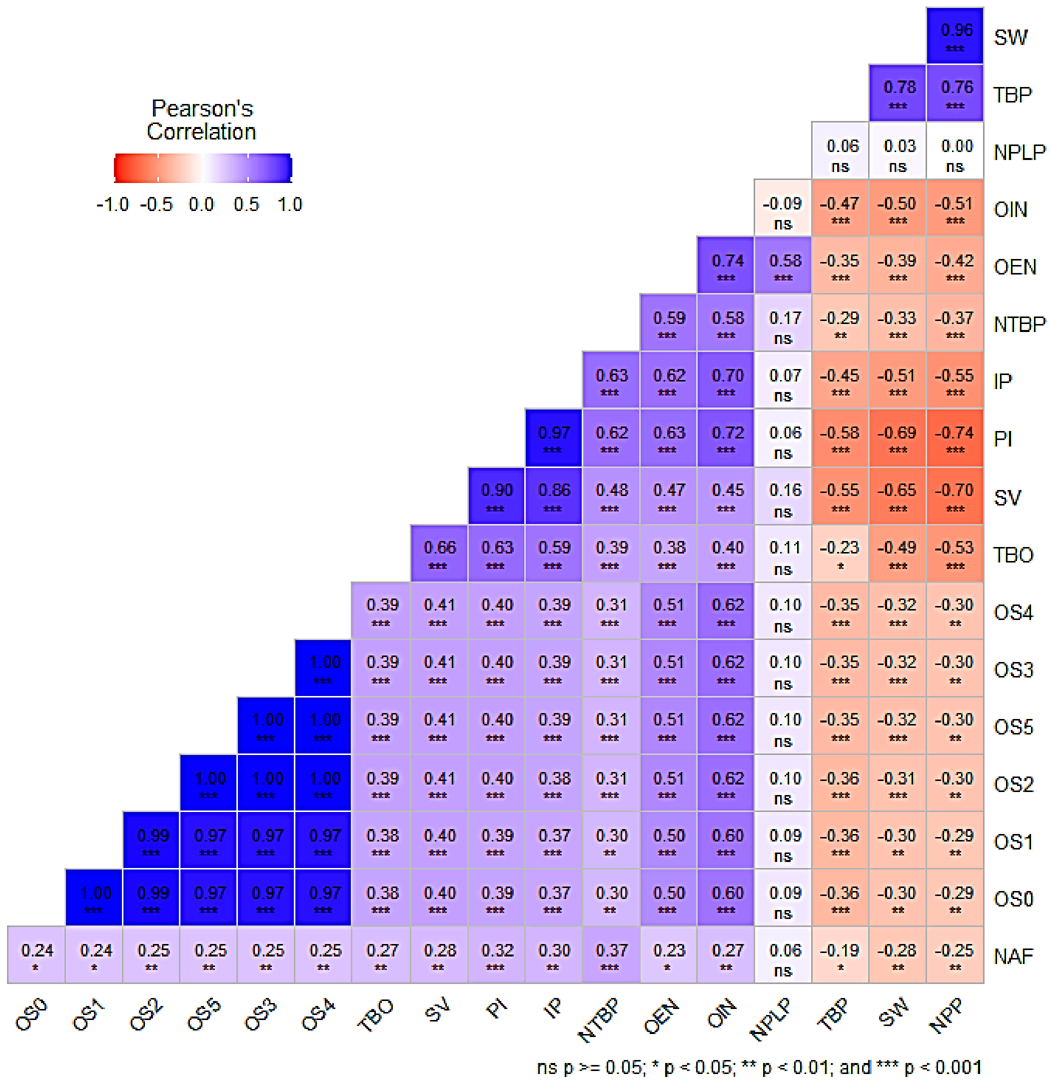

Figure 2

Pearson’s correlation matrix of agronomic traits and Orobanche parasitism index in lentil genotypes. NAF: Number of aborted flowers. NTBP: Number of broomrape plants. NPLP: Number of plants per pot. NPP: Number of pods per plant. OS0 to OS5: Orobanche stages, from stage 0 to stage 5. PI: Parasitism index. OIN: Orobanche incidence. OSV: Orobanche severity. SW: Seed weight. TBO: Total biomass of Orobanche. TBP: Total biomass of lentil plants. IP: Performance index. OEN: Emerged Orobanche number per plant.

