



**Full Length Article**

## Release of a New Faba Bean Variety "Chourouk" Resistant to the Parasitic Plants *Orobanche foetida* Poir. and *Orobanche crenata* Forsk. in Tunisia

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### Abstract

Chourouk is a new faba bean variety (*Vicia faba* L. var. Minor) selected for resistance to broomrapes. It was developed from a cross between orobanche resistant breeding line selected by INRAT "XBJ90.03-20-1-1-1-1-1-D" and "19TB", selected by INRA France for its resistance to *Ascochyta blight* and low tannin content and registered in the Tunisian plant variety catalogue. In field highly infested with *Orobanche foetida*, Chourouk produced 1.71 t/ha grain yield, representing 4.2 and 6.5 times the yield recorded on the susceptible checks Bachaar (0.41 t/ha) and Badi (0.26 t/ha) cultivars. It is more productive (42.5%) than the resistant check Najeh cultivar (1.2 t/ha). In non-infested field, *O. foetida* caused only 31.8% average yield losses for Chourouk against 55.5%, 86.2% and 87.3% for the resistant check Najeh and the susceptible ones Bachaar and Badi, respectively. It showed also good resistance level against *O. crenata* and *O. foetida* under controlled conditions. A maximum of 11.3% of *O. foetida* seeds germination rate was recorded for Chourouk against 71.6% for Badi and 6% for Najeh. The difference in the behavior recorded for Chourouk under field and controlled conditions compared to Najeh suggests that other resistance mechanisms are involved in the resistance and merit to be studied in the future. © 2018 Friends Science Publishers

**Keywords:** Chourouk; *Orobanche crenata*; *Orobanche foetida*; Resistance; Tunisia; *Vicia faba*

### Introduction

The broomrapes (*Orobanche* spp. and *Phelipanche* spp.) are troublesome holoparasitic plants, completely dependent on their hosts for their nutritional requirements; water and nutrients. *Orobanche crenata*, *O. cumana*, *O. foetida*, *O. minor*, *Phelipanche aegyptiaca* and *P. ramosa* are the most economically damaging species and cause severe yield losses on many crops especially in the Mediterranean region. In Tunisia, *O. foetida*, *O. crenata*, *O. cumana*, and *P. ramosa* were found parasitizing many cultivated crops (Kharrat *et al.*, 1992; Kharrat, 2002; Amri *et al.*, 2012, 2013). *O. crenata* was mentioned for a long time as a serious problem for many legume crops in Tunisia, but *O. foetida* has been described as an emerging problem for legume production especially faba bean (Kharrat *et al.*, 1992; Kharrat, 2002). Nowadays, *O. foetida* and *O. crenata* have become major problems not only for faba bean but also for many other legume crops such as chickpea (*Cicer arietinum* L.), lentil (*Lens culinaris* Medik.), grass pea

(*Lathyrus sativus* L.), medick (*Medicago truncatula* and *M. scutellata*), common vetch (*Vicia sativa* L.) and narbon vetch (*Vicia narbonensis* L.) (Nadal *et al.*, 2008; Trabelsi *et al.*, 2016).

Climate change resulted recently in increasing broomrape damages on many potential and strategic crops with an ever-increasing infestation level of many fields discouraging farmers to grow any more susceptible crops particularly legumes. The current infested area in Tunisia is estimated to cover more than 50,000 ha located in the main grain legume production area (Fig. 1). Several potential methods and strategies; chemical, agricultural and biological were used and divulged to farmers but unfortunately for several reasons, all strategies resulted in incomplete protection and broomrape remains as an uncontrolled agricultural problem. The most effective way to fight orobanche is through an integrated management approach based mainly on the selection of genetic material carrying tolerance to orobanche. During the last decades, several varieties/lines were developed and released in many

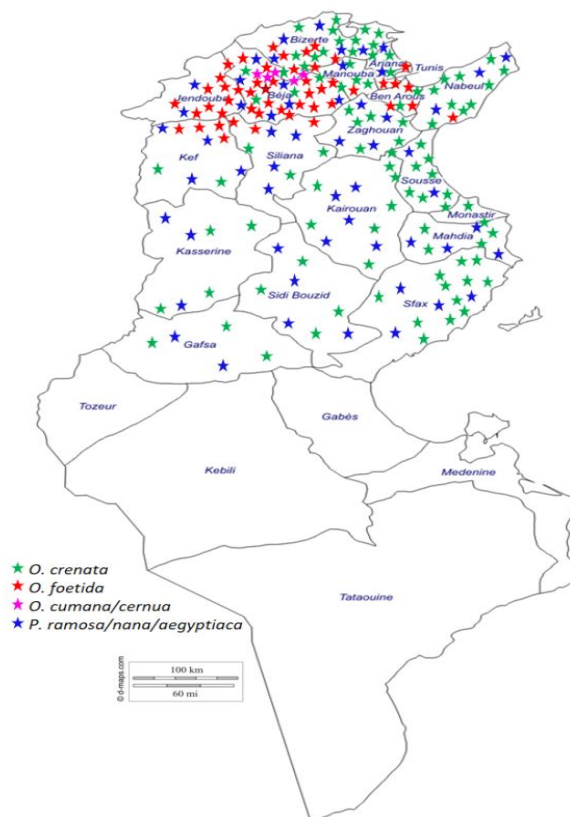
countries; Baraca, VF1071, VF136 and VF172 in Spain, Giza 4, Giza 842, Sakha, Misr1 (667/153/87), Misr3(X-1722) and Cairo 4, Cairo 5 and Cairo 25 in Egypt (Abdalla and Darwish, 2008; Attia et al., 2013; Rubiales et al., 2014). Numerous studies showed that different mechanisms were involved in the resistance such as low number of underground and emerged orobanche, reduced seed germination stimulant production, host plant root architecture and physical barrier (Perez-De-Luque et al., 2005; 2006a; Abbas et al., 2010; 2011; Fernandez-Aparicio et al., 2014; Trabelsi et al., 2015, 2017).

