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Resistance to *Orobanche crenata* Forsk. in lentil (*Lens culinaris* Medik.): exploring some potential altered physiological and biochemical defense mechanisms

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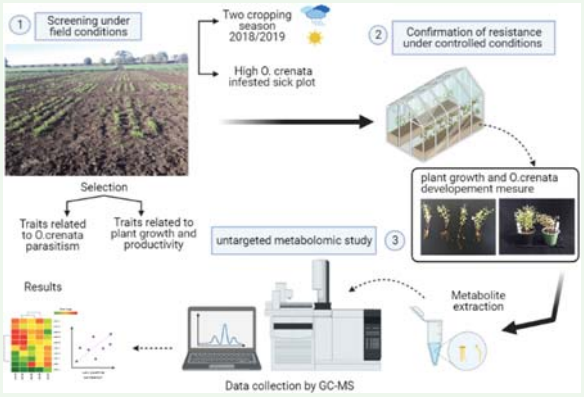
ABSTRACT

Management of broomrape (*Orobanche crenata* Fosk.) that causes important damages on lentil production becomes a veritable concern in the Mediterranean region. Eighty lentil accessions were evaluated for resistance to *O. crenata* under field and controlled conditions. Both genotypes ILL6415 and ILL7723 expressed the highest resistance level under field and pot experiment with low *Orobanche* infestation and relatively high seed yield (50.1 g m^{-2}). Such resistance was associated with physiological and biochemical changes in metabolites profiling. In total, 109 and 115 metabolites were identified in the lipophilic phase of both ILL6415 and ILL7723, respectively, against only 92 metabolites recorded for susceptible check Zaaria. Significant differences were observed in metabolite concentrations (fatty acids, sterols alkanes) between roots and shoots of susceptible and resistant infested plants. Accumulation of α-linolenic acid and arachidic acid was more pronounced in the resistant genotypes ILL6415, ILL7723 which could be associated with resistance pathways involved in the resistance to *O. crenata*.



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Orobanche crenata; *Lens culinaris* Medik; resistance; metabolomics

1. Introduction

Lentil (*Lens culinaris* Medik.) is the third-most important cool-season grain legume in the world after chickpea and pea. It is widely grown as a rainfed crop on 3.85 million hectares area and accounted for 6% of the total global pulses production with 3.59 million tonnes and average yield of 0.93 tn ha^{-1} (Sehgal et al. 2021). The major production regions are South Asia and China (44.3%), North America (41%), Central and West Asia and North Africa – CWANA (6.7%), Sub-Saharan Africa (3.5%) and Australia (2.5%) (Kumar et al. 2013). Lentil has been cultivated for more than 10,000 years for its important agronomic and socioeconomic roles worldwide (Erskine et al. 2011). With high protein level and an important amount of carbohydrates, fibers, minerals and antioxidant compounds, lentil is considered as one of the most nutritious legume crop (Singh and Singh 2014). It is an important staple food crop particularly among the poor populations and smallholder farmers (Çarman 1996). As other legumes crops, lentil is considered as a pivotal component for sustainable agriculture due to its ability to fix nitrogen in the soil, which makes practical to use it in rotation with cereals (Shah et al. 2003). Morocco is the second major producer of lentil in Africa with more than 40,000 ha cultivated area (Taha et al. 2018), and total annual production of 30,670 tons (Idrissi et al. 2020). It account about 14% of the total legume cultivated area in the country. In Morocco, Lentil is mainly cultivated under rainfed conditions in the dry areas where drought is the major abiotic constraints limiting the crop production and productivity due to low and irregular rainfall (Idrissi et al. 2020). Attack by the parasitic weed *O. crenata* seems to be the most important biotic stress limiting the

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production and the development of the crop especialy in Zaair region which is the most important production area in the country. In fact, *O. crenata* is an holoparasitic plants completely dependent on the host for its nutritional requirements. Its is considered as a serious threat that causes important damages and yield losses on many legume crops in the Mediterranean region and Sub-Sahan Africa (Amri et al. 2021). During the last decades, attack by this parasite has burden lentil production and productivity in Morocco (Kumar et al. 2015; Abu-Irmaileh and Labrada 2017; Idrissi et al. 2020). The continuous spread of this threat and important damages that causes on lentil and other host crops often force farmers to give up growing these crops (Amri et al. 2019). Recent assessment conducted in Morocco reported 51% estimated infested legumes cultivated areas with an average yield loss of 30–40% (Abu-Irmaileh and Labrada 2017).

Several control methods have been tested but none of them resulted in complete and successful control of the parasite (Abbes et al. 2019). Breeding for resistance is considered as the most economically feasible and environment friendly control method (Amri et al. 2019). This strategy has shown promising success and many resistance sources were identified in faba bean, Chickpea, sunflower and tomato (Kharrat et al. 2010; Nefzi et al. 2016; Amri et al. 2019; Bai et al. 2020; Cvejić et al. 2020). However, breeding for resistance to broomrape remains complicated because of the limited source of the resistance and the low heritability of the genes and QTLs associated with that resistance (Pérez-De-Luque et al. 2005; Amri et al. 2021). Indeed, a better understanding and development of solid knowledge about the resistance mechanisms involved and the interaction between the host and the parasite will help to improve the resistance level and develop new breeding material (Pérez-De-Luque et al. 2007; Trabelsi et al. 2017; Abbes et al. 2020). Lentil is poorly competitive with *O. crenata*, which can cause complete yield loss under high infested conditions. Unfortunately, studies on lentil resistance to *O. crenata* and the interaction between host and parasite are still insufficient. A recent scientific report showed that the metabolomics profile of the parasite is different from that of the host, which means that the parasite may have a self-regulating in metabolism (Amir 2016; Clermont et al. 2019). Some other studies reported that environmental stresses cause changes in primary and secondary plant metabolisms which depend on plant resistance strategies (Hasanuzzaman et al. 2013).

In this study, we aimed to evaluate the response of a lentil germplasm collection to *O. crenata* parasitism under field and controlled conditions and investigate the potential metabolic differences between identified susceptible and resistant genotypes.

2. Materiel and methods

2.1. Plant material and field trials

In total, 80 lentil genotypes were subjected to field evaluation and screening for resistance to *O. crenata* (Table 1). Germplasm was provided by the International Center of Agricultural Research in dry areas (ICARDA) genebank. The trial was conducted during two consecutive cropping seasons 2017/2019 in a high *O. crenata* infested sick plot at ICARDA Merchouch research station – Morocco. Different genotypes were planted end of November according to an alpha lattice design with two replications. For each genotype, 30 seeds were planted in 1 m row with 30 cm inter-row spacing. The local cv. Bakria which is reported to be moderately susceptible to *O. crenata* was used as check. Hand weeding was done when necessary and neither herbicide nor fertilizer were applied. The following parameters were recorded before and at crop maturity, Number of days to flowering (D2F), days to *Orobanche* emergence (D2OE), *Orobanche* incidence (OIN), *Orobanche* severity (OSV), Emerged *Orobanche* number per plant (EON), Emerged *Orobanche* dry weight per plant (EODW), biological yield g m^{-2} (BY) and seed yield g m^{-2} (SY), the harvest index (HI) and parasitism index (PI). The PI was calculated according to the following formula:

$$PI = OIN*OSV/100$$

OIN: Percentage of lentil plants showing at least one emerged shoots of orobanche per row.
OSV: level of damage (1–9 scale) caused by the parasite on lentil growth and seed production (Abbes et al. 2007).