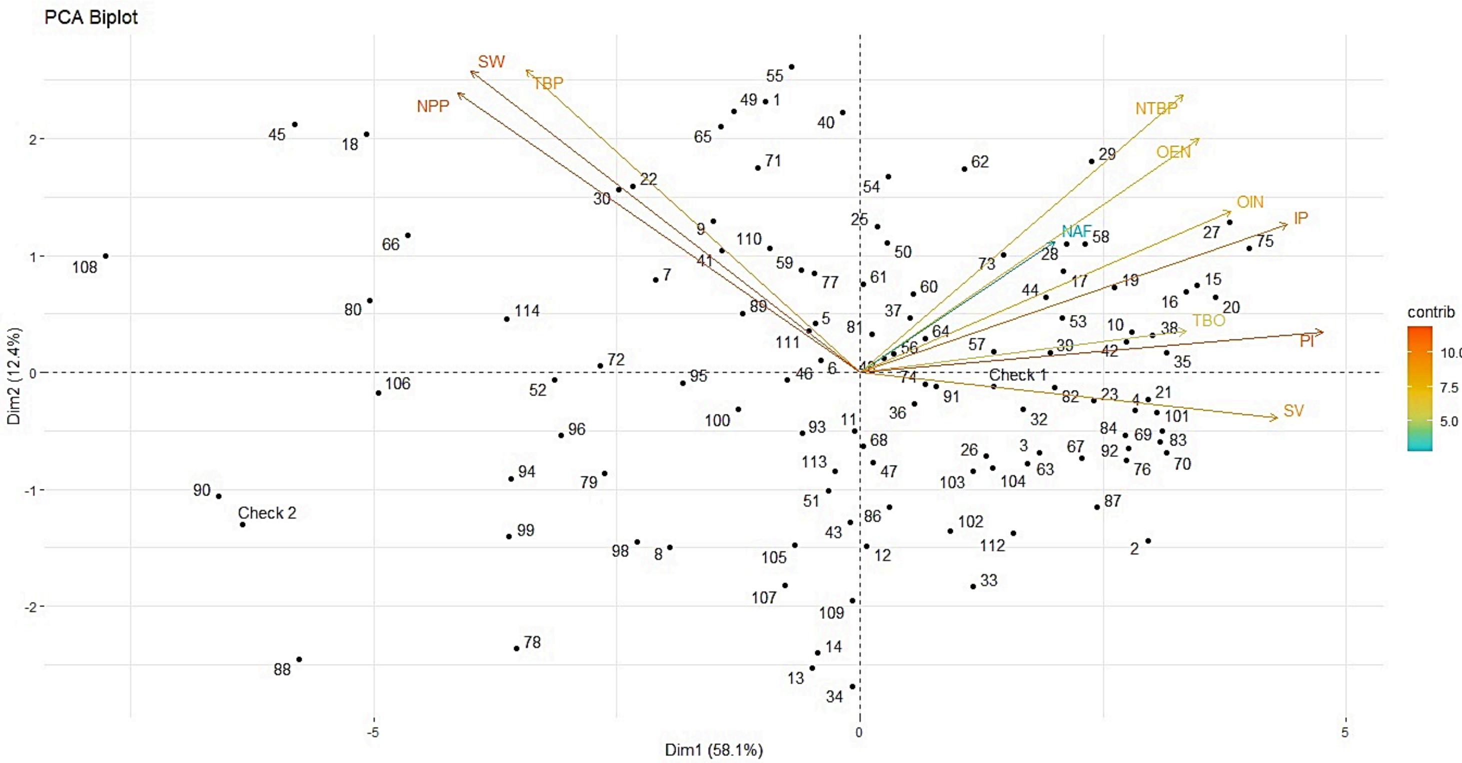

Figure 3

Principal component analysis (PCA) biplot of Orobanche-tolerant lentil genotypes based on all measured traits. NAF: Number of aborted flowers. NTBP: Number of broomrape plants. NPLP: Number of plants per pot. NPP: Number of pods per plant. PI: Parasitism index. OIN: Orobanche incidence. OSV: Orobanche severity. SW: Seed weight. TBO: Total biomass of Orobanche. TBP: Total biomass of lentil plants. IP: Performance index. OEN: Emerged Orobanche number per plant.