In Tunisia, a breeding program aiming to the development of faba bean varieties tolerant to *O. foetida* was initiated during the past several years at the Institut National de la Recherche Agronomique de Tunisie (INRAT). The first partially resistant faba bean cultivar ('Najeh') was released in 2009 and registered it in the Tunisian Official Catalogue of Plant Variety (Kharrat et al., 2010). In 2014, a new faba bean small seeded variety "Chourouk" (XAR-VF00.13-10-2-3-1-2-1) was registered also in the Tunisian National Plant Variety Catalogue (JORT N°42, May 26<sup>th</sup>, 2015; COV N° 117, March 02<sup>nd</sup>, 2015). It was developed from a cross performed by INRAT (National Institute for Agricultural Research of Tunisia) at Ariana, Tunisia in 2000 and selected after several years of single plant selection in different orobanche infested plots. The purpose of the present research work was to report the performances of the newly developed variety Chourouk under field and controlled conditions.

## Material and Methods

### Cross Information and Selection Process

The new released faba bean variety Chourouk (XAR-VF00.13-10-2-3-1-2-1) was obtained from a cross performed at INRAT, Ariana - Tunisia in 2000 between the orobanche resistant breeding line selected by INRAT "XBJ90.03-20-1-1-1-1-1-D" used as a female parent and the male parent "19TB", a white flower breeding line selected by INRA France for its resistance to *Ascochyta* blight and low tannin content. The main objective of the cross was to develop new faba bean material resistant/tolerant to orobanche and major fungal diseases. The female parent XBJ90.03-20-1-1-1-1-1-D was developed from a cross performed at Beja (INRAT) - Tunisia in 1990 using the line Sel.88Lat.18025 which carries resistance genes to *O. crenata* and coming from the Egyptian line Giza 402 (F-402). A single plant selection was followed since F2 for 6 generations and was conducted in highly infested *O. foetida* plot at Oued Beja experimental station under insect-proof tunnel (8 × 64m). The process of purification with negative selection (elimination of plants shown emerged broomrapes) was conducted during several years in isolated in small insect proof cages (4.5 × 8m). The progeny of individual selected plants was sown in a single



**Fig. 1:** Distribution of different *Orobanche* and *Phelipanche* species in Tunisia

row 2.5 m length and 0.5 m apart in presence of frequently repeated susceptible (cv. Badi) and resistant (cv. Najeh) faba bean checks. Selection was based on the resistance to major fungal diseases and mainly the resistance level to *O. foetida*. Only free emerged orobanche plants with high pod setting were selected and the same process was applied and repeated during six subsequent cropping seasons. The process of purification with negative selection (elimination of plants shown emerged broomrapes) was conducted during several years in small insect proof cages (4.5 × 8m) and started in F7:8 (2007–2008 cropping season). The best line (row) showing the highest resistance level to orobanche and based on the number of emerged orobanche shoots and agronomic traits especially pod setting and grain yield were selected and harvested in single plants (at least 16 single plants) that were kept in pods without threshing. The remaining plants for the same selected row were bulked. During the next cropping season, the selected rows (single plants) were planted each in isolated small 4.5x8 m insect-proof cage. Sixteen different single plants were sown separately in 16 different 4 m rows with 0.5 m inter-rows spacing. For each row/single plant 4 different pods (3-4 seeds) were chosen for their uniformity and sown separately in one 4 m row. At the end of the cropping season (harvesting time) and among the 16 single plants/row, only

the homogeneous ones showing the best resistant level and good agronomic traits were selected and harvested in 8-10 single plants that were kept in pods without threshing and were used to do the same process the subsequent cropping season. The remaining plants from the same selected rows were bulked after eliminating those showing emerged broomrapes for conducting preliminary yield trials. The same procedure was repeated in subsequent years in a field naturally and highly infested with *O. foetida* for purification, generation advance process and seed increase.

