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Screening of Faba Bean (*Vicia faba* L.) Germplasm Accessions against Grey Mould Disease Caused by *Botrytis fabae* Sard

Ibrahim Elkhalil Benzohra[1], Hakima Belaidi[2]

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ABSTRACT

Background: Faba bean (*Vicia faba* L.) is thirst most important grain legume in the world and the first one in Algeria. The chocolate spot disease caused by *Botrytis fabae* Sard (BF), is the major constraint of this culture in Algeria when caused a destructive damages. **Methods:** The aim of this study is to find the sources of resistance for Seven varieties of faba beans (Giza 02, Giza 40, Giza 461, Sakha 02, Sakha 03, Precoce de Seville), by using the detached leaflet inoculation test for resistance to the two isolates from BF represent two different regions of northwest Algeria (Mascara and Relizane).

Result: A significant difference (P <0.05) was observed in the reaction of the varieties which manifest themselves by a different reaction vis-à-vis the isolates of BF. Total sensitivity was observed in the four (4) varieties Giza02, Sakha01, Sakha02 and Sakha03, two (2) varieties (Giza40 and Ziban), are tolerant, while the variety named 'Precoce de Seville' showed significant resistance to both isolates from BF. These results showed a similar pathogenic behavior of two isolates of BF and variability in the level of reactions of the varieties of beans. The variety 'Precoce de Seville' showed promising results to be valued and cultivated in order to reduce the damage caused by this disease and reduce the use of chemicals.

Key words: *Botrytis fabae*, Detached leaves, Resistance, Screening, Sensitivity, *Vicia faba* L.

The faba bean (*Vicia faba* L.), is a Fabaceae native to Central Asia, domesticated for 10,000 years (Duc, 1997; Kumar *et al*., 2019) and it is practiced in about 58 countries (Singh *et al*., 2012). It is the fourth most important grain legume in the world behind peas, chickpeas and beans (Yahia *et al*., 2012; Luo *et al*., 2014). This crop has been part of our agricultural systems for a long time; its global area is more than 2,5 million hectares of which more than 50% is located in China, 20% in North Africa and less than 10% in Europe (Abu Amer *et al*., 2011). World production has reached up to 4.92 MT (FaoStat, 2020).

In Algeria, the faba bean remains the most important food crop, covering an area of 40222 hectares with a total production of 54878 tonnes (FaoStat, 2020). Unlike in the world, the faba bean in Algeria is the first place among other grain legumes (Maatougui *et al*., 1994).

Chocolate spot, caused by the fungus *Botrytis fabae* Sard., is the major disease of faba bean (*Vicia faba* L.) in Algeria (Maatougui *et al*., 1994; Ranjana *et al*., 2019). The chemical control against this disease is possible but is not environmentally friendly (Bouhassan *et al*., 2003). An effective control is a research of resistance sources in faba bean germplasm collection and transferred to commercial cultivars via breeding (Villegas-Fernandez *et al*., 2012; Gautam *et al*., 2013). In this case, this mode of control based on plant screening tests like cut-twig, detached leaves, greenhouse and field screenings (Boumaaza *et al*., 2018; Bouhassan *et al*., 2004a).

The present study has the objective to evaluate the resistance sources in seven faba bean gemplasm accessions against two *Botrytis fabae* isolates using detached leaves screening method.

[1]Department of Phoeniciculture, Experimental Station for Biophysical Environment, Bechar-Centre for Scientific and Technical Research on Arid Regions (CRSTRA), Campus Universitaire B.P. 1682 RP, Biskra, Algeria.

[2]Laboratoire Ecodéveloppement des Espaces, Department of Environmental Sciences, Djillali Liabes University of Sidi Bel Abbes, Algeria.

Corresponding Author: Ibrahim Elkhalil Benzohra, Department of Phoeniciculture, Experimental Station for Biophysical Environment, Bechar - Centre for Scientific and Technical Research on Arid Regions (CRSTRA), Campus Universitaire B.P. 1682 RP, Biskra, Algeria. Email: ibrahimelkhalil@live.fr

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Plant material

Seven (07) varieties of different origins were used in the inoculation test: Giza 02, Giza 40, Sakha 01, Sakha 02, Sakha 03, Ziban and Precoce de Seville (Table 1).

Fungal material

Two different strains (BF1 and BF2) of *Botrytis fabae* were used in this screening test, with BF1 from the Mascara region and BF2 from Relizane. The two strains used in this study were obtained by isolation from faba bean leaves with typical chocolate spot symptoms. The *Botrytis fabae* strains were identified by macroscopic (colony color, sclerotia size, *etc*.)

and microscopic (size of conidia, etc.) characteristics, to distinguish it from the other species *Botrytis cinerea* which can causes the same symptoms of chocolate spot disease (Table 2). The BF strains were cultivated on the PDA culture medium (Potato Dextrose Agar, Fischer Ltd., France), at temperature of 22 ± 2°C (Bouhassan *et al*., 2003; 2004a, b).