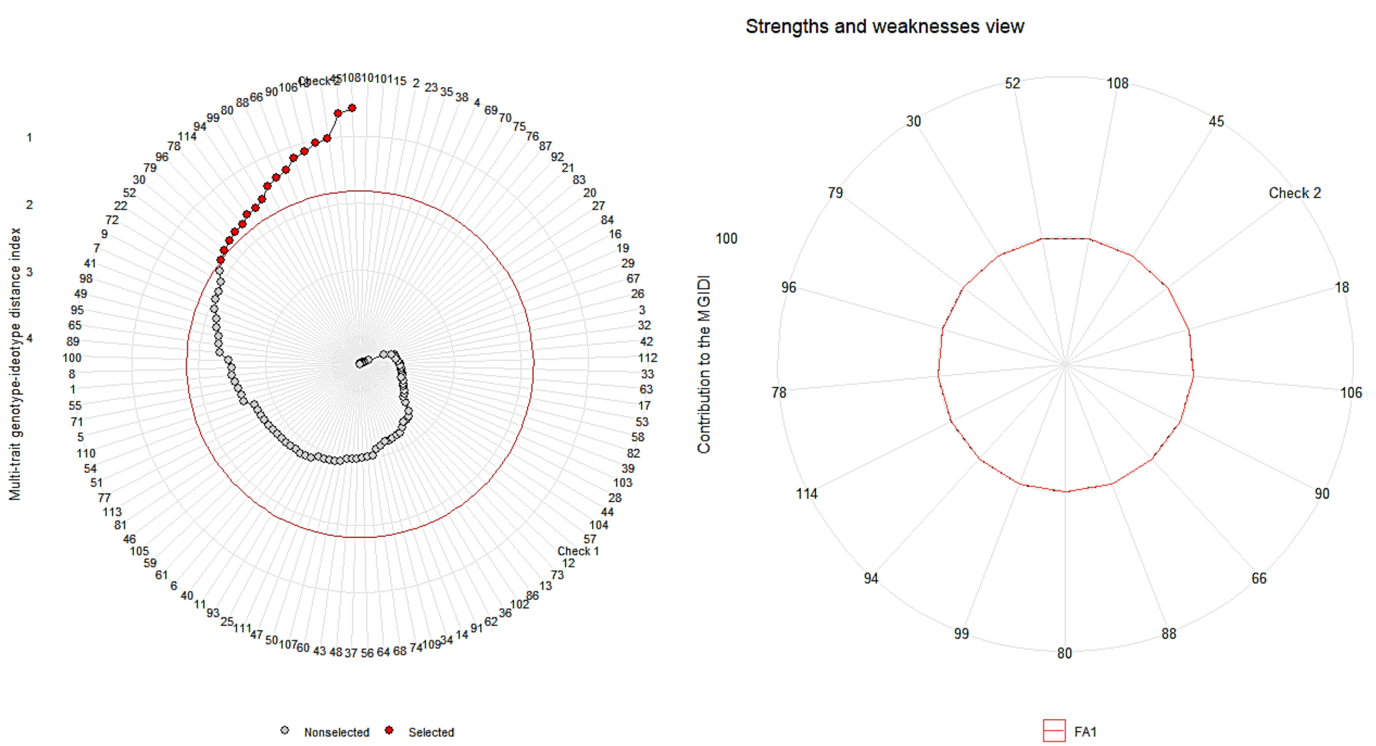

Figure 4

Evaluation of strengths and weaknesses of Orobanche-tolerant genotypes and ranking of genotypes based on MGIDI, using parasitism index (PI) and number of pods per plant (NPP). FA1 Factor analysis 1

