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Investigation of some biochemical mechanisms involved in the resistance of faba bean (*Vicia faba* L.) varieties to *Orobanche* spp.

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Abstract: Broomrapes are weedy root parasitic plants that cause important damage to the faba bean production. Genetic resistance is considered as the most desirable control method. In this study, we investigated some of the mechanisms involved in the resistance to *Orobanche crenata* and *O. foetida* for two Tunisian resistant varieties Najeh and Chourouk in comparison with the susceptible variety Badi. The results showed that, for Najeh and Chourouk, the *Orobanche* infestation did not significantly influence the plant growth as indicated by inconsiderable changes in the shoot and root dry weights, pod setting and chlorophyll and carotenoid contents. In comparison to Badi, both resistant varieties showed a reduced *Orobanche* attachments/tubercles number and dry weights, low H_2O_2 and malondialdehyde (MDA) accumulation with increased levels of peroxidase activity in the roots. An *Orobanche* infestation was found to induce a significant decrease in the total lipid content and lipid unsaturation in the Badi leaves only. For resistant varieties, no significant changes were observed in these two parameters, which may indicate conservation and stability in the membrane fluidity. The resistance of Najeh and Chourouk was mainly associated with a relatively more efficient enzymatic antioxidative response and reduced lipid peroxidation that helped the plants to avoid the damaging effect of an *Orobanche* attack. Therefore, these two varieties could be used as a component of an integrated control strategy to improve the plant growth and productivity under an *Orobanche* infestation.

Keywords: antioxidant; fatty acids; broomrapes; genetic resistance

Broomrapes (*Orobanche* spp. and *Phelipanche* spp.) are obligate parasites that lack chlorophyll and the ability to synthesise their own assimilates. Theses parasites are completely dependent on their hosts

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for their nutritional requirements. Broomrapes penetrate the host tissues by mechanical action and enzymatic digestion and/or the alteration of host cell walls by secreted enzymes. These enzymes make the host cell walls become fragile and facilitate the progression of parasite intrusive cells into the host root (Nun et al. 2003; Veronesi et al. 2007).

A broomrape infestation causes oxidative stress as a biotic stress factor and leads to the generation of reactive oxygen species (ROS) (Vránová et al. 2002; Demirbas & Acar 2008). ROS are highly reactive and cytotoxic components. They can damage proteins, carbohydrates, DNA and RNA, lipids, and can result in cell death. One of the consequences of ROS activity is the oxidative damage to the membrane integrity due to lipid peroxidation processes (Apel & Hirt 2004). Changes in the membrane lipid composition may affect the fluidity and the intrinsic membrane protein activities as a result of an alteration in the lipidic environment in which they are embedded (Quartacci et al. 2000). The response of plants is complex and involves changes in their morphology, physiology and metabolism. To cope with the oxidative damage initiated by the ROS formed under biotic and abiotic stress, plants possess a complex antioxidant defence system including non-enzymatic and enzymatic antioxidants (Türkan & Demiral 2009). Enzymatic ROS scavenging mechanisms in plants include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and glutathione peroxidase (GPX). SOD acts as the first line of defence against ROS, dismutating a superoxide anion radical to H_2O_2. APX, GPX, POX, and CAT subsequently decompose H_2O_2. POX and CAT belong to the important enzymes removing the ROS in plants (Apel & Hirt 2004).

Broomrapes cause important yield losses in many crops worldwide. In Tunisia, *Orobanche foetida* Poiret and *Orobanche crenata* Forssk. are considered to be the major agricultural problem of the faba bean production, causing yield losses that can attain more than 90% in highly infested fields (Abbes et al. 2007a; Amri et al. 2019). Several control methods were tested including cultural, chemical, biological, genetic resistance and other innovative techniques (Fernández-Aparicio et al. 2011; Bouraoui et al. 2016; Abbes et al. 2019), but none of them have resulted in the complete successful control of the parasite.

In Tunisia, research activities on *Orobanche* spp. were intensified during the last two decades and resulted in the development and release of the new, moderately resistant faba bean varieties Najeh and Chourouk (Abbes et al. 2007a; Kharrat et al. 2010; Trabelsi et al. 2015; Amri et al. 2019). These two varieties are carrying partial resistance to both *O. crenata* and *O. foetida* and significantly contributed to limiting the damage caused by these parasitic weeds. Previous studies on these two varieties reported that several mechanisms were involved in the resistance to both *O. crenata* and *O. foetida* (Abbes et al. 2009a, b; Trabelsi et al. 2016, 2017). These mechanisms were associated to the low *Orobanche* spp. seed germination stimulants production resulting in a low germination rate, a limited attachment number of germinated seeds and the reduced growth of established tubercles. All these factors result in a low number of *Orobanche* emerged shoots in the host plant. A deep host plant root system could help in obtaining less infestation (Abbes et al. 2007b). Previous studies also reported that the slow and limited tubercles growth after attachment to the host root system was related to the low soluble invertase activity, low osmotic potential of the infested roots and the organic nitrogen deficiency of the host phloem sap (Abbes et al. 2009a, b). The aim of this study was to assess the effect of an *Orobanche* spp. infestation on the biochemical and physiological parameters in resistant and susceptible faba bean varieties. This was carried out based on the growth parameters, the chlorophyll content, the level of lipid peroxidation and antioxidant enzymes activities as well as the contents of the total lipids and fatty acids composition.