2.2. Pot experiment

Out of the 80 tested genotypes, five lines selected for their resistance to *O. crenata* were subjected to a confirmation pot experiment under controlled conditions with two *Orobanche* treatments (infested and non-infested). The five selected lines all with two released varieties (Bakria and Zaaria) were planted in 2 l pots. Infested pots were inoculated with *O. crenata* seeds (collected during previous seasons on faba bean plants) at a density of 20 mg.kg^{-1} soil. Four replications were considered for each genotype/treatment and planting was performed mid-November with 3–4 seeds per pot. Pots were watered when necessary to keep plants at good soil moisture. After emergence, the number of lentil plants was reduced to only one plant per pot. At the end of the experiment, the 56 days aged plants were uprooted from pots. The host root system all with *O. crenata* attachments were washed carefully and the following parameters were determined. *O. crenata* attachments were counted and classified according to their development stage (Abbes et al. 2011) to underground/non-emerged *Orobanche* tubercles (NEO) and emerged *Orobanche* shoots (EON) per plant. Lentil shoot (SDW) and root (RDW) dry weight were also determined for the same plants.

Table 1. Lentil genotypes used to identify the resistance levels under field conditions during cropping seasons 2017–2018 and 2018/2019.

Region	Country of origin/Entry IG number
Americas	MEX (5645-ILL351, 502), ARG (4605, 268), CAN (4738), CHL (468, 361), URY (4778), ICA (10952, 10912),
Europe	ESP (4926, 5628-ILL915, 5653), FRA (6528), ITA (5418) DEU (4881), POL (705), UKR (82), UNK (4345, 6415), RUS (4830, 597, 4819), CZE (4409), YUG (2230-ILL624), GRC (304), HRV (4915),
North and Sub-Saharan Africa	ETH (5639-ILL207), DZA (4781), MAR (7726, Chakkouf, Bakria, Zaaria), SDN (1861),
Asia	IND (4164), IRN (223, 257), AFG (213), JOR (5384, 5562, 5244), LBN (5626-ILL840, 840, 191), PSE (4606), NPL (3485, 3487), PAK (6350), SYR (6870, 6848, 490), TUR (590, 71), ARM (619),
Others	LIRL-22-107, LIRL-21-187, 6002, 6021, 8009, 9951, 88527, 6025, LIRL-22-109, 9850, 8090, 7213, LC960254, 8195, 7982, 7984, 7685, 7701, 6783, 7668, 8622, 7934, 6015, 8068, 7723, 7532

2.3. Metabolites analyses

Untargeted metabolomics profiling was performed using gas chromatography–mass spectrometry (GC–MS). The method used in this study for organic extraction and transesterification from shoots and roots of both infested and non-infested plant is sited by (Mutale-joan et al. 2020) with some modifications. Samples were grinded in liquid nitrogen then 300 mg from each sample dissolved in 4 mL chloroform/methanol mixture (2/1, v/v). 10 µL of internal standard Dodecane (Sigmaaldrich; Density, 0.75 g/mL) were added into the mixture, which were placed to a heat block (Labet International, Edison, USA) pre-set at 85°C and left for 2 h. After the mixture was placed to an ultra-sound bath (Branson ultrasonic Sonifier 450, Danbury, USA), the sonication was carried for 60 min. One milliliter of H_2O was added to the vials and the mixtures were thoroughly vortexed. Then the organic phase was transferred into a new vial and the chloroform was evaporated under a stream of nitrogen gas. For acid transesterification, 500 µL of methanol/sulfuric acid (6%, v/v) was added to dried organic material, then the mixture was heated, sonicated and dried as described above. 750 µL of chloroform and 250 µL of distilled water were added for phase separation. The organic phase was collected and conserved at −20°C for further analysis. The gas chromatography (GC) (Agilent 7890A Series) coupled to mass spectrometry (MS) was used for the identification of apolaire compounds. A volume of 4 µL of each tested samples were injected into the 123-BD11 column (15 m × 320 µm × 0.1 µm) by 1/4 split mode using helium as carrier gas at 3 $mL.min^{-1}$. Briefly, the temperature was set at 230 and 150°C in the ion source and the MS transfert line, respectively. The oven temperature was set to started at 30°C and to reach 360°C at the end. The identification of metabolite was carried by the comparison of their mass spectra (MS) with NIST 2014 MS Library.

2.4. Statistical analysis

Statistical analyses were done using SPSS software and R script. Analysis of variance (ANOVA) for field data was done considering a linear mixed model with blocks and repetitions as random factors and seasons and genotypes as fixed factors. Person correlation coefficients between all morphological traits were computed and tested for their statistical significance using prcomp function and Mix-Omics package. PCA and heatmap was generated using R studio, visualization of corrplot and ggplot packages, integrated into the R sofware. One way anova and Duncan's test was studied using SPSS software.

3. Results

3.1. Field screening for resistance to O. crenata in lentil

Results showed a high variability within the lentil germplasm collection in the response to *O. crenata* parasitism. Field screening and evaluation showed significant differences ($P \leq 0.05$) between the tested genotypes for D2F, D2OE, PI, BY, SY and HI. No significant differences were recorded for EON and EODW. The cropping season showed significant effect on D2F, D2OE, EON, EODW, SY and HI and no significant effect on PI and BY. Except for PI, the interaction genotype*cropping season was significant for all other parameters (Table 2). Out of the 80 tested accessions, only five accessions showed a good resistance level to *O. crenata*.

The correlation matrix (Figure 1) shows that traits related to lentil productivity such as SY and HI were negatively correlated with traits related to *O. crenata* infestation level D2OE ($r = -0.20^{**}$), PI ($r = -0.37^{**}$) and EON ($r = -0.34^{**}$). Interestingly negative correlations were found also between SY and HI ($r = -0.42^{**}$) and D2F ($r = -0.56^{**}$) (Figure 1). However, a high positive correlation was found between D2F and D2OE, D2OE and EODW, SY and BY, BY and D2OE, EON and PI (Correlation coefficients varied between 0.17 and 0.68, $p \leq 0.001$). The correlations between different traits were confirmed at a higher dimension using PCA which was used to select the best resistant genotypes. Five genotypes (ILL6415, ILL1861, LIRL21187, ILL7723, and ILL4830) were identified with a good level of resistance to *O. crenata* under field conditions.