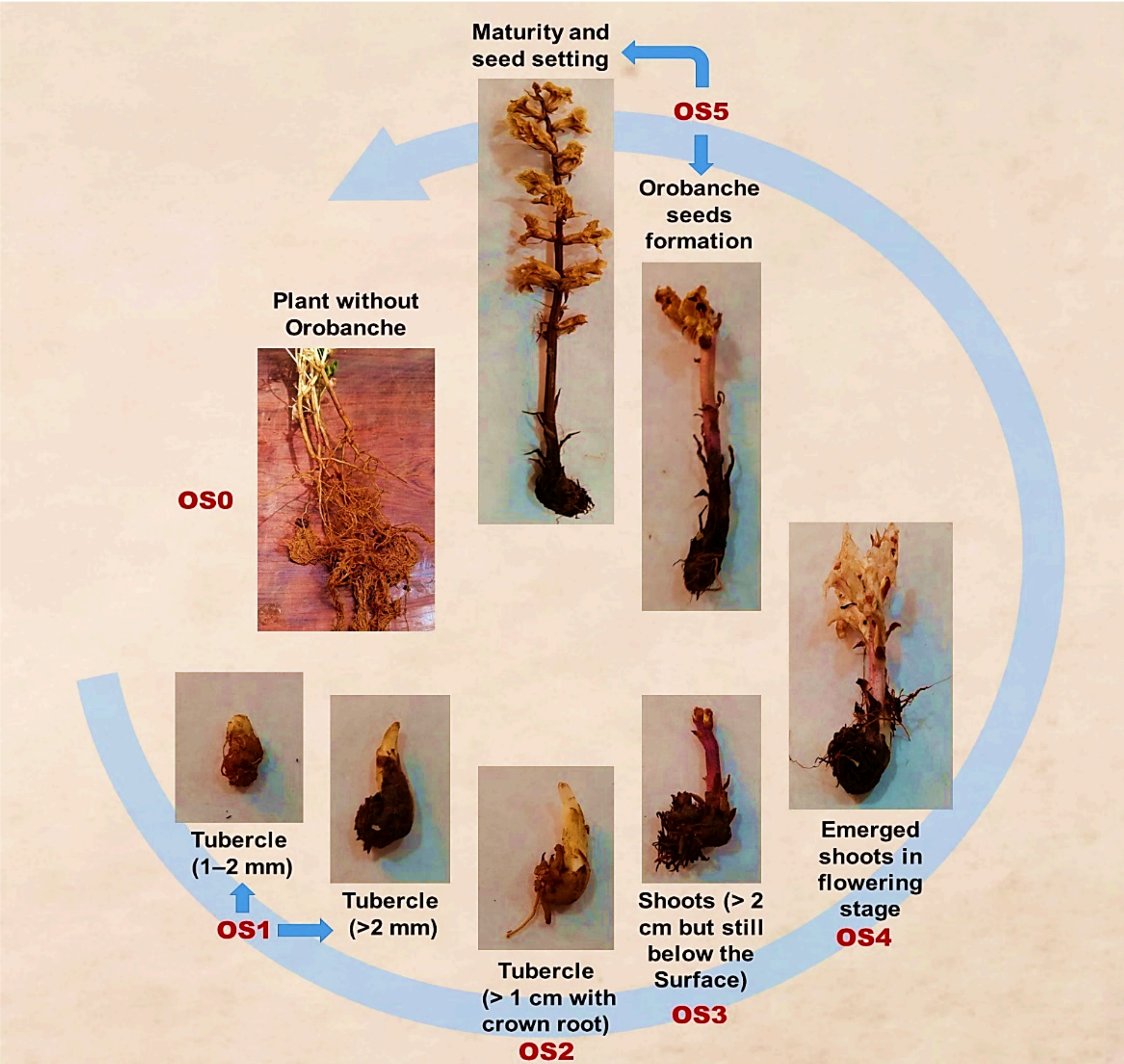

Figure 5

Visual representation of Orobanche development stages from OS0 to OS5 in lentil genotypes