### Field Evaluation Yield Trials

One location preliminary yield trial was conducted since cropping season 2009-2010 in *O. foetida* infested field at Oued Beja research station. Then multi-locations advanced yield trials (2010-2011 and 2011-2012) were conducted either in infested fields or in non-infested field for yield traits performance. The trials were conducted according to a randomized complete block design (RCBD) with three replications under different environmental conditions at Oued Beja (36°43'N; 9°13'E, sub-humid) and Oued Meliz (36°28' N, 8°29'E, semi-arid) research stations. The trial included as checks all Tunisian registered small seeded varieties (cvs. "Badi" and "Bachaar" susceptible to orobanche and "Najeh", partially resistant to orobanche).

At Oued Beja, two trials were conducted and planted at the same day in both free and *O. foetida* infested field, however at Oued Meliz, the trials were implemented in free orobanche soil. The seeds of each genotype were sown in 4 rows plot of 4 m length and 0.5 m inter row spacing at a density of 24 seeds per m<sup>2</sup>. Sowing took place during the last week of November. No herbicides were applied on the trials after plant emergence and only hand weeding was carried out. The monthly rainfall and average temperature records for the two cropping seasons (2010-2011 and 2011-2012) collected from the METHOS meteorological are presented in Table 1.

Resistance/tolerance to orobanche was evaluated based on several parameters recorded at different development stages of the host plants. Number of days to orobanche emergence (NDOE) recorded for each plot/variety when at least one orobanche shoot appears at the soil surface. The NDOE was calculated as the number of days from sowing to orobanche emergence. Before harvesting, both orobanche infestation incidence (percentage of faba bean plants presenting at least one emerged orobanche shoot per plot) and orobanche severity using a 1 to 9 scale (where 9 represents the highest degree of susceptibility) were recorded (Abbes *et al.*, 2007). At harvesting time, the number of total faba bean plants and emerged orobanche shoots and dry weight were recorded only for the two central rows. Based on the collected data orobanche shoot number (ONP) and dry weight (ODWP) per faba bean plant were calculated. Finally, the grain yield (gm<sup>-2</sup>) was recorded on the same two central rows for each plot.

### Pot Experiment

Seeds of faba bean genotypes (Chourouk, Najeh and Badi) were surface sterilized with calcium hypochlorite (5%) during 15 min then rinsed five times with sterilized distilled water. Artificial inoculation was carried out by mixing uniformly 20 mg of *O. foetida* or *O. crenata* per kg of soil. Faba bean seeds previously disinfected were transferred in 5 L capacity pots (free and infested by fetid or crenate broomrapes). Five pots per genotypes were used for each treatment. Pots were placed under natural conditions at Ariana during 2011-2012 cropping season. Pots were irrigated with tap water when necessary. Three month later, at the pod setting stage, faba bean plants were uprooted from the soil and washed carefully. The total orobanche tubercles number (TONP) and their dry weight (TODWP) per plant were determined. TODWP was recorded after being dried in an oven at 70°C during 72 h.

### Mini-rhizotron Experiment

Faba bean genotypes were evaluated in mini-Rhizotron cocultures carried out in quadratic plastic dishes (120 × 120 × 17 mm, Greiner) as described by Trabelsi *et al.* (2016). *O. foetida* seeds were surface sterilized for 5 min in sodium hypochlorite (2%) and rinsed five times with sterile distilled water. For each genotype six quadratic plastic Petri dishes were filled with sterilized sand and covered with moistened sterilized glass fiber filter paper. An amount of 20 mg of orobanche seeds were used for each Petri dish and spread carefully over the glass fiber filter paper surface. Faba bean seeds previously disinfected as described for pots experiment were sown in water agar and incubated in darkness conditions at 22 ± 3°C for germination. Seedlings were transferred to the plastic Petri dishes. The whole was placed in containers covered with aluminum foil and maintained under natural light at 22 ± 3°C and in humidity 78% in the green house. Petri dishes were watered with modified nutrient solution (Vincent, 1970) with reduced amount of nitrogen. The germination rate of orobanche seeds was determined closely to the faba bean roots 45 days after inoculation under a binocular microscope. Broomrape attachments were recorded also after 66 days.

### Statistical Analysis

ANOVA was performed using the SPSS statistical program v.23 and differences among treatments for all measurements were compared at  $P=0.05$  and by using Duncan's Multiple-Range Test.

### Results

At Oued Beja and Oued Meliz experimental stations and in absence of orobanche infestation, Chourouk produced an average grain yield of 2.65 t/ha, whereas the others faba

**Table 1:** Min, Max and average temperature (°C) and rain (mm) monthly recorded at Oued Beja research station during 2010/11 and 2011/12 cropping seasons

Cropping seasons	Temp./Rain	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Average	Total
2010-11	Temp. min	17.3	13.6	10.7	6.2	5.6	5.1	6.4	9.5	12.3	15.2	10.2	-
	Temp. max	30.7	26.7	20.8	17	15.9	15.5	18.3	23.5	26.4	31.5	22.6	-
	Rain (mm)	42.7	82	56.8	61.6	63.2	138.4	58.3	38.6	43.4	7.4	-	592.4
2011-12	Temp. min	18.4	13.5	11.5	7.6	6.9	3.9	6.5	10	11.2	17.9	10.7	-
	Temp. max	32.3	25.5	20.6	16.3	14.9	12.5	19.1	22.3	27.8	36.3	22.8	-
	Rain (mm)	20.3	113.8	110.2	188.9	76.3	187.2	65.4	136.8	2.2	0	-	901.1