Test of artificial inoculation

The faba bean seeds used were sterilized with sodium hypochlorite (2%) for 3 minutes and then rinsed 3 times with sterile distilled water. Then, they had sown in 10 cm × 5 cm pots, containing a sterile peat, at the rate of 2 seeds / pot. After 15 days, leaflets can be removed to do the inoculation test at the rate of 2 leaflets for each repetition.

Table 1: List of faba bean germplasm accessions with their origin.

Germplasm	Germplasm type	Origin
Giza 02	Différentielle	ICARDA[a]
Giza 40	Différentielle	ICARDA[a]
Sakha 01	Différentielle	ICARDA[a]
Sakha 02	Différentielle	ICARDA[a]
Sakha 03	Différentielle	ICARDA[a]
Ziban	Population	CRSTRA[b]
Précoce de Séville	Standard	Aci-Alg[c]

a : International Center of Agricultural Research on Dry Areas (ICARDA), Biqaa, Lebanone ; b : Centre for Scientific and Technical research on Arid Regions (CRSTRA), Biskra, Algeria; c: Aci Algeria: Seed Crop Firm.

To prepare the inoculum of two BF isolates, the colonies of isolates were immersed in sterile distilled water taken and then scraped off with a sterile glass spatula. The suspension is adjusted to 3 × 10^6 conidia/ml using Malassez-cell (Bouhassan *et al*., 2004b). Symptoms were assessed 15 days after inoculation of the bean leaflets. The severity of the disease is scored according to the rating scale of Bouhassan *et al*. (2004b), (1, 2, 3, 4 and 5), which respectively represent 0%, 10%, 20%, 50% and 100% of the surface of the necrosis on leaflet. This scale is based on the percentage of the area of the infection relative to the entire area of the leaflet. With faba bean leaflets inoculated, we are classified into interval classes, the leaflets of seedlings of varieties marked from 1.0 to 2.9 are considered resistant and those which are coded from 3.0 to 5 are sensitive (Bouhassan *et al*., 2004b).

Statistical analysis

Thevariances (σ^2), averages (x) and standard deviation (σ) of manyrepetitionswere analyzed by the software XLStat (Addin Soft, USA). The no parametric testof K samplesof Kruskal-Wallis ($P_{0.05}$ et $P_{0.01}$), was used in this statistical analysis.

All symptom ratings were collected only after 7 days of inoculation. A significant difference (P <0.05) was observed in the reaction of the varieties to two strains of *Botrytis fabae* (BF1 and BF2), (Table 3). According to the results of the screening test based on the rating of symptoms from the percentage of symptom intensity, the results are as follows:

Table 2: Macro and microscopic characteristics to distinct between *Botrytis fabae* and *B. cinerea* (Zhang *et al*., 2010).

Characteristics	*Botrytis fabae*	*Botrytis cinerea*
Types of colonies	Cotony	flutty
Sclerotia colour	Black	black
Sclerotia number per petri dish	500-1100	30 - 150
Sclerotia diameter (mm)	0.4 - 3 × 0.3 - 2.1	0.7 - 27.5 × 0.5 - 12
Conidia diameter (µm)	12.9 - 22.7 × 10.5 - 18.5	7.7 - 12.2 × 7.3 - 9.1
Conidiophore measures (µm)	1005 - 1885 × 14 - 17	935 - 1745 × 14 - 17

Table 3: Reaction of 07 faba bean varieties against *Botrytis fabae*.

Faba bean varieties	Scale notations [a] ($x \pm \delta$)			
	BF1	Reaction	*BF2*	Rraction
Giza 02	3.66[c]±0.5	S	3.66[c]±0.5	S
Giza 40	2 .33[d]±0.5	S	4 .66[d]±0.5	S
Sakha 01	3 .66[c]±0.5	S	3 .66[c]±0.5	S
Sakha 02	3[b]	S	2.33[b]±0.5	R
Sakha 03	3[b]	S	1.66[a]±0.5	R
Ziban	3.33[c]±0.5	S	2.33[b]±0.5	R
Precoce de Seville	1.66[a]±0.5	R	1.66[a]±0.5	R
Global reaction	3.23	2.85		
LSD	16,35*	20,09**		
P	0,012	0,003		

*: Significant effect at 5%; **: Significant effect at 1%; a: This reaction was found by the interval's of rating scales reported by Bouhassan *et al.*, 2004a. We have two interval's: 1-2.9: resistant variety (R) ; 3-5 : v. sensible (S); [a, b, c, d] : Homogenate groups ; BF1, BF2 : *B. fabae* isolates; x: mean observation of reaction; δ: Standard deviation; LSD: Least significant difference; *P*: Probability.