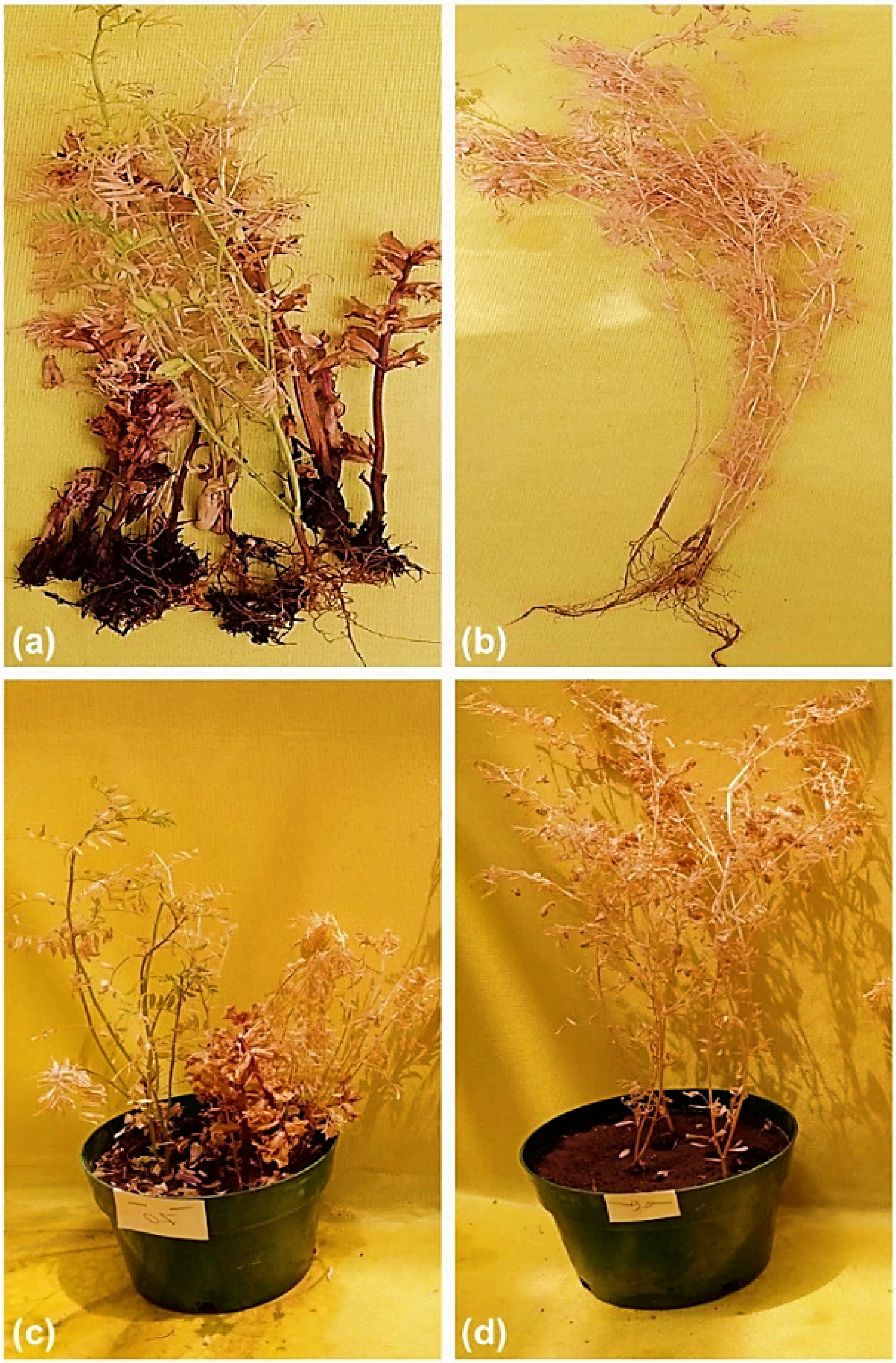

Figure 6

Comparison of Orobanche emergence in tolerant (b,d) and sensitive (a,c) lentil genotypes