**Table 2:** Grain Yield (t/ha) recorded for the studied genotypes under free orobanche fields at both research stations Oued Beja (OB) and Oued Meliz (OM) during 2010/11 and 2011/12 cropping seasons

	OB 2010/11	OB 2011/12	OB Average	OM 2010/11	OM 2011/12	OM Average	OB & OM Average
Badi	2.19±0.43 <sup>a</sup>	1.59±0.34 <sup>a</sup>	1.89	3.60±1.35 <sup>a</sup>	2.89±0.39 <sup>a</sup>	3.24	2.56
Bachaar	3.8±0.49 <sup>c</sup>	1.67±0.22 <sup>a</sup>	2.74	4.7±1.16 <sup>a</sup>	2.87±1.47 <sup>a</sup>	3.78	3.26
Najeh	3.0±0.16 <sup>b</sup>	1.65±0.09 <sup>a</sup>	2.33	3.55±1.21 <sup>a</sup>	2.12±0.88 <sup>a</sup>	2.84	2.58
Chourouk	2.66±0.47 <sup>ab</sup>	2.22±0.54 <sup>a</sup>	2.44	3.51±0.78 <sup>a</sup>	2.2±0.66 <sup>a</sup>	2.85	2.65

Data are three replication means ± SD

Data with the same letter per column are non-significantly different at P=0.05 (Duncan test)

**Table 3:** Grain Yield (t/ha), reduction percentage (%) as compared to free Orobanche field recorded for the studied genotypes under highly *O. foetida* infested field at Oued Beja research station during 2010/11 and 2011/12 cropping seasons

	OB 2010-11	OB 2011-12	Average	Percentage compared to Najeh (%)	Reduction (%) as compared to OB free orobanche field		
					2010-11	2011-12	Average
Badi	0.44±0.22 <sup>a</sup>	0.08±0.06 <sup>a</sup>	0.26	21.7 (-78.3)	79.9±10.2 <sup>b</sup>	94.8±3.8 <sup>b</sup>	87.3
Bachaar	0.64±0.23 <sup>a</sup>	0.18±0.05 <sup>a</sup>	0.41	34.1 (-65.9)	83.2±6 <sup>b</sup>	89.2±2.8 <sup>b</sup>	86.2
Najeh	2.09±0.39 <sup>b</sup>	0.32±0.3 <sup>a</sup>	1.2	100	30.4±13 <sup>a</sup>	80.6±18.3 <sup>b</sup>	55.5
Chourouk	2.34±0.41 <sup>b</sup>	1.08±0.45 <sup>b</sup>	1.71	142.5 (+42.5)	12±15.5 <sup>a</sup>	51.7±20.2 <sup>a</sup>	31.8

Data are three replication means ± SD

Data with the same letter per column are non-significantly different at P=0.05 (Duncan test)

bean varieties Badi, Bachaar and Najeh respectively yielded 2.56, 3.26 and 2.58 t/ha (Table 2). Under highly *O. foetida* infestation level in Oued Beja experimental station, Chourouk showed a relatively high grain yield production. For cropping seasons 2010/11 and 2011/12, the average yield 1.71 t/ha was recorded for Chourouk against 1.2, 0.41 and 0.26 t/ha respectively recorded for the resistant check Najeh and both susceptible checks Bachaar and Badi (Table 2). The yield produced by Chourouk under orobanche infested field represents 4.2 and 6.5 times the yield recorded for the susceptible checks Bachaar and Badi, respectively. Compared to the resistant check Najeh, the new variety Chourouk produced 42% more grain yield under high orobanche infestation level. The decreases in yield registered on Bachaar and Badi were respectively 65.9% and 78.3% as compared to Chourouk (Table 3).

The evaluation of the 4 varieties at Oued Beja experimental station during the two cropping seasons in *O. foetida* naturally infested field and in free-orobanche field (sowing was done in the same date) showed that Orobanche induced 31.8% mean grain yield loss for the new variety Chourouk, whereas the losses were 55.8% for Najeh, 86.2% for Bachaar and 87.3% for Badi. In *O. foetida* infested field, the new variety Chourouk showed significantly low infection level as compared to the susceptible checks Bachaar and Badi (Fig. 4). During the two cropping seasons

2010/2011 and 2011/2012, an average orobanche incidence of 66.7% was observed for Chourouk against 97.5% and 94.2% recorded respectively for Bachaar and Badi. A relatively low orobanche severity attack (4) was recorded for Chourouk against 4.8 for Najeh and 7.2 for both Bachaar and Badi (Table 4). Also, for the new variety Chourouk orobanche emergence occurred 3-4 days after Bachaar and Badi and 1-1.5 days before Najeh (Table 5). Under field conditions, only one emerged orobanche spikes per plant was observed in average for the variety Chourouk during the two cropping seasons against 2.4 and 2.1 spikes recorded respectively for the susceptible checks Bachaar and Badi. Average emerged orobanche dry weight per plant of 2.9 g, 6 g and 5.5 g were observed respectively for the new variety Chourouk and both susceptible checks Bachaar and Badi. The resistant check Najeh showed a lower infestation level as compared to chourouk with 0.7 emerged spike per plant and 1.8 g orobanche dry weight per plant.