PCA revealed that the first three principal components (PC) explained 81% of the total original variation. The first PC, which explained 38.8% of the total variation, showed negative correlation with PI ($r = -0.63$) and positive correlation with BY ($r = 0.57$), SY ($r = 0.89$) and HI ($r = 0.85$). This PC corresponds to the list of genotypes with, high PI and low BY and SY such as ILL5645 and ILL82. The second PC, which described 24.6% of the total variability, is characterized with a positive correlation with EON ($r = 0.76$) and EODW ($r = 0.76$) and opposed the genotypes with high EON and EODW such as ILL5418 and ILL705 to those with low number such as ILL6415 and ILL7723. The third PC explained 17.6% of the total data variability and it is positively correlated with D2F ($r = 0.62$) and EODW ($r = 0.64$) which corresponds to genotypes with high D2F and D2OE (Figure 2).

A cluster analysis, using all collected data was performed to cluster the different studied genotypes based on their resistance level. Four groups were identified (Figure 2). The first cluster contains 46 genotypes that presented a high susceptibility to *O. crenata* with high PI values ranging from 5.53 to 9 and low HI and seed production levels (SY ≤ 36.5 g m^{-2}). The second cluster with only six genotypes, showed a moderate susceptibility to *O. crenata* expressed by high D2OE, a PI varying from 6.9 to 8.75, moderate emerged *Orobanche* number and dry weight. The level of seed production for the genotypes in this cluster varied from 6.22 to 39.1 g m^{-2} with a maximum HI of 0.12. Cluster three consisted of 22 genotypes showing intermediate values for the remaining morphological traits. Only six genotypes

Table 2. Analysis of variance of morphological traits of lentil genotypes under *O.crenata* infestation conditions.

	D2F	D2OE	PI	EON	EODW	BY	SY	HI
Genotypes (G)	19.9**	3.2**	1.9**	1.2^{ns}	1.1^{ns}	1.4*	2.3**	2.6**
Cropping Season (CS)	197.5**	386.5**	0.8^{ns}	103.8**	60.3**	0^{ns}	10.31**	69.1**
G*CS	6**	2.5**	0.8^{ns}	1.67**	1.4*	1.5**	2**	2.3**

D2F: Days to flowering, D2OE: Day to Orobanche emergence, IP: Parasitism index, EON: Emerged Orobanche number, EODW: Emerged Orobanche dry weight, BY: Biological yield, SY: Seed yield, HI: Harvest index;** significant at the 0.01 level;* significant at the 0.05 level, ^{ns}not significant.

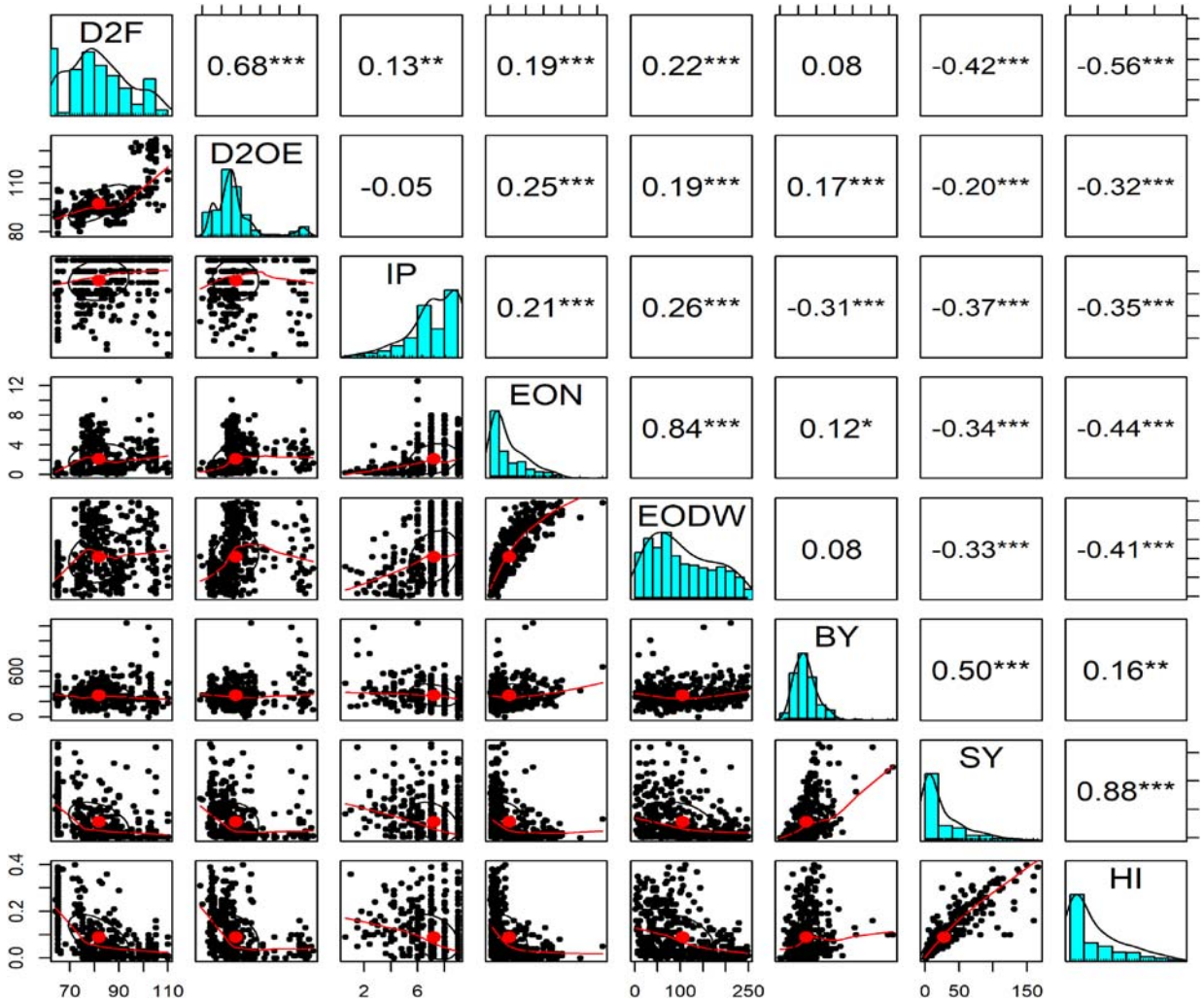

Figure 1. Correlation analysis among morphological parameters of lentil in response to *O.crenata* infection under field conditions during the cropping seasons 2017–2018 and 2018–2019. **D2F:** Days to flowering; **D2OE:** Days to *Orobanche* emergence; **IP**: Parasitism index; **EON:** Emerged *Orobanche* number; **EODW:** Emerged *Orobanche* dry weight; **BY:** Biological yield; **SY:** Seed yield; **HI**: Harvest index. ***Correlation is significant at the 0.001 level; ** Correlation is significant at the 0.01 level; * Correlation is significant at the 0.05 level.

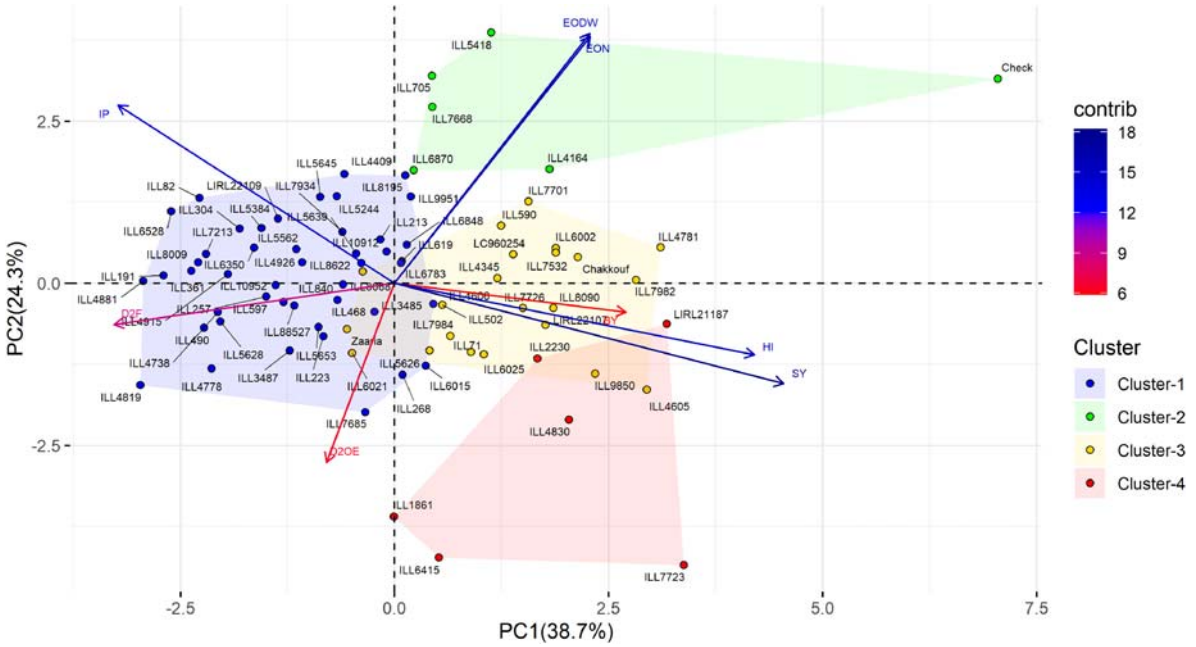

Figure 2. Biplot of the first two dimensions of the principal component analysis (PCA) for the 80 genotypes based on their morphological traits under *O. creneta* infection in field condition. **D2F:** Days to flowering; **D2OE:** Days to *Orobanche* emergence; **IP:** Parasitism index; **EON:** Emerged *Orobanche* number; **EODW:** Emerged *Orobanche* dry weight; **BY:** Biological yield; **SY:** Seed yield; **HI:** Harvest index; **contrib:** variables contribution to the PCA Cluster analysis based on Euclidean distance using the Ward method was performed to cluster the different studied genotypes based on their resistance level.

Table 3. Mean, maximum and minimum of morphological traits of the four clusters grouping the 80 lentil genotypes under *O. crenata* infection.

	Cluster 1			Cluster 2			Cluster 3			Cluster 4		
	Min	max	mean	Min	max	mean	Min	max	mean	Min	Max	mean
D2F	80.7	103.0	89.9	72.4	211.0	73.7	73.0	92.0	83.2	85.0	101.0	90.9
D2OE	92.0	113.3	100.2	90.5	105.3	93.2	87.5	170.8	97.2	101.0	117	107.2
IP	5.5	9	8.1	6.9	8.5	7.0	5.1	8.0	7.3	2.8	5.9	5.1
EON	0.1	3.8	1.6	1.6	6.1	2.6	0.2	2.8	1.8	0.4	3.07	1.5
EODW	0.1	2.41	1.1	1.4	3.2	1.6	0.3	2.1	1.2	0.2	1.84	1.0
BY ($g.m^{-2}$)	98.2	431.3	243.6	211	452.5	294.6	170.8	532.3	250.4	365.8	723.7	396.3
SY ($g.m^{-2}$)	0.7	36.5	10.2	6.2	39.1	34.3	7.7	64	32.0	30.4	103.8	46.7
HI	0.0	0.1	0.03	0.02	0.1	0.04	0.1	0.2	0.1	0.1	0.2	0.1

D2F: Days to flowering, D2OE: Day to *Orobanche* emergence, IP: Parasitism index, EON: Emerged *Orobanche* number, EODW: Emerged *Orobanche* dry weight, BY: Biological yield, SY: Seed yield, HI: Harvest index

with the highest resistance level were grouped in the fourth cluster. This group was characterized by relatively the lowest PI ranging from 2.77 and 5.87. The best recorded SY were observed for the genotypes of this cluster such as ILL7723, ILL4615, ILL1861and LIRL21187. Genotypes from cluster four showed the best resistance level against *O. crenata* infectation and relatively high seed production (Table 3).

3.2. Pot experiment and confirmation of the resistance under controlled conditions

Five genotypes from cluster 4, ILL1861, ILL4830, ILL4615, ILL7723 and LIRL21187, were selected to be subjected to a confirmation experiment and evaluation of the impact of the parasite on host development under controlled conditions. Result showed a highly significant difference between the genotypes ($p < 0.001$) under both infested and free *Orobanche* conditions. Compared to non-infested control plants, biomass production (shoots and roots) was significantly decreased by *O. crenata* for all the tested genotypes except ILL6415 (Figure 3(A)).

However, no significant differences were observed between the studied genotypes for the average number of TON ($p > 0.05$) that varied from 3.5 to 7.2, and the NEO ($p > 0.05$) wish varied from 3.2 to 5.9. Only EON showed significant differences between genotypes ($p \leq 0.05$) wish varied from 0.4 to 0.7 (Table 4).

Significant differences were observed between tested genotypes under both treatments (infested and non-infested) ($p < 0.001$) for the SDW (Table 5). Also, results showed that *O. crenata* significantly affected the RDW ($p \leq 0.05$). The genotypes*treatment interaction was highly significant for SDW ($p < 0.001$) and not significant for RDW ($p > 0.05$). Compared to non infested plants, an important decrease of SDW (90%) was observed for genotypes Bakria, Zaaria and ILL4830. Moderate to low SDW decreases were observed for the genotypes ILL6415 (53.8%) and ILL7723 (66%). The same genotypes showed a relatively low RDW decrease with respectively 19.2% and 22.2% against a maximum of 72% recorded for Zaaria and ILL4830.

3.3. Qualitative untargeted metabolomic analysis

To investigate the impact of *O. crenata* parasitism on the metabolomic profile of susceptible and resistant lentil genotypes and to gain more information and knowledge about the chemical and biochemical mechanisms involved in the resistance mechanisms, a metabolic profiles analysis was performed using GC–MS for three lentil genotypes characterized by different resistance levels; two resistant genotypes (ILL6415, ILL 7723) and one susceptible genotype (Zaaria). For the resistant genotype ILL6415, a total of 109 metabolites were identified in the lipophilic phase of both infested and non-infested plants, in which 59 were observed only in non-infested plants and 54 metabolites were found in the infested plants. For the genotype ILL7723, 115 metabolites were detected in which 66 were annotated in the non-infested plants and 49 in the infested plants. For the susceptible check Zaaria, 92 metabolites in total were recorded including 48 metabolites in non-infested plants, and 44 in the infested plants. Results showed that compared to non infested plants the number of identified metabolites decreased in shoots of infested plants. In total, 33, 33 and 13 metabolites were recorded in the infested shoots of ILL6415, ILL7723 and Zaaria, respectively against 38, 45 and 35 recorded in the non-infested shoots. Similar decreases were observed in the infested roots for both resistant genotypes ILL6415 (17 peaks), ILL7723 (16 peaks) respectively against 21 metabolites recorded in the non-infested roots. Conversely to resistant genotypes, results showed an increase of the number of metabolites in the infested roots of the susceptible check Zaaria (31 peaks) against only 13 observed in non-infested plants. The annotated metabolites in the shoot and the root belong to several biochemical groups such as fatty acid, sterol, alkane, alcene, organic compounds and others (Figure 4(C) and Figure 5(C)).

The metabolites concentration found in the shoot and the root of infested and non-infested genotypes were used to perform a PCA and to build heat-maps to identify the pattern between the samples and gain clear details about the metabolic changes (Figures 4 and 5). The PC1 and PC2 of the shoot metabolites explain 78% and 17.6% of the total variance respectively. For both genotypes ILL6415 and ILL7723, the non-infested and infested pant functions were close to each other. Instead, PC1 and PC2 separate the function of the non-infested and infested Zaaria (Figure 4(B)). Differently to the shoot, PC1 and PC 2 explain 36.3% and 25.3% of the total root metabolite variations. The PC plot separates markedly the functions of non-infested root of ILL6415, ILL7723 and Zaaria from the infested root functions (Figure 5(B)). Out of 19 abundant and identified metabolites detected, only linolenic acid, arachidic acid and abietic acid are markedly accumulated in the shoot of the resistant infested genotypes ILL6415. Four metabolite levels including stigmasterol, methyl 2-hydroxytetracosanoate, lignoceric acid and stigmastan-3,5-diene decreased compared with the non infested plants. On the other hand, the metabolite concentrations do not show significant changes between the treatments in the shoot of the resistant genotype ILL7723. While linolenic acid, g-sitosterol and melissic acid exhibit increased concentrations in the shoot of the infested

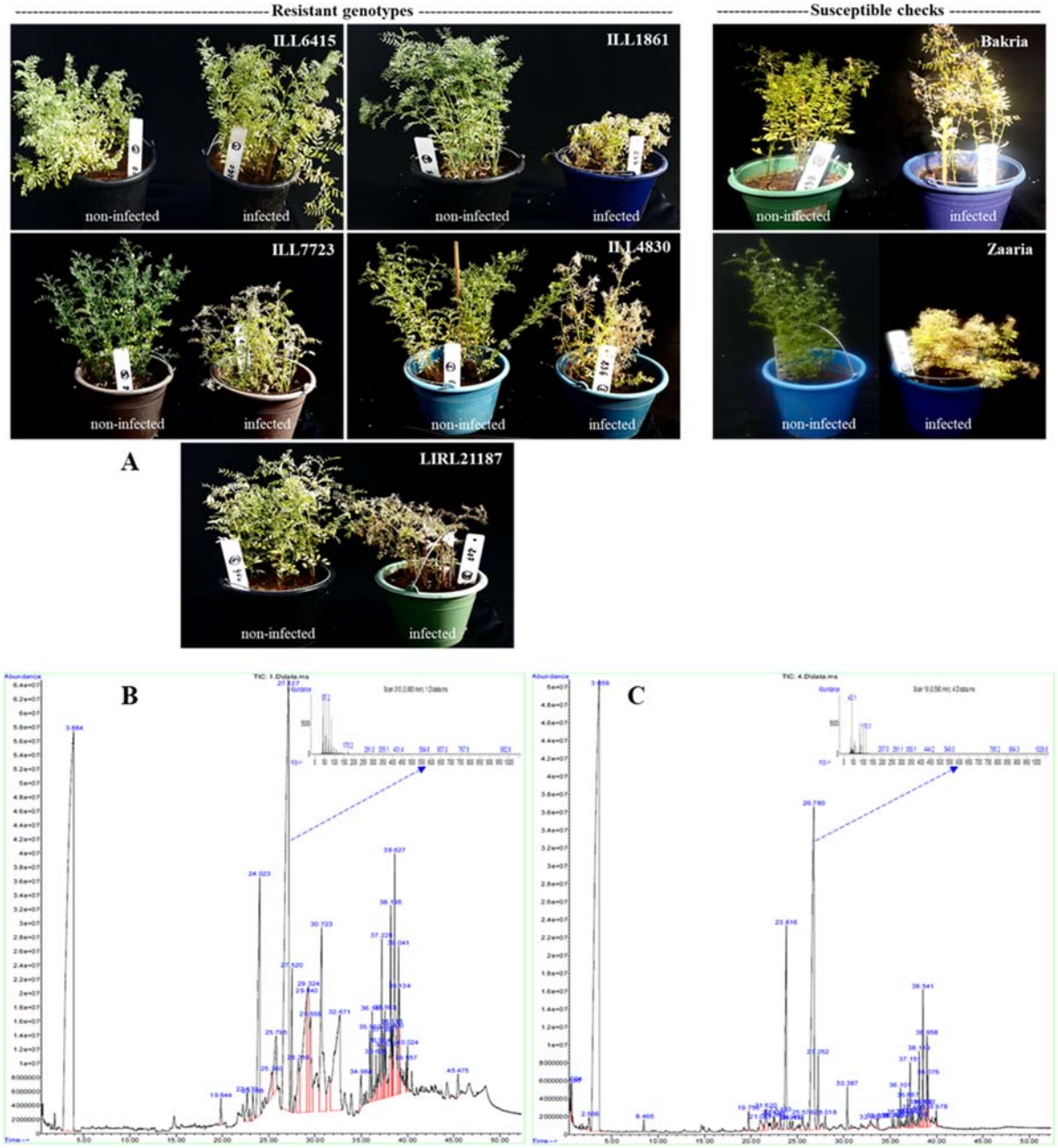

Figure 3. Growth variation between infected and no-infected plants for the five selected genotypes and the susceptible checks (A) GC–MS chromatograms of the shoot of the resistant genotype (ILL6415), (B): non-infected (C): Infected.

Table 4. The attachment number of *O. crenata* in selected lentil genotypes and the checks cultivars.

	Total Orobanche Number per Plant (TON)	Emerged Orobanche Shoots (EON)	Non-Emerged Orobanche Tubercles (NEO)
ILL 6415	4.1 ± 0.8 ^{b}	0.4 ± 0.2 ^{b}	3.3 ± 1.0 ^{a}
ILL1861	7.1 ± 0.8 ^{a}	0.6 ± 0.3 ^{a}	5.1 ± 1.4 ^{a}
LIRL21187	5.1 ± 1.9 ^{ab}	0.7 ± 0.3 ^{ab}	3.9 ± 2.3 ^{a}
ILL7723	5.8 ± 2.5 ^{a}	0.7 ± 0.4 ^{a}	3.8 ± 2.4 ^{a}
ILL4830	7.2 ± 3.4 ^{ab}	0.4 ± 0.2 ^{ab}	5.9 ± 2.9 ^{a}
Zaaaria	4.3 ± 1.8 ^{ab}	0.6 ± 0.3 ^{ab}	3.2 ± 1.5 ^{a}
Bakria	3.5 ± 1.8 ^{b}	0.6 ± 0.3 ^{b}	2.8 ± 0.9 ^{a}
Total	4.8 ± 2.0	0.6 ± 0.1	3.7 ± 1.8

Data are means ± SE. Means with the same letters within a column are not significantly different at $p > 0.05$. TON: Total *Orobanche* number, EON: emerged *Orobanche* number, NEO: underground *Orobanche* number.

susceptible genotypes Zaaria compared with the non-infested plants (Figure 4(A)).

Moreover, concentrations of g-sitosterol, stigmastan-3,5-diene, telfairic acid, docosanoic acid, stigmasterol and arachidic acid significantly increased in the infested root of ILL6415, when a significant reduction of palmitic acid, oleic acid, lignoceric acid and methyl 2-hydroxy-tetracosanoate were found in the same infested roots. Stearic acid, telfairic acid and montanic acid were more abundant in the infested root of ILL7723 than the non-infested root, for the same genotype only melissic acid and palmitoloic acid were reduced in the infested root compared to the non-infested root. For the susceptible genotype Zaaria, tricosanoic acid and pentacosanoic acid were significantly accumulated in the infested root (Figure 5(A)).

4. Discussion

4.1. Field evaluation

The holoparasitic weed *O.crenata* is known for its devastating effect on productivity of many crop species, especially legume crops such as faba bean, chickpea, grass pea and lentil

Table 5. Mean ± standard error (SE) of shoot (SDW) and root dry weight (RDW) of the different infested and non-infested genotypes under controlled conditions.

	Shoot dry weight (SDW) (g)			Root dry weight (RDW) (g)		
	non-infected	infected	decreases (%)	non-infected	infected	decreases (%)
ILL 6415	2.7 ± 0.8 ^{bc}	1.2 ± 0 .0 ^{d}	53.8	1.0 ± 1.3 ^{a}	0.8 ± 0.9 ^{ab}	19.2
ILL1861	2.7 ± 0.8 ^{cd}	0.4 ± 0.0 ^{bcd}	84	1.2 ± 0.8 ^{a}	1.1 ± 0.2 ^{a}	66
LIRL21187	3.5 ± 1.4 ^{ab}	0.9 ± 0.0 ^{d}	73	0.6 ± 0.3 ^{a}	0.4 ± 0.1 ^{ab}	29.4
ILL7723	1.7 ± 0.3 ^{bcd}	0.6 ± 0.2 ^{ab}	66	0.7 ± 0.6 ^{a}	0.5 ± 0.0 ^{ab}	22.2
ILL4830	2.4 ± 0.5 ^{bcd}	0.2 ± 0.1 ^{d}	90	0.7 ± 0.5 ^{a}	0.2 ± 0.0 ^{b}	66.1
Bakria	5.0 ± 1.6 ^{a}	0.5 ± 0.1 ^{abc}	90	0.9 ± 0.2 ^{a}	0.4 ± 0.2 ^{ab}	48.9
Zaaria	2.7 ± 1.3 ^{bc}	0.2 ± 0.0 ^{cd}	90	0.5 ± 0.2 ^{a}	0.1 ± 0.0 ^{b}	72.0

Data are means ± SE Means with the same letters within a column are not significantly different at $p > 0.05$

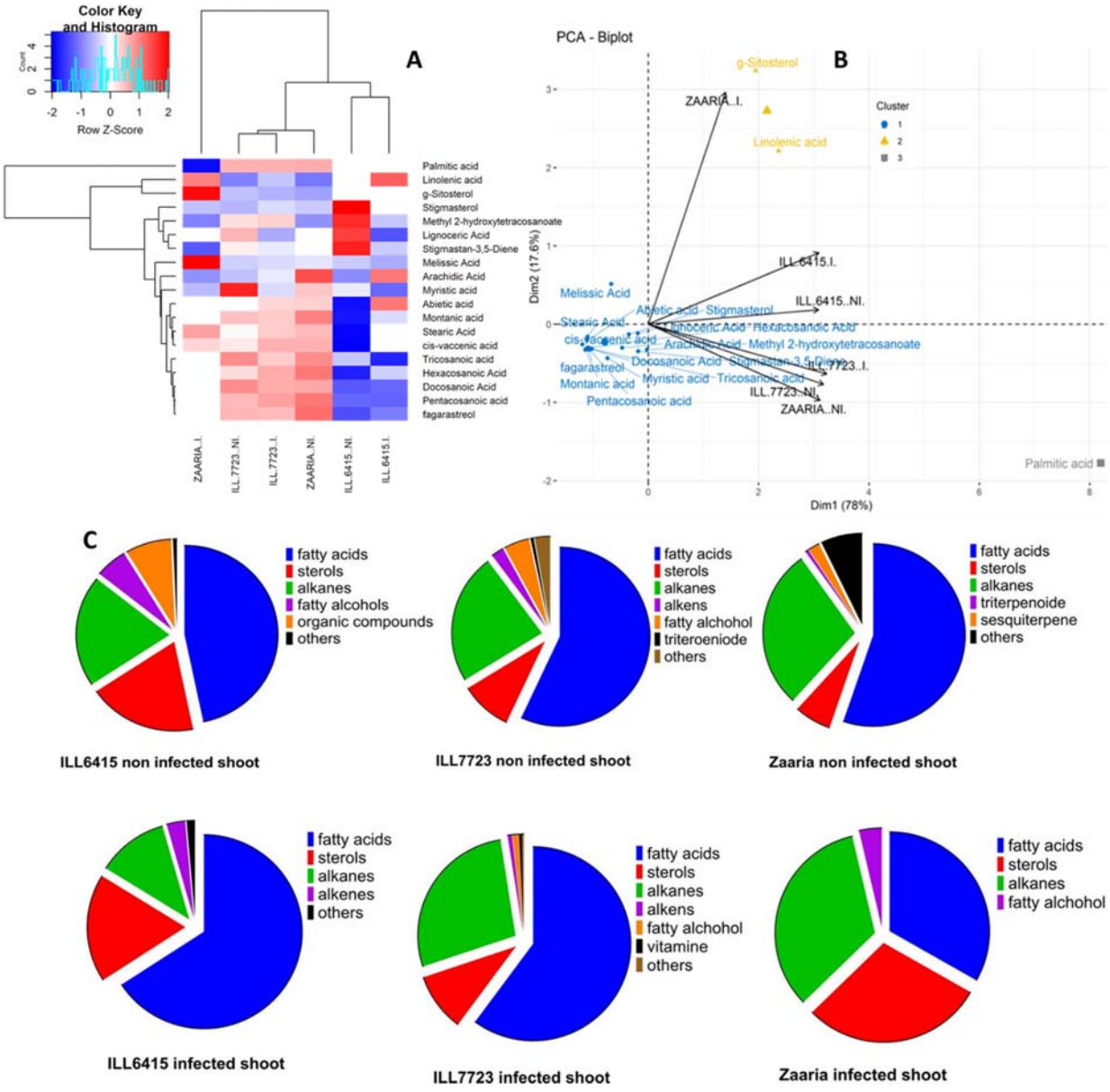

Figure 4. Heat map and principal component analysis (PCA) of metabolite levels detected in infested and non-infested lentil genotypes (centered and scaled) of shoot metabolites (A and B). Metabolite concentrations were row normalized to highlight differences among treatments. Relative abundance ranges from blue (lower than the average percentage value) to red (higher than the average percentage value). The Compounds identified in the different apolar extracts derived from infested and non-infested shoot were grouped in structurally related families (C).

(Amri et al. 2012; Millán et al. 2015; Nefzi et al. 2016; Abdallah et al. 2020). In our study, we evaluated the performance of a lentil germplasm collection under *O. crenata* open field infested conditions. The 80 tested genotypes showed significant differences in their response to *O. crenata* parasitism, this variation could be associated with the genetic variation within the population. Out of the 80 tested genotypes, ILL4615, ILL7723, ILL1861, ILL4830, ILL9850 and LIRL21187 showed a good resistance level. Similar results were reported in previous studies, using different lentil populations and studying their behavior against the *O. crenata* parasitism (Ennami et al. 2017; Mbasani-Mansi et al. 2019). Regarding our results, early flowering was positively correlated to D2OE ($r = 0.68^{***}$) and negatively correlated with SY ($r = -0.20^{***}$). The early flowering, which has been described to have a positive effect on plant potential

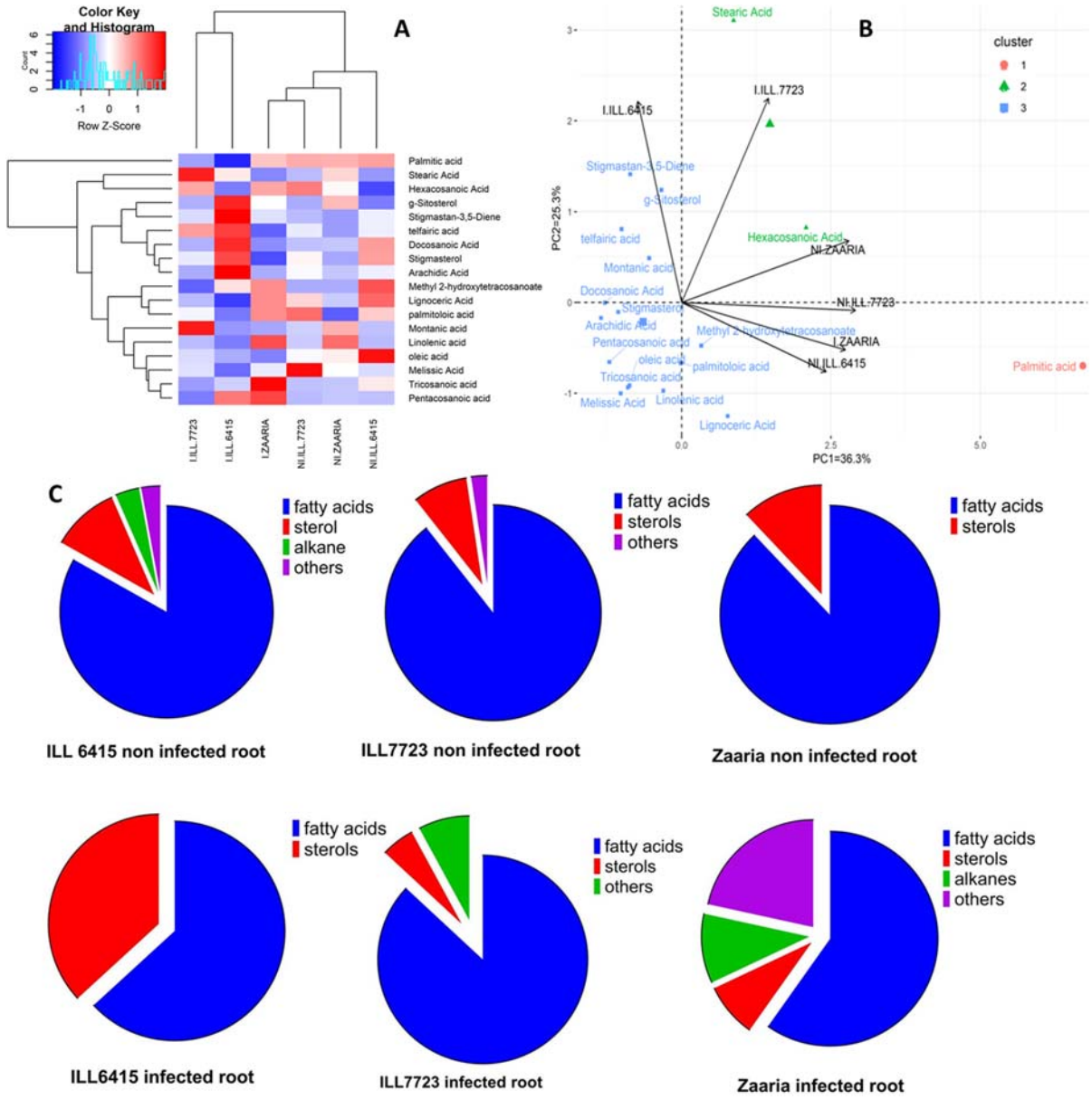

Figure 5. Heat map and principal component analysis (PCA) of metabolite levels detected in infested and non-infested lentil genotypes (centered and scaled) of root metabolites. Metabolite concentrations were row normalized to highlight differences among treatments. Relative abundance ranges from blue (lower than the average percentage value) to red (higher than the average percentage value). The Compounds identified in the different apolar extracts derived from infested and non-infested roots were grouped in structurally related families (C).

yield through the prolongation of reproductive phase as well as seed filling period (Sundaram et al. 2019), is associated with genes that are highly affected by the environmental conditions (Yaish et al. 2011). It is tightly controlled by some complex genetic and metabolic pathways such as salicylic acid and gibberellin pathways as well as some other phytohormones involved in plant growth (Dezar et al. 2011; Jing et al. 2020). The root parasitic plants are metabolically dependent on their hosts (Clermont et al. 2019), which suggest that the early metabolism adjustments associated with early flowering will stimulate the release of germination stimulants in the rhizosphere of the host root system resulting in an early attachment and development of the parasite on the host plant. such early infestation results with an early parasitism impact affecting the host metabolism and thus its physiological and phenological behavior which explain the correlation found in the current study between D2F, D2OE and SY (Amri et al. 2021). Parasitism Index was positively correlated with EON and EODW, which reflects the severe negative effect of *O. crenata* parasitism on lentil growth and seed production. Similar results were reported in previous studies performed on faba bean (Abbes et al. 2011; Trabelsi et al. 2015; Trabelsi et al. 2016), lentil (Ennami et al. 2017), chickpea (Nefzi et al. 2016) and grass pea (Abdallah et al. 2020).

The score plot, the biplot and cluster analysis are graphical representations used for a better visualization of PCA results, and allow the identification of genotypes with the desirable characteristics. In general, whatever the genotypes are located close to the trait that's mean a higher value for the corresponding trait. The genotypes ILL4605, ILL4830, LIR22107, ILL6415 and ILL7723 clustered together in cluster 4 showed the lowest PI levels and the highest SY and BY. The same genotypes are located to the negative side of PC2 which means they have low values of EON and EODW. On the other hand, genotypes such as ILL82, ILL304, ILL5385 clustered together in cluster 1, showed the highest PI and lowest SY and BY, and presented as the most susceptible genotypes to *O. crenata*.

4.2. Pot experiment

The pot experiments were conducted under controlled conditions to confirm field results and assess the underground infestation which was difficult to consider under field conditions. Data related to EON, NEO SDW and RDW recorded for different tested genotypes showed that the growth and biomass production have decreased in infested plants for all the genotypes compared to the non-infested plants. These results showed that out of the five tested genotypes, only two genotypes ILL6415 and ILL7723 showed good resistance level under controlled conditions which confirm the results observed under open field conditions. Such resistance was expressed by the lowest shoot and root dry weight reduction and a low number of total *O. crenata* shoots and tubercules. similar results were reported in previous studies performed on faba bean and lentil (Ennami et al. 2017; Abdallah et al. 2020) who reported a large variation in infestation intensity under pots conditions and field trials.

4.3. Qualitative untargeted metabolomic analysis

In this part of the study, the two resistant genotypes ILL 6415 and ILL7723 with the susceptible genotype Zaaria were selected to conduct a qualitative metabolomic analysis to explore the biochemical mechanisms involved in the resistance against *O. crenata*. The principal components analysis (PCA) is commonly used in non-targeted metabolomics studies to perform metabolite correlation networks (Arbona et al. 2013). Based on PCA for shoot metabolites performed for both resistant genotypes ILL6415 and ILL7723 and compared to the control samples *Orobanche* parasitism was found to have a less effect on metabolites changes compared to the susceptible genotype Zaaria (Figure 4(B)). However, the parasitism was found to have a markedly effect on metabolites change in the root metabolite for all genotypes compared to their respective non-infested control plants (Figure 5(B)). The changes in metabolomic profile was reported also by Clermont et al. (2019) between the facultative parasite *T. versicolor* and the obligate holoparasite *P. aegyptiaca* with the host species *M. Truncatula* and *A. thaliana*, respectively. Similarly, other authors reported that the parasitism by *P. aegyptiaca* and *O. foetida* has affected respectively tomato (Amir 2016) and faba bean (Trabelsi et al. 2017; Abbes et al. 2020) metabolites when compared to non infested plants.

The results demonstrated that *O. crenata* parasitism may modify the shoot and the root metabolites of the host plants, especially for susceptible genotypes. Indeed, out of the detected metabolites, the increase of α-linolenic acid in the shoot of the resistant genotype could be related to a specific biochemical pathway of resistance, this essential fatty acid is an important component of the cell membrane that maintain the membrane integrity and functionality, prevents the membrane rigidification under stress conditions and enhance the production of ROS via the activation of Ca^{2+}-ATPase (Delavault et al. 2017; Jia et al. 2020). Besides, α-linolenic acid is a precursor of jasmonic acid (JA) synthesis, a key rules in mediating resistant responses of plants under biotic and abiotic stresses by eliciting the production of alkaloid, terpenoid, coumarins, phytoalexins and taxane compound that are widely known by their functional value to plant under stress and regulating plant growth and development (Delavault et al. 2017). However, the accumulation of arachidic acid in the shoot and the root of the resistant genotype ILL6415 may serve as a defensive agent to fight the parasite attack, because several phytohormones and secondary metabolites are derived from this compound (Scharenberg et al. 2019). This long-chain fatty acid could be related to the wax, cutin and suberin production pathways, which help the plant to reduce water loss and protect the plant surface (Mutale-joan et al. 2020). Similar accumulation in α-linolenic acid and arachidic acid were reported by Jia et al. (2020) in drought-tolerant *Populus simoniicv* and drought-susceptible *P. deltoides* under drought conditions stress. Otherwise, clear difference was observed in root sterol content between the resistant and susceptible genotypes and between infested and non-infested plants. Wang et al. (2012) suggested that plant cells involve mechanical defense using sterols to make a physical barrier against the nutrient efflux to prevent nutrient and loss against bacterial and pathogens attack. In contrast to previous studies that focused more on polar metabolite variations including starch, amino acid and some secondary metabolite in response to parasitism in different plants (Abbes et al. 2009; Amir 2016; Clermont et al. 2019), the current study put the light-on fatty acids and sterols as important metabolites that can serve as regulatory pathways to conduct defense mechanisms in lentil and support the suggestion of the ability of the host plant to regulate phloem composition depending onthe host–parasite interaction (Jokinen and Irving 2019). Further, much remains unknown about the host–parasite biochemical interactions and a lot of work is still needed in exploring novel metabolic pathways and associated gene expression, genomic and proteomic analysis.

5. Conclusion

The broomrape *O. crenata* is one of the major problems limiting the production and the development of lentil in Morocco and many other countries in the region. Identification and development of resistant germplasm remains the best option to control this parasite. In this study, potential sources of resistance were identified out of 80 genotypes screened and evaluated under high *O. crenata* infested field and controlled conditions. Both genotypes ILL6415 and ILL7723 showed the highest level of resistance to the crenate broomrape. Such resistance was associated with complex physiological and biochemical mechanisms such as an increase of some specific metabolite biosynthesis that contribute in improving the host immunity and fight against the parasite. These mechanisms, if combined with other potential physical and/or chemical mechanisms, through classical breeding and/or genetic engineering approches, could help in improving the resistance to *O. crenata* in lentil.

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Author contribution

YE, RM, MA., designed the research. YE, KH, HE, MA: performed the experiments. YE, KH, OB, RM, AD, ZA, NES, MA, contributed materials/analysis tools. YE, MA, wrote the paper. YE, HE, AD, ZA, NES, SK, RM, MA, revised the paper. All authors approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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