

EVALUATION OF SOME FABA BEAN GENOTYPES UNDER INFECTION BY CHOCOLATE SPOT DISEASE

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ABSTRACT

*This investigation was carried out at Etay El-Baroud Agricultural Research Station farm, El-Beheira Governorate during two winter seasons of 2021/2022 and 2022/2023, to explore new genotypes more resistant to chocolate spot disease than the local cultivars and clearing the correlation between the yield productivity and physiological traits. Eight new diverse faba bean genotypes (GB6, GB11, GB12, GB13, GB16, GB17 GB18 and GB20) and six faba bean cultivars (Sakha 1, Sakha 4, Giza716, Giza 843, Misr 1, and Giza 40) were used in this study. Genotypes were arranged in randomized complete block design with three replicates. The new genotypes GB 16, GB17, GB18 and GB20 were more resistant to chocolate spot disease under both of artificial infection with *Botrytis fabae* virulent isolate (*B. f N*) and under natural infection in the open field. These genotypes possessed high mean values for yield and its components criteria in the second season only. The resistant new genotypes (GB 17 and GB 20) have surpassed the resistant cultivars (Sakha 1 and Sakha 4) in photosynthetic pigments content (Chlorophyll a, b). There was a positive correlation between decreasing chocolate spot disease severity and the increasing of total phenols, peroxidase activity and proline content. The two-way hierarchical cluster analysis showed that disease severity, number of pods plant⁻¹, seed yield ardab fad⁻¹, Chl a, Total Chl., peroxidase and shoot dry weight after 70 days are related characters.*

Key words: *Vicia faba*, Genotypes, Chocolate spot disease *fabae*, Chlorophyll a and b, Total phenols, Peroxidase, Proline.

INTRODUCTION

Faba bean (*Vicia faba* L.) is an important crop for human nutrition as a good source of vegetarian protein and for soil fertility through biological N₂-fixation (Sharaan *et al* 2004). It plays an important role in world agriculture, owing to its high protein content, ability to fix atmospheric nitrogen, capacity to grow and yield well on marginal lands (Al-Ghamdi 2007). Its seed contains minerals (iron, zinc, calcium) and vitamins (B1, B2, C). Also, it is used as a fodder and forage crop (Rubiales 2010). *Vicia faba* ranks fourth primary dietary legume in the world (Graham and Vance 2003). In Egypt, the average cultivated area during 2017 to 2021 was 89041 fad, with an average seed yield of 9.2 ardab/fad, and total production approximately 127 thousand metric tons which covers about 32.6% of the total national consumption (Bulletin of Statistical Cost Production & Net Return (2022). Unfortunately, this strategic crop production is attacked by

many diseases which considered as a limiting factor for its cultivation, including foliar diseases.

Chocolate spot disease caused by (*Botrytis fabae* Sard.) is considered one of the most destructive diseases and causes considerable losses in faba bean yield in North Delta region of Egypt, particularly during wet seasons where optimum conditions of temperature (18-20°C) and relative humidity (90-100%) cause its spread and severity (El-Banoby *et al* 2013). In the Nile Delta, yield loss due to chocolate spot disease reaches 60-80% among susceptible cultivars and 34% among tolerant ones (Bouhassan *et al* 2004 and Sahile *et al* 2008). Infected plants usually have fewer pods which reduces their yield potential. In unprotected crops the disease can be expected to reduce yields by 30-50 percent in bad conditions (Mbazia *et al* 2016). In addition, seeds from badly affected plants may have a reddish-brown discoloration, which lowers their market value. Symptoms initially appear on leaves as reddish-brown spots that develop into chocolate-colored lesions. These can enlarge into extensive necrotic zones that lead to severe premature defoliation and stem damage. If the disease develops at flowering time, it can cause complete crop failure (Harrison 1988 and Crop Pro 2019). The infection decreases the total carbohydrates, nitrogen, nucleic acid, and protein contents of the yielded seeds (