Under controlled conditions in pot experiment, the new variety Chourouk showed a high level of resistance to both *O. crenata* and *O. foetida* species (Table 6). Compared to cv. Badi for which 28.7 (*O. foetida*) and 8.4 (*O. crenata*) tubercles per plant were counted in average, only 1.6 and 0.4 tubercles in average per plant, respectively, were recorded for the new variety Chourouk with almost the same behavior as the resistant check Najeh (0.4 for *O. foetida* and 0.8 tubercles for *O. crenata*).

**Table 4:** Orobanche incidence and severity recorded for the studied genotypes under highly *O. foetida* infested field at Oued Beja research station during 2010/11 and 2011/12 cropping seasons

	Orobanche incidence (%)			Orobanche severity (1 - 9)		
	2010/11	2011/12	Average	2010/11	2011/12	Average
Badi	100±0 <sup>b</sup>	95±5 <sup>b</sup>	97.5	6.3±1.2 <sup>b</sup>	8±1 <sup>b</sup>	7.2
Bachaar	100±0 <sup>b</sup>	88.3±16.1 <sup>ab</sup>	94.2	7±2 <sup>b</sup>	7.3±0.6 <sup>ab</sup>	7.2
Najeh	60±26.5 <sup>a</sup>	53.3±33.3 <sup>a</sup>	56.7	3.7±1.2 <sup>a</sup>	6±1 <sup>ab</sup>	4.8
Chourouk	50±20 <sup>a</sup>	83.3±15.3 <sup>ab</sup>	66.7	3±0 <sup>a</sup>	5±2 <sup>a</sup>	4

Data are three replication means ± SD

Data with the same letter per column are non-significantly different at  $P=0.05$  (Duncan test)

**Table 5:** Number of days to Orobanche emergence, Orobanche tubercle number and dry weight per plant recorded for the studied genotypes under highly orobanche infested field at Oued Beja research station during 2010/11 and 2011/12 cropping seasons

	Number of days to orobanche emergence (NDOE)			Orobanche number per plant (ONP)			Orobanche dry weight per plant (ODWP)		
	2010/11	2011/12	Average	2010/11	2011/12	Average	2010/11	2011/12	Average
Badi	136±1.7 <sup>a</sup>	134.7±1.2 <sup>a</sup>	135.3	2.2±0.4 <sup>c</sup>	1.9±0.5 <sup>a</sup>	2.1	6.5±0.2 <sup>b</sup>	4.6±2.1 <sup>a</sup>	5.5
Bachaar	137±3 <sup>a</sup>	135.3±1.2 <sup>a</sup>	136.2	1.6±0.2 <sup>b</sup>	3.3±3.4 <sup>a</sup>	2.4	4.7±1.2 <sup>b</sup>	7.4±7.4 <sup>a</sup>	6
Najeh	143.7±2.3 <sup>b</sup>	138±4.4 <sup>a</sup>	140.8	0.5±0.2 <sup>a</sup>	0.9±0.8 <sup>a</sup>	0.7	2.1±1.5 <sup>a</sup>	1.5±1.2 <sup>a</sup>	1.8
Chourouk	141.7±5 <sup>ab</sup>	136.7±3.1 <sup>a</sup>	139.2	0.4±0.2 <sup>a</sup>	1.7±0.7 <sup>a</sup>	1	1.2±0.6 <sup>a</sup>	4.5±2 <sup>a</sup>	2.9

Data are three replication means ± SD

Data with the same letter per column are non-significantly different at  $P=0.05$  (Duncan test)

**Table 6:** Total Orobanche tubercles number and dry weight per plant recorded for the studied genotypes in pot experiment

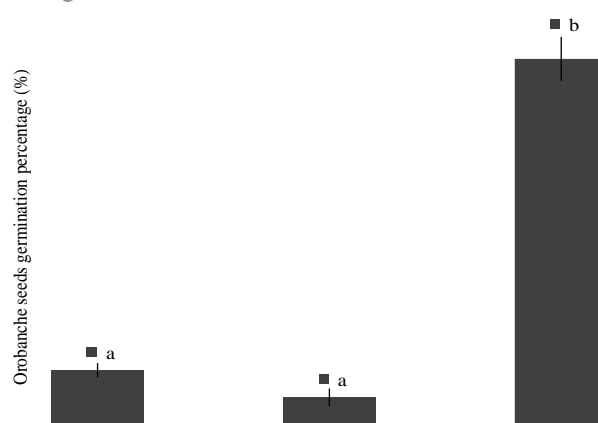
	Total Orobanche number per plant (TONP)		Total Orobanche dry weight per plant (TODWP)	
	<i>O. foetida</i>	<i>O. crenata</i>	<i>O. foetida</i>	<i>O. crenata</i>
Badi	28.7±9.3 <sup>b</sup>	8.4±1 <sup>b</sup>	2.98±0.95 <sup>b</sup>	2.42±0.53 <sup>b</sup>
Najeh	0.4±0.4 <sup>a</sup>	0.8±0.6 <sup>a</sup>	0.01±0.01 <sup>a</sup>	0.07±0.06 <sup>a</sup>
Chourouk	1.6±0.9 <sup>a</sup>	0.4±0.2 <sup>a</sup>	0.52±0.33 <sup>a</sup>	0.05±0.03 <sup>a</sup>