MATERIALS AND METHODS

Plant culture. Three Tunisian small-seeded faba bean (*Vicia faba* Linnaeus) varieties were used in this study; the susceptible control variety Badi and the partial resistant varieties to both *O. foetida* and *O. crenata*, Najeh (Kharrat et al. 2010) and Chourouk (Amri et al. 2019). *O. foetida* Poiret and *O. crenata* Forsk. seeds were collected in 2015 from mature spikes in infested faba bean fields, respectively, from Beja and Ariana (Tunisia).

Both the faba bean and *Orobanche* seeds were superficially sterilised by soaking in calcium hypochlorite (1%) for 15 min and were washed twice with sterilised water. The broomrape seeds (20 mg/kg of soil) were mixed with a sterilised soil in a 10 L capacity pot. Five pots were prepared for each variety. Two

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faba bean seeds were then sown directly into each pot. Two weeks after the faba bean emergence, the seedlings were thinned to one per pot. The pots containing the broomrape-free soil were used as controls. The plants were grown under greenhouse conditions at 20 ± 3 °C with a humidity of 70% and a 16 h photoperiod and watered as needed.

At the maturity stage (four months after sowing), the faba bean plants were uprooted. The total number of broomrape attachments per host plant were counted and classified according to their developmental stage (1 – attachment of haustorium to host root; 2 – small tubercles without root development; 3 – tubercles with crown roots without shoot formation; 4 – underground tubercles with shoot formation; 5 – emergence of spikes) (Labrousse et al. 2001). In addition, the shoot height and pod number were measured. The dry weight of the host shoot and the root and broomrape attachments were determined following incubation of the fresh tubercles in an oven at 80 °C for 72 hours. At the maturity stage, tissue samples were also collected from host and parasite for the physiological and biochemical analysis.

Determination of chlorophyll and carotenoid contents. The chlorophyll content in the leaves (100 mg FW) was extracted in 80% chilled acetone and estimated by the method of Arnon (1949). The carotenoid concentration, in the same extract, was calculated using the McKinney formula (1941).

Determination of hydrogen peroxide H_2O_2 content. The hydrogen peroxide was measured using the method described by Velikova et al. (2000) with a slight modification. An amount of 0.5 g of fresh leaf and root was extracted in 3 mL trichloroacetic acid TCA (1%) at 4 °C and centrifuged for 10 min at 12 000 × g. For the assay, 0.75 mL of the supernatant was collected and mixed with 0.75 mL of a phosphate buffer (10 mM, pH 7) and 1.5 mL of 1 M potassium iodide solution (KI). The optical density was measured at 390 nm. The H_2O_2 contents were determined according to the standard range.

Extraction of total protein content and determination of antioxidant enzymes activity. The samples were homogenised on ice with a mortar and pestle in a 50 mM potassium-phosphate buffer (pH = 7.8), containing 0.1 mM Na_2-EDTA, 1 mM PMSF and 0.05 g of polyvinylpyrrolidone (PVP). This mixture was centrifuged at 12 000 × g for 15 min at 4 °C. The supernatant was stored at –20 °C for the peroxidase (GPOX) and superoxide dismutase (SOD) assays. The total protein concentration was determined according to the Bradford method (Bradford 1976) using the Bio-Rad protein assay reagent.

The peroxidase activity (GPOX, EC1.11.1.7) was assessed according to the Anderson et al. (1995) method which consists of measuring the guaiacol oxidation in the presence of H_2O_2. The increase in absorbance due to formation of tetra guaiacol was recorded at 470 nm.

The superoxide dismutase (SOD, EC1.15.1.1) activity was assayed by monitoring the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Lee et al. (2001). The reaction mixture contained a 50 mM phosphate buffer (pH = 7), 75 µM of NBT, 10 mM methionine and 2.7 µM riboflavin and an appropriate aliquot of the enzyme extract. The reaction was started by placing the tubes under a fluorescent lamp for two min. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition in the reduction of the NBT as monitored at 560 nm.

Determination of lipid peroxidation and membrane permeability. The level of lipid peroxidation in the plant tissues was determined by the 2-thiobarbituric acid (TBA) reactive metabolites chiefly malondialdehyde (MDA) as described previously by Buege and Aust (1972). In brief, 0.25 g of tissue was homogenised in 5 mL of 0.1% (w : v) trichloroacetic acid. This mixture was centrifuged at 10 000 g for 5 minutes. To 1 mL of the supernatant, 4 mL of 20% (w : v) trichloroacetic acid containing 0.5% (w : v) of thiobarbituric acid were added. After 30 min of heating at 95 °C, the obtained mixture was quickly cooled in an ice bath and centrifuged at 10 000 g for 10 min. The absorbance of the supernatant was measured at 532 nm. The value was corrected for the nonspecific absorption at 600 nm. The level of lipid peroxidation is expressed as nmol of MDA formed using an extinction coefficient of 155 mM/cm.