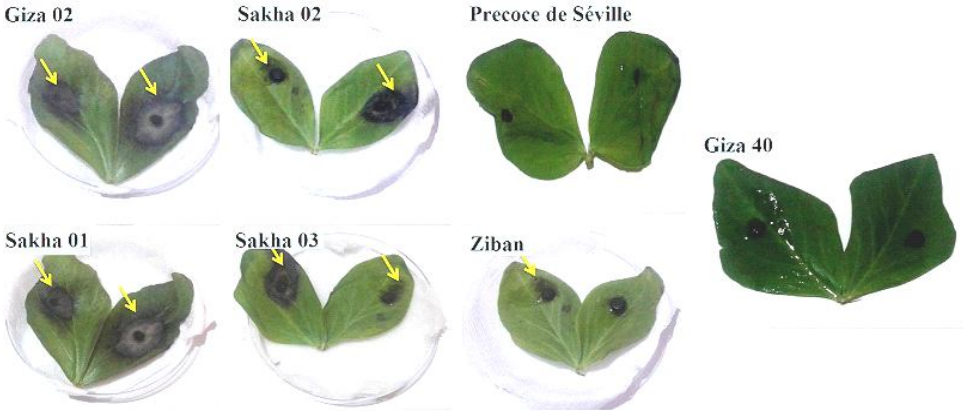


Fig 1: Reaction of 7 faba bean varieties against *Botrytis fabae* isolates with typicals symptoms of chocolate spot disease.

Total sensitivity was observed in the four (4) varieties Giza02, Sakha 01, Sakha 02 and Sakha 03, followed by total death of the leaflets after 12 days of inoculation; Two (2) varieties (Giza 40 and Ziban) showed tolerance towards strains of *Botrytis fabae* (Fig 1). The standard variety 'Precoce de Seville' showed significant resistance to both strains of *Botrytis fabae* (Fig 1, Table 3).

In comparison between two BF isolates (BF1 and BF2), no difference was observed in their pathogenicity on all seven faba bean varieties. All these varieties showed the same reaction of resistance/sensitivity to chocolate spot disease caused by these two isolates.

The importance of genetic control through the use of resistant varieties against pest pests has been reported by several authors worldwide (Bouhassan *et al.*, 2004a, b; Ahmed *et al*., 2016; Wolde and Mitiku, 2018; Rubiales *et al*., 2012; Sheikh *et al*., 2015).

Our essay is the first research work carried out in Algeria on this topic, that it is based on the research of the sources of resistance of seven (07) varieties of faba bean (Giza 02, Giza 40, Sakha 01, Sakha 02, Sakha 03, Ziban and Precoce of Seville), against the chocolate spot disease caused by *Botrytis fabae* Sard. A similar study was carried out in Spain by Madeira *et al*. (1993) and Sillero and Rubiales (2014), in Poland by Goral and Walentyn-Goral, 2012 and in Morocco by Bouhassan *et al*. 2004b.

A significant difference was observed in the reaction of faba bean varieties against two strains of *Botrytis fabae*, by 04 susceptible varieties (Giza02, Sakha01, Sakha02 and Sakha03), two tolerant varieties (Giza40 and Ziban) and only one resistant variety (Precoce de Seville). This difference has been reported in some studies around the world with other faba bean cultivars (Villegas-Fernandez *et al*., 2012; Wolde and Mitiku, 2018; Abd-Rabh *et al*., 2013; Bouhassan *et al*., 2004a,b; Terefe *et al*., 2015). El-Komy *et al*. (2015) reported in their work the total sensitivity to *Botrytis fabae* of two varieties of faba bean that we used in our essay (Giza 02 and Sakha 02).These results are very close to those obtained in our test. In Tunisia, Rhaeim *et al*. (2013) noted the limited number of resistant varieties to *Botrytis fabae* despite the large number of varieties tested (67 varieties). The same remark was observed in our results and in other authors (Abd-Rabh *et al*., 2013; Wolde and Mitiku, 2018).

Although the majority of the authors in the bibliography have chosen the field screening test, but certain authors such as El-Komy *et al*. (2015), Bouhassan *et al*. (2004a, b) and Madeira *et al*. (1993), confirmed the similarity of results of this type of screening with other tests like cut-twig, greenhouse and detached leaves.

CONCLUSION

The objective of this study was to find the sources of resistance on seven (07) varieties of faba beans against two chocolate spot stains caused by *Botrytis fabae* Sard., The major constraint of faba bean cultivation in Algeria and other countries.

A remarkable difference was observed in the reaction of varieties of beans by only one variety (Precoce de Seville) which showed promising resistance and two tolerant varieties (Giza 40 and Ziban), but the other four varieties are very sensitive to this disease (Giza 02, Sakha 01, Sakha 02 and Sakha 03).

The standard variety 'Precoce de Seville' which has shown significant, encouraging and promising resistance must be confirmed by another field screening test before proposing it to farmers for cultivation, by which it can be reduced the use of chemical fungicides and preserving the environment.

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