Data are three replication means ± SD

Data with the same letter per column are non-significantly different at  $P=0.05$  (Duncan test)

In mini-rhizotron experiment, low *O. foetida* seed germination was observed in the proximity of Chourouk and Najeh roots compared to Badi (Fig. 2). *O. foetida* seeds germination rate of 71.6% was recorded for the susceptible check Badi against only 11.3% and 6% observed for Chourouk and Najeh, respectively. After 66 days' experiment, 27.4 tubercles were recorded on the susceptible check Badi against only 1.4 and 0.2 tubercles recorded on Chourouk and Najeh, respectively (Fig. 3).

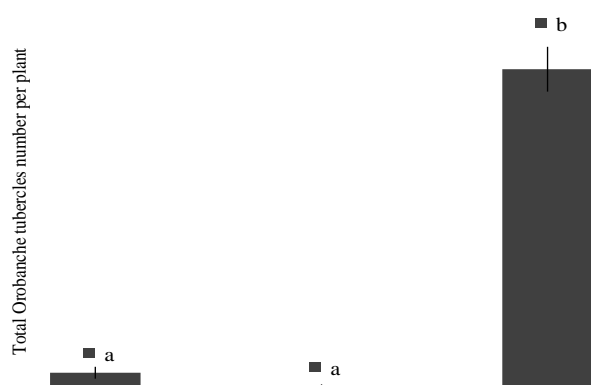
Chourouk is an early flowering variety. It flowers 6 to 8 days earlier than cvs. Bachaar and Badi and 1 to 2 days than cv. Najeh. For maturity, Chourouk is earlier than cv. Badi. It has indeterminate growth with an average height around 1 m and well convenient for mechanical harvesting. The variety has a weak anthocyanin pigmentation on the stem and the flower standard and presents a black melanin spot on the wings. The leaflet has average width and presents a central position of the maximum width. The pods are pubescent and in general short to medium (6-8 cm) containing 3 or 4 regular, beige and black hilum seeds (Fig. 4). Chourouk's 100 seed weight is almost the same as for cv. Najeh (60-65 g), slightly higher than cv. Bachaar (55-60 g) and cv. Badi (50-55 g). Chourouk has average resistance to major fungal diseases; ascochyta blight, chocolate spot and rust.

**Fig. 2:** Orobanche seed germination percentage (%) recorded for the new released variety Chourouk compared to both susceptible (Badi) and resistant (Najeh) checks under controlled conditions in mini-rhizotron experiment

## Discussion

The new variety "Chourouk" showed a quite high resistance level to orobanche under both field and controlled conditions. Under highly orobanche infested field, was





**Fig. 3:** Total Orobanchae tubercles number per plant recorded 66 days after inoculation for Chourouk, Badi and Najeh in mini-rhizotron experiment. Data are three replication means  $\pm$  SD; Data with the same letter per column are non-significantly different at  $P=0.05$  (Duncan test)



**Fig. 4:** Pod aspect and appearance of the new released variety Chourouk compared to Badi, Bachaar and Najeh

significantly superior in grain yield and parameters related to orobanche resistance such as emerged orobanche number and dry weight, incidence and severity compared to the susceptible faba bean varieties (Badi and Bachaar). Immunity for broomrape infection in faba bean is inexistent and only partial resistance is reported (Rubiales, 2014). Various mechanisms of resistance has been reported through numerous studies such bio-chemicals related to germination stimulants or inhibitors production and physicals (Rubiales *et al.*, 2003; Perez-De-Luque *et al.*, 2005; 2006a, b; Abbas *et al.*, 2009a, b, 2010, 2011; Fernandez-Aparicio *et al.*, 2014; Trabelsi *et al.*, 2015, 2016, 2017). The performance of Chourouk for resistance is quite similar than Najeh, registered in 2009 in Tunisia. Results showed that under controlled conditions, the new variety Chourouk seems to be slightly less resistant to both orobanche species than cv. Najeh but no significant differences were observed between both varieties for the previous studied resistance parameters.