The membrane permeability of the leaves was determined as follows: the leaf tissue (100 mg) was vibrated for 30 min in deionised water, followed by the measurement of the conductivity of the medium (EC_1). Then, the samples were boiled for 15 min and the final conductivity (EC_2) was measured. The percent leakage of the electrolytes was calculated using the following Formula (1) :

$$\text{The percent leakage of electrolytes} = \frac{EC_1}{EC_2} \times 100 \quad (1)$$

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Lipid extraction and determination of fatty acid composition. The lipids were extracted according to the method of Allen and Good (1971). The leaf tissues were fixed in boiling water for 5 min to denature the phospholipases and homogenise them in a chloroform:methanol mixture (2 : 1, v : v). The homogenate was centrifuged at 3 000 g for 15 minutes. The lower chloroform phase containing the lipids was aspirated and evaporated at 40 °C under vacuum using a rotary evaporator or with nitrogen gas. The residue was immediately dissolved again in 2 mL of toluene:ethanol mixture (4 : 1, v : v) for conservation. The fatty acids from the total lipids (TL) were methylated by the method of Metcalfe et al. (1966). The fatty acid methyl esters of the total lipids were analysed by gas chromatography using a chromatograph (model 4890D, Hewlett Packard, USA) equipped with an Innowax capillary column (30 m × 0.53 mm i.d.) maintained isothermally at 210 °C. For measuring the amounts of the fatty acids, heptadecanoic acid (17 : 0) was added as an internal standard. The calculation of the fatty acid quantities was performed using an integrator.

Statistical analysis. The statistical analyses were performed using the SPSS software (Version 15.0). The differences among the treatments for all the measurements were compared at $P = 0.05$ using Duncan's multiple-range test. At least three repetitions were conducted for each experiment.

RESULTS

Orobanche parasitism impact on host plant development. For both *Orobanche* species, the total Orobanche attachments/tubercles number and DW per host plant were significantly lower for both the tested resistant varieties, Najeh and Chourouk, compared to what was recorded for the susceptible variety Badi (Table 1). All the attachments attained emerged spikes (S5) except for Chourouk with *O. crenata* and for Najeh with *O. foetida*. Out of the total *O. crenata* and *O. foetida* attachments, the number per plant for the susceptible control, Badi, were 64.41 and 38.09%, respectively, having reached the S5 stage. For the same variety, the total *Orobanche* spp. number, the non-emerged *Orobanche* number and the total *Orobanche* DW were significantly higher with *O. crenata* than with *O. foetida*. No significant differences were observed between the two resistant varieties for these parameters. No necrosis of the attachments/tubercles was observed in this experiment.

Regarding the faba bean plant development, the following parameters: shoot height, shoot and root DW and pod number per plant were recorded in the infested and non-infested plants (Table 2). For both Orobanche species, no significant differences, in these parameters, were observed between infested and non-infested plants for the two resistant varieties Najeh and Chourouk. However, the plants were significantly affected by *Orobanche* spp. parasitism for the susceptible control Badi for which the lowest shoot and root dry weight was recorded under both Orobanche species. No pod development was observed for Badi infested with *O. crenata* and only 0.02 pod/plant was recorded under the *O. foetida* infestation. For the shoot height, a significant decrease was only observed in the Badi plants infested with *O. foetida* (Table 2).

Compared to the non-infested conditions, *Orobanche* significantly decreased the chlorophyll and carotenoid contents in the infested plants for the susceptible variety Badi. However, no significant difference was observed for the resistant varieties Najeh and Chourouk (Table 3).

Hydrogen peroxide content. The results did not show any significant increase in the H_2O_2 content in

Table 1. The number and total dry weight (g) of *O. crenata* and *O. foetida* harvested on the roots of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

	Variety	Total *Orobanche* plants	Non-emerged *Orobanche* plants	Emerged *Orobanche* plants	*Orobanche* DW (g)
O. crenata	Chourouk	0.40^{c*}	0.40^{c}	0.00^{b}	0.05^{c}
	Najeh	0.80^{c}	0.20^{c}	0.60^{b}	0.07^{c}
	Badi	11.80^{a}	7.60^{a}	4.20^{a}	3.60^{a}
O. foetida	Chourouk	1.60^{c}	1.20^{c}	0.40^{b}	0.52^{c}
	Najeh	0.40^{c}	0.40^{c}	0.00^{b}	0.01^{c}
	Badi	8.40^{b}	3.20^{b}	5.20^{a}	2.40^{b}

The plants were sampled four months after infestation; *the data with the same letter in the column are not significantly different ($P = 0.05$, Duncan's test), $n = 5$; DW – dry weight

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Table 2. The effects of the *O. crenata* and *O. foetida* infestation on the shoot height (cm), root and shoot DW (g) and pod number on the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

Variety		Shoot height (cm)	Shoot DW (g)	Root DW (g)	Pod number
Chourouk	Control	41.40^{a*}	8.69^{a}	3.04^{a}	3.80^{a}
	O. crenata	38.60^{ab}	7.60^{abc}	2.20^{abc}	3.60^{a}
	O. foetida	40.40^{a}	6.72^{abc}	1.67^{abc}	3.40^{a}
Najeh	Control	37.60^{ab}	7.17^{abc}	2.97^{a}	3.00^{a}
	O. crenata	31.60^{bc}	6.44^{bc}	1.39^{bc}	3.20^{a}
	O. foetida	31.20^{bc}	5.83^{c}	1.90^{abc}	3.00^{a}
Badi	Control	38.60^{ab}	8.14^{ab}	2.51^{ab}	3.20^{a}
	O. crenata	32.60^{bc}	3.14^{d}	0.99^{c}	0.00^{b}
	O. foetida	28.20^{c}	2.32^{d}	1.06^{c}	0.02^{b}

The plants were sampled four months after the infestation; *the data with the same letter in the column are not significantly different (P = 0.05, Duncan's test), n = 5

Table 3. The chlorophyll and carotenoid contents (mg/g FW) in the leaves of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties, non-infested and infested by *O. crenata* or *O. foetida*

Variety		Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Chourouk	Control	28.20^{a}	13.21^{a}	41.41^{a}	3.14^{a}
	O. crenata	22.49^{ab}	9.71^{ab}	32.19^{ab}	2.46^{ab}
	O. foetida	22.71^{ab}	9.68^{ab}	32.32^{ab}	2.49^{ab}
Najeh	Control	15.29^{bc}	8.09^{bc}	23.38^{bc}	1.85^{bc}
	O. crenata	18.81^{b}	8.29^{bc}	27.09^{b}	2.05^{b}
	O. foetida	15.84^{bc}	7.28^{bcd}	23.11^{bc}	1.82^{bc}
Badi	Control	21.49^{ab}	10.63^{ab}	32.11^{ab}	2.29^{b}
	O. crenata	9.33^{d}	4.08^{d}	13.41^{d}	0.92^{d}
	O. foetida	7.38^{d}	5.31^{d}	12.69^{d}	1.09^{cd}

The plants were sampled four months after the infestation; *the data with the same letter in the column are not significantly different (P = 0.05, Duncan's test), n = 3

response to the *Orobanche* parasitism for the two resistant varieties Chourouk and Najeh, whereas a significant increase was recorded for the susceptible variety Badi. Such an increase was more pronounced in the roots (132.8 and 69%) than in the leaves (33.3 and 66.7%), respectively, under the *O. crenata* and *O. foetida* infestation (Figure 1).

Antioxidant enzymes. For all the three varieties, no significant differences were observed between the non-infested plants in the root tissues for the GPOX activity. For the same plants, this activity was lower in the leaves than in the roots with significant differences between the tested varieties. Najeh showed a higher GPOX activity level compared to what was observed for Chourouk. The *Orobanche* parasitism increased the GPOX activity, which was only observed in the root tissues of both resistant varieties Najeh (36.05 and 70.89%) and Chourouk (34.11 and 43.84%), respectively, under the *O. crenata* and *O. foetida* infestation (Figure 2). No significant differences were observed between the infested and non-infested Badi plants for both the root and shoot tissues.

The SOD activity was not affected by the Orobanche parasitism in both the root and leaf tissues in all the faba bean varieties, except for the Najeh root tissues infested by *O. crenata* where a slight decrease was observed in comparison to the control plants (Figure 3).

Lipid peroxidation and membrane permeability. For both resistant varieties, Najeh and Chourouk, the leaves MDA content did not significantly vary between the infested and non-infested plants with both Orobanche species. However, for Badi, the MDA content has significantly increased with *O. crenata* (97.47%) and with *O. foetida* (43.41%) compared to the control plants. No differences were recorded in the roots for all the studied faba bean varieties and the *Orobanche* species (Figure 4).

The two *Orobanche* species *O. crenata* and *O. foetida* showed significant increases in the elec-

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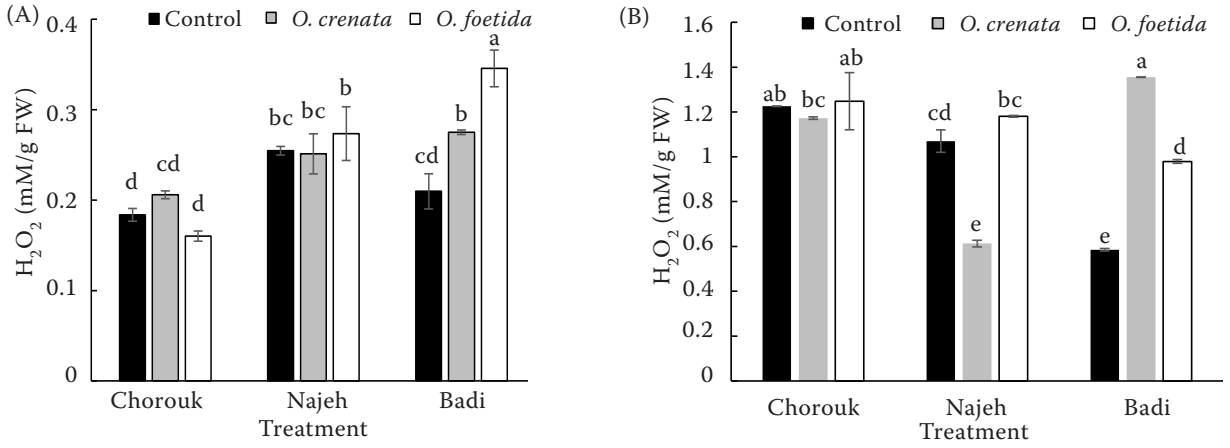

Figure 1. The effect of the *Orobanche* infestation on the hydrogen peroxide H_2O_2 (mM/g FW) in the roots (A) and leaves (B) of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

The plants were sampled four months after the infestation; the data are the means ± SE; the data with the same letter are not significantly different (P = 0.05, Duncan's test), n = 3; FW – fresh weight