Despite, the better resistance level observed for cv. Najeh, the new variety Chourouk was significantly more

productive under field conditions with 42.5% higher grain yield. Previous studies demonstrated that the resistance observed for cv. Najeh is allied to low orobanche seed germination stimulant production by the roots (Abbas *et al.*, 2006, 2007; Trabelsi *et al.*, 2017) and a deeper root system and their architecture that can escape to orobanche attachments and guarantee nutrients uptake for the host plant (Amri *et al.*, 2007 non-published data). Also, it is important to mention that low or high orobanche seeds germination rate could not be taken as an imperative indicator of resistance/susceptibility to orobanche for faba bean and other legumes species. Thus, some resistant legume species presented the same orobanche seeds germination percentage or even higher than those observed on a susceptible accession (Abbas *et al.*, 2006; 2008; Nefzi *et al.*, 2016; Trabelsi *et al.*, 2017).

The partially resistance level of the new developed variety Chourouk under field and controlled conditions and its yield performance was the main reason for its registration in the Tunisian plant variety catalogue. Chourouk was developed through a classical breeding approach which needs to be confirmed by new molecular tools. Further advanced studies are recommended to explore the resistance mechanisms involved, especially that the behavior of Chourouk is slightly different under field and controlled conditions than Najeh suggesting that other potential resistance mechanisms could be taken place after orobanche germination and attachment on the root system and that merit to be identified.

## Conclusion

Facing the exponential increase of orobanche infested areas, the introduction of this new variety into farmer fields is extremely urgent in order to (i) limit the damages and yield losses and secure yield for farmers under orobanche parasitism and (ii) to contribute to the rehabilitation of grain legumes especially faba bean in many favorable regions in Tunisia where, due to orobanche problem, many farmers abandoned growing faba bean and other legumes and substituted them by other crops, especially cereals, which are not hosts of orobanche. Also, the new developed variety Chourouk could be introduced into Tunisian and other national faba bean breeding programs in the region as source of resistance to *O. crenata* and *O. foetida* and subjected to advanced and comparative studies using new tools in order to increase knowledge on the different resistance mechanisms involved in this plant-pathogen interaction.

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## References

- Abbes, Z., M. Kharrat and W. Chaïbi, 2006. Study of the interaction between *Orobanche foetida* and faba bean at root level. *Tunis. J. Plant Prot.*, 1: 55–64
- Abbes, Z., M. Kharrat, P. Delavault, P. Semier and W. Chaïbi, 2007. Field evaluation of the resistance of some faba bean (*Vicia faba* L.) genotypes to the parasitic weed *Orobanche foetida* Poiret. *Crop Prot.*, 26: 1777–1784
- Abbes, Z., M. Kharrat and W. Chaïbi, 2008. Seed Germination and tubercle development of *Orobanche foetida* and *Orobanche crenata* in presence of different plant species. *Tunis. J. Plant Prot.*, 3: 101–109
- Abbes, Z., M. Kharrat, P. Delavault, W. Chaïbi and P. Simier, 2009a. Nitrogen and carbon relationships between the parasitic weed *Orobanche foetida* and susceptible and tolerant faba bean lines. *Plant Physiol. Biochem.*, 47: 153–159
- Abbes, Z., M. Kharrat, P. Delavault, W. Chaïbi and P. Simier, 2009b. Osmoregulation and nutritional relationships between *Orobanche foetida* and faba bean. *Plant Signal. Behav.*, 4: 1–3
- Abbes, Z., M. Kharrat, K. Shaaban and B. Bayaa, 2010. Comportement de différentes accessions améliorées de féverole (*Vicia faba* L.) vis-à-vis d'*Orobanche crenata* Forsk. et *Orobanche foetida* Poir. *Cah. Agric.*, 19: 194–199
- Abbes, Z., F. Sellami, M. Amri and M. Kharrat, 2011. Variation in the resistance of some faba bean genotypes to *Orobanche crenata*. *Pak. J. Bot.*, 43: 2017–2021
- Abdalla, M.M.F. and D. S. Darwish, 2008. Investigations on faba bean (*Vicia faba* L.) Cairo 4, Cairo 5 and Cairo 25, new varieties tolerate to *Orobanche*. *Egypt. J. Plant Breed.*, 12: 315–320
- Amri, M., Z. Abbes, S. Ben Youssef, M. Bouhadida, H. Ben Salah and M. Kharrat, 2012. Detection of the parasitic plant, *Orobanche cumana* on sunflower (*Helianthus annuus* L.) in Tunisia. *Afr. J. Biotechnol.*, 11: 4163–4167
- Amri, M., Z. Abbes, M. Bouhadida and M. Kharrat, 2013. First Report of the Parasitic Plant *Phelipanche ramosa* on berseem clover (*Trifolium alexandrinum* L.) in Tunisia. *Tunis. J. Plant Prot.*, 8: 127–132
- Attia, S.M., M.M. El-Hady, H.A. Saber, M.A. Omer, S.A. Khalil, M.A. Samia, A.A.M. Ashrei Rehab, A.M. Abd-Elrahman, M.A.M. Ibrahim Zeinab, E. Ghareeb, T.S. El-Marsafawy, E.H. El-Harty, E.A.A. El-Emam, F.H. Shalaby, A.G. Helal, A.M. El-Garhy, E.M. Rabie, M. Abdeen, M. El-Noby, K.M.M. Yamani, H.T. Abd El-Aal, M.A. Ibrahim, R.A. Abo Mostafa, W. El-Rodeny, K.M. MorsyNoher, A. Mahmoud, A.F. El-Sayedand and H.A. Ghannam, 2013. Misr 3, a New *Orobanche* Tolerant Faba Bean Variety. *Egypt. J. Plant Breed.*, 17: 143–152
- Fernandez-Aparicio, M., T. Kisugi, X. Xie, D. Rubiales and K. Yoneyama, 2014. Low strigolactone root exudation: a novel mechanism of broomrape (*Orobanche* and *Phelipanche* spp.) resistance available for faba bean breeding. *J. Agric. Food Chem.*, 62: 7063–7071
- Kharrat, M., M.H. Halila, K.H. Linke and T. Haddar, 1992. First report of *Orobanche foetida* Poiret. on faba bean in Tunisia. *Fabis Newslett.*, 30: 46–47
- Kharrat, M., 2002. Etude de la virulence de l'écotype de Béja d'*Orobanche foetida* sur différentes espèces de légumineuses. In: *Proceedings of the 2<sup>nd</sup> Seminar of REMAFEVE/REMALA Network*, p: 89. Hammamet, Tunisia
- Kharrat, M., Z. Abbes and M. Amri, 2010. A new faba bean small seeded variety "Najeh" tolerant to orobanche registered in the Tunisian catalogue. *Tunis. J. Plant Prot.*, 5: 125–130
- Nadal, S., M.T. Moreno and B. Román, 2008. Control of *Orobanche crenata* in *Vicia narbonensis* by glyphosate. *Crop Prot.*, 27: 873–876
- Nefzi, F., I. Trabelsi, M. Amri, M. Kharrat and Z. Abbes, 2016. Response of some chickpea (*Cicer arietinum* L.) genotypes to *Orobanche foetida* Poiret parasitism. *Chil. J. Agric. Res.*, 76: 170–178
- Perez-De-Luque, A., D. Rubiales, J.I. Cubero, M.C. Press, J. Scholes, K. Yoneyama, Y. Takeuchi, D. Plakhine and D.M. Joel, 2005. Interaction between *Orobanche crenata* and its host legumes: unsuccessful haustorial penetration and necrosis of the developing parasite. *Ann. Bot.*, 95: 935–942
- Perez-De-Luque, A., C.I. Gonzalez-Verdejo, M.D. Lozano, M.A. Dita, J.I. Cubero, P. Gonzalez-Melendi, M.C. Risueno and D. Rubiales, 2006a. Protein crosslinking, peroxidase and  $\beta$ -1,3-endoglucanase involved in resistance of pea against *Orobanche crenata*. *J. Exp. Bot.*, 57: 1461–1469
- Perez-De-Luque, A., M.D. Lozano, J.I. Cubero, P. Gonzalez-Melendi, M.C. Risueno and D. Rubiales, 2006b. Mucilage production during the incompatible interaction between *Orobanche crenata* and *Vicia sativa*. *J. Exp. Bot.*, 57: 931–942
- Rubiales, D., A. Pérez-de-Luque, D.M. Joel, C. Alcántara and J.C. Sillero, 2003. Characterisation of resistance in chickpea to *Orobanche crenata* (broomrape). *Weed Sci.*, 51: 702–707
- Rubiales, D., 2014. Legume breeding for broomrape resistance. *Czech J. Genet. Plant Breed.*, 50: 144–150
- Rubiales, D., F. Flores, A.A. Emeran, M. Kharrat, M. Amri, M.M. Rojas-Molina and J.C. Sillero, 2014. Identification and multi-environment validation of resistance against broomrapes (*Orobanche crenata* and *Orobanche foetida*) in faba bean (*Vicia faba*). *Field Crops Res.*, 166: 58–65
- Trabelsi, I., Z. Abbes, M. Amri and M. Kharrat, 2015. Performance of faba bean genotypes with *Orobanche foetida* and *Orobanche crenata* infestation in Tunisia. *Chil. J. Agric. Res.*, 75: 27–34
- Trabelsi, I., Z. Abbes, M. Amri and M. Kharrat, 2016. Response of some selected faba bean (*Vicia faba* L.) genotypes to *Orobanche* spp. in Tunisia. *Plant Prod. Sci.*, 19: 562–573
- Trabelsi, I., K. Yoneyama, Z. Abbes, X. Xie, M. Amri, M. Kharrat and K. Yoneyama, 2017. Characterization of strigolactones produced by *Orobanche foetida* and *Orobanche crenata* resistant and susceptible faba bean genotypes and effect of phosphorous, nitrogen, and potassium deficiencies on strigolactone production. *S. Afr. J. Bot.*, 108: 15–22
- Vincent, J.M., 1970. *A Manual for the Practical Study of Root Nodule Bacteria*. Oxford: Blackwell Scientific

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