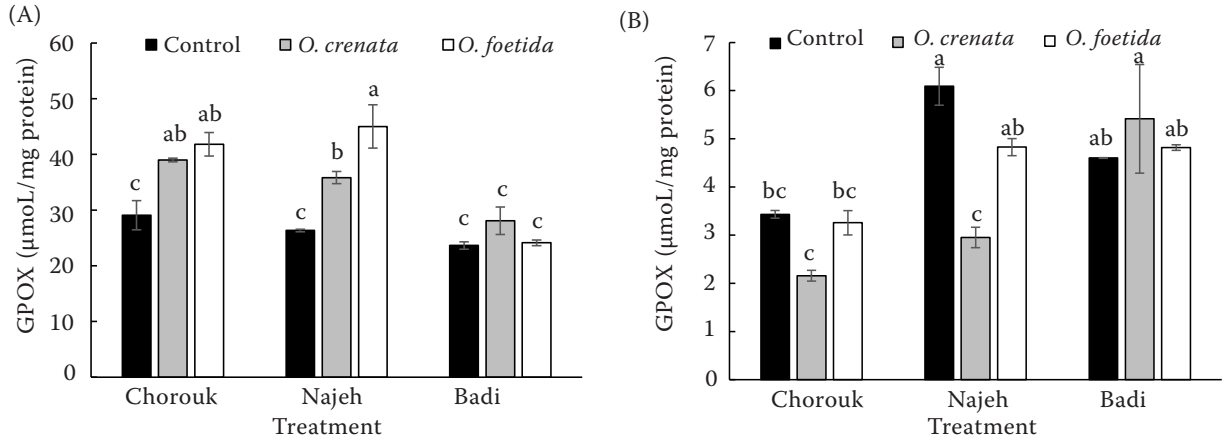

Figure 2. The effect of the *Orobanche* infestation on the membrane guaiacol peroxidase GPOX activity (µmoL/mg protein) in the roots (A) and leaves (B) of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

For explanation see Figure 1

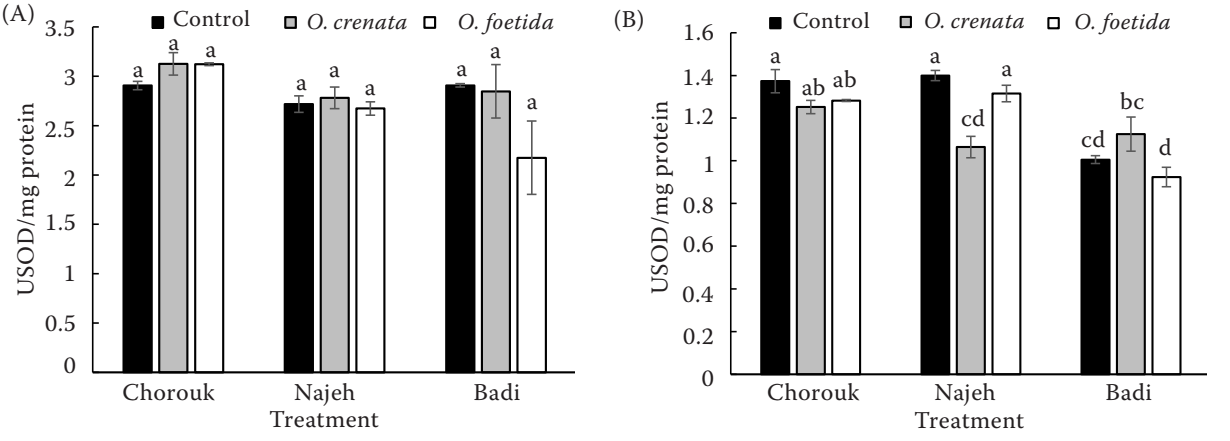

Figure 3. The effect of the *Orobanche* infestation on the membrane superoxide dismutase SOD activity (USOD/mg protein) in the roots (A) and leaves (B) of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

For explanation see Figure 1

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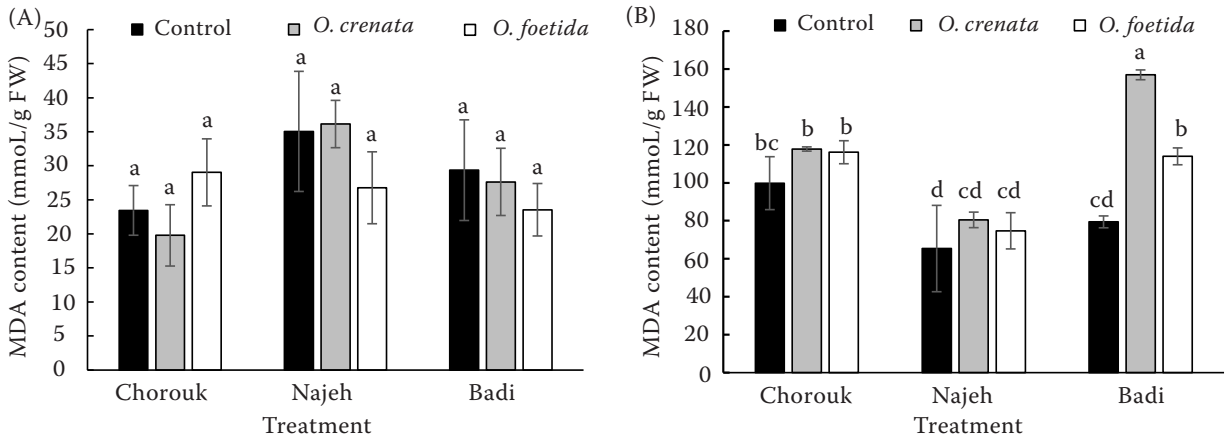

Figure 4. The effect of the *Orobanche* infestation on the lipid peroxidation (mmol/g FW) in the roots (A) and leaves (B) of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

For explanation see Figure 1

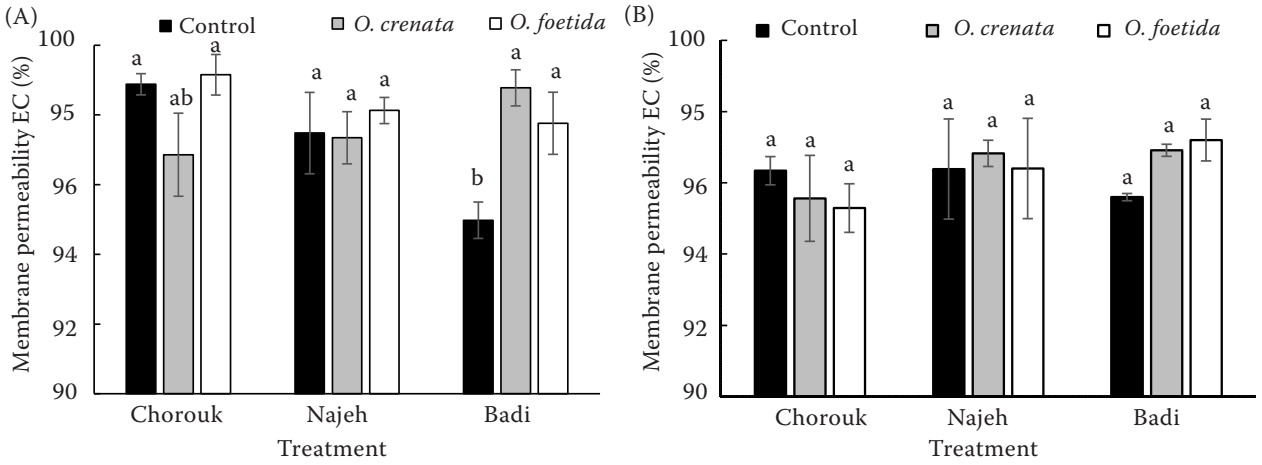

Figure 5. The effect of the *Orobanche* infestation on the membrane permeability EC (%) in the roots (A) and leaves (B) of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

For explanation see Figure 1

trolyte leakage only of 3.81 and 2.78%, respectively, in the roots of the Badi infested plants. For the same plants, the leaves membrane permeability was also moderately affected (Figure 5).

Total lipid content and fatty acid composition in faba bean plants. The two *Orobanche* species did not induce significant changes in the TL content in the leaves for both resistant varieties, Najeh and Chourouk, against a significant decrease in the susceptible variety Badi infested by *O. foetida* (62.31%) (Figure 6).

For the three faba bean varieties, linolenic acid was the predominant fatty acid in the non-infested plant leaf tissues, while both palmitic and stearic acids were found to be the predominant fatty acids under the infested conditions for the susceptible variety Badi (Table 4). No differences were observed for both resistant varieties for which the linolenic acid remained the predominant fatty acid.

O. crenata resulted in a significant increase in the percentage in the linolenic acid in the Chourouk plants and in the oleic and linoleic acids in the Najeh plants. Significant decreases were observed in the stearic acid for the Chourouk plants and palmitic and other fatty acids in the Najeh plants infested with *O. crenata*. No changes were observed for the other fatty acids in both varieties. The parasitism by *O. foetida* induced, in the Chourouk plants, a significant increase in the percentage in the oleic and other fatty acids and a significant decrease in the stearic acid. However, this parasite only increased the percentage of the linoleic acid in the Najeh

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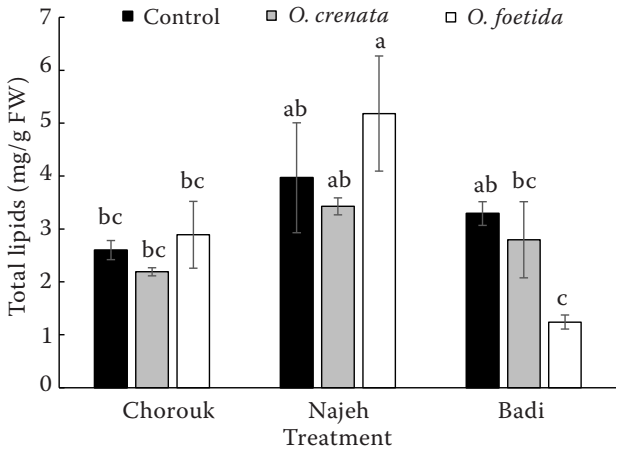

Figure 6. The effects of the Orobanche infestation on the total lipids (TL) (mg/g FW) in the leaves of the resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

For explanation see Figure 1

infested plants. The rest of the fatty acids did not change significantly under the *O. foetida* infestation for both varieties. For Badi, the two *Orobanche* species increased the percentage of the palmitic and stearic acids and decreased the percentage of the oleic, linoleic and linolenic acids (Table 4).

The *O. crenata* and *O. foetida* parasitism decreased percentage of the unsaturated fatty acids in the Badi leaves by 41.06 and 25.45%, respectively. Compared to the non-infested control plants, no significant changes were observed for the resistant varieties (Table 4).

DISCUSSION

Broomrapes cause serious damage to the faba bean production in Tunisia as we as in many other Mediterranean countries. The use of resistant varieties as the main component of an integrated control strategy seems to be the best way to control this parasitic weed. The results showed that the two resistant faba bean varieties, Najeh and Chourouk, showed low infestation levels compared to the susceptible variety Badi. *Orobanche* parasitism did not significantly affect the growth of these two varieties, particularly the shoot and root DW, pod formation and chlorophyll and carotenoid contents. These results confirm those reported on the faba bean (Trabelsi et al. 2016; Amri et al. 2019) and on the chickpea (Nefzi et al. 2016). It is widely known that a broomrape-host interaction results in drastic chlorosis. Previous studies indicated that chlorophyll amounts decreased by 50% in tomato plants subsequent to an *Orobanche* spp. infestation (Harb et al. 2004; Mauromicale et al. 2008). On the other hand, Demirbas and Acar (2017) showed that the shoot and root growth were considerably inhibited due to a broomrape infestation, but without change in the chlorophyll content.

In this study, the H_2O_2 contents increased under the *Orobanche* infestation on the susceptible variety Badi, whereas no significant changes were observed for the resistant varieties Najeh and Chourouk in comparison to the non-infested control plants. These results showed that the *Orobanche* infestation caused more oxidative damage in the Badi plants

Table 4. The effects of the *Orobanche* infestation on the fatty acid composition (TL %) in the leaves of resistant (Chourouk and Najeh) and susceptible (Badi) faba bean varieties

Variety		C16 : 0	C18 : 0	C18 : 1	C18 : 2	C18 : 3	Others	Unsaturation
Chourouk	Control	23.47^{bc*}	28.97^{b}	5.19^{cde}	6.64^{de}	32.19^{bc}	3.54^{c}	44.63^{de}
	O. crenata	27.09^{ab}	16.57^{cd}	4.43^{de}	5.71^{de}	41.35^{a}	4.85^{bc}	52.12^{bc}
	O. foetida	24.47^{bc}	21.45^{c}	9.84^{b}	9.88^{bcd}	29.17^{cd}	5.18^{b}	49.08^{bcd}
Najeh	Control	27.90^{ab}	14.85^{de}	8.43^{bc}	9.03^{cd}	31.84^{bc}	7.95^{a}	49.49^{bcd}
	O. crenata	20.04^{cd}	19.68^{cd}	14.57^{a}	13.76^{ab}	27.34^{cd}	4.61^{bc}	55.88^{b}
	O. foetida	26.70^{b}	17.94^{cd}	4.67^{cde}	15.36^{a}	27.99^{cd}	7.33^{a}	48.09^{cd}
Badi	Control	18.64^{e}	10.98^{e}	15.55^{a}	12.00^{abc}	35.88^{ab}	6.94^{a}	64.11^{a}
	O. crenata	30.74^{a}	38.81^{a}	3.59^{e}	3.70^{e}	15.39^{e}	7.77^{a}	23.05^{f}
	O. foetida	23.99^{abc}	29.53^{b}	7.83^{bcd}	7.17^{de}	23.34^{d}	8.13^{a}	38.66^{e}

The plants were sampled four months after the infestation; *the data with the same letter in the column are not significantly different (P = 0.05, Duncan's test), n =3; C16 : 0 – palmitic acid; C18 : 0 – stearic acid; C18 : 1 – oleic acid; C18 : 2 – linoleic acid; C18 : 3 – linolenic acid; the "others" include minor fatty acids such as lauric acid C12 : 0, myristic acid C14 : 0, palmitoleic acid C16 : 1, arachidic acid C20 : 0 and behenic acid C22 : 0

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than in the two resistant varieties, which was concordant with the increases observed for the MDA and electrolyte leakage in the susceptible variety, as the oxidative damage indicators. Several reports indicated that *Orobanche* resistant plants showed H_2O_2 accumulation in the root cortex close to the site of penetration (Pérez-De-Luque et al. 2006). According to Garcia-Limones et al. (2002) and Mellersh et al. (2002), the rapid generation of H_2O_2, one type of ROS, has been considered as one of the earliest cellular responses in both compatible and incompatible plant-pathogen interactions. In our study, the non-accumulation of H_2O_2 in the tissues of the resistant plants is correlated with an important increase in the GPOX antioxidant activity known as the main H_2O_2 scavenging enzyme. Consequently, it could be suggested that a synergic action between the H_2O_2 production and GPOX activity to enhance the lignification process exists, which reinforces the plant cell wall to limit the parasite penetration and simultaneously consumes the H_2O_2 overproduction to protect the plant tissues against the infection generated oxidative damages. This phenomenon was reported in the legume plant reaction against *Orobanche* spp. (Pérez-De-Luque et al. 2009) and fungal infections (Djebali et al. 2011). Indeed, peroxidases are able to oxidise various substrates in the presence of H_2O_2. They are ascribed with a variety of functional roles, including lignification and cell wall phenol deposition, suberisation, hormone catabolism, developmental related processes, defence against pathogens and response to other stresses (Penel et al. 1992). In this study, the GPOX activity increased owing to the *Orobanche* infestation in the root tissues of Najeh and Chourouk varieties only. The high GPOX activity in the resistant varieties indicate the plant ability or capacity to reduce the level of ROS produced under the *Orobanche* infestation. This suggests that tolerance to *Orobanche* spp. could be induced by enhancing the antioxidant capacity of the plants. The induction of antioxidant enzymes has been reported to be among plant defence mechanisms by conferring mechanical and/or chemical barriers in host tissues against *Orobanche* spp. (Goldwasser et al. 1999; Castillejo et al. 2004). Increased POX activities have been reported in *Phelipanche aegyptiaca* Pers. infested *Arabidopsis* spp. plants (MOR et al. 2008), *P. ramosa* infested Arabidopsis plants (Demirbas & Acar 2017), resistant genotypes of vetch plants (Goldwasser et al. 1999), *O. crenata* infested pea genotypes (Castillejo et al. 2004) resistant sunflowers (Demirbas & Acar 2008) and *Pisum sativum* Linnaeus plants (Pérez De-Luque et al. 2005). In addition, the increase in the level of antioxidant enzymes activities, such as phenylalanine ammonia-lyase PAL and polyphenol oxidase PPO, has been reported in several pathosystems. For example, Al-wakeel et al. (2013) showed that the infestation of tomato plants with *P. ramosa* stimulated these activities when compared to their respective levels in healthy plants.

SOD constitutes the first defensive line against oxidative stress (Apel & Hirt 2004). Our results indicated no significant differences for the SOD activity in all the resistant and susceptible varieties in response to the *O. crenata* and *O. foetida* infestation. Similarly, Demirbas and Acar (2017) showed that the SOD activity did not change during a broomrape infestation in *Arabidopsis* spp. plants. On the other hand, increases were reported in the SOD activity in 1–9 day-old (Demirbas & Acar 2008) and 67 day-old (Olesea 2019) resistant sunflowers following an *O. cumana* infestation. In the present study, it can be concluded that, among H_2O_2-eliminating enzymes, GPOX responds to the *Orobanche* spp. infestation in the resistant varieties. The lower SOD activity to the *Orobanche* infestation in these varieties might be compensated by the enhanced GPOX activity.

Excess ROS leads to the peroxidation of unsaturated lipids and reduces the membrane integrity in plants (Apel & Hirt 2004). The MDA content, product of lipid peroxidation, has been considered a biochemical indicator of oxidative damage (Apel & Hirt 2004). In this study, the MDA and electrolyte leakage levels increased with the *Orobanche* infestation in the Badi plants, but did not change significantly in the infested Najeh and Chourouk plants compared to the non-infested control plants. Similarly, according to Demirbas and Acar (2017), the MDA content increased 5 times with a *P. ramosa* infestation in Arabidopsis plants. The effect of the *Orobanche* infestation on the membrane integrity could be related to changes in the lipid content and fatty acid profiles. An *Orobanche* infestation was found to induce a significant decrease in the TL content only in the Badi leaves infested by *O. foetida*. With *O. crenata*, this decrease was not significant. However, for the resistant varieties, no significant changes were observed in the TL content. A decrease in the TL content has been reported in other pathosystems such as the faba bean – *O. crenata* (Mostafa et al. 1982) and *Brassica juncea* – *Cuscuta*

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reflaxa (Mishra & Sanwal 1992). Contrary, Sharma et al. (1985) reported a higher lipid content in the host plants *Medicago sativa* Linnaeus., *Helianthus annuus* Linnaeus., *P. sativum* and *Lantana camara* Linnaeus infested with *C. reflaxa*. The decreased lipid levels in Orobanche infested Badi plants may be due to higher degradation of the lipids.

The faba bean leaf tissues showed that the linolenic acid was the predominant fatty acid. Arbaoui & Link (2008) and Ryu et al. (2017) reported that linolenic acid is the main fatty acid in the leaves of different faba bean genotypes. The variation in the fatty acid profile of the faba bean leaves, as a result of infestation by *O. crenata* and *O. foetida*, was significant. In the susceptible Badi plants, the saturated fatty acids increased with a significant decrease in the unsaturated fatty acids upon the infestation. Similar results have been reported by Mishra & Sanwal (1992), who showed that the percentage of unsaturated fatty acids in *B. juncea* decreased 23% upon infestation by *Cuscuta*. However, the percentage unsaturation did not change or increase in the two resistant varieties in response to the *Orobanche* attack. Moreover, it has been suggested that the high level of unsaturation may be required to maintain the degree of fluidity needed for the diffusion of lipophilic compounds and/or may confer a suitable geometry to the lipid molecules. Thus, the unchanged lipid unsaturation observed in the resistant plants submitted to the Orobanche infestation may indicate the conservation and stability in the membrane fluidity. The accumulation of the polyunsaturated acids (18 : 3) was higher in the resistant varieties Najeh and Chourouk than in the susceptible control Badi. Hence, higher desaturase enzymes activities may be observed in the resistant varieties than in Badi leaves. Nevertheless, this hypothesis should be tested by further analyses on the enzyme activities in the three varieties.

CONCLUSION

It may be concluded that the use of resistant faba bean varieties significantly decreased negative effect of the *Orobanche* parasitism. This was mainly correlated with an efficient enzymatic antioxidative defence system, mainly with the involvement of the GPOX activity that (*i*) controlled the ROS overproduction via H_2O_2 consumption which was manifested in a stability of lipid peroxidation and electrolyte leakage, and (*ii*) strengthened the cell wall via the lignification process which protects plant from oxidative damage and limits the *Orobanche* infection capacities. Therefore, these two varieties could be used as a component of an integrated control strategy to improve plant growth under an *Orobanche* infestation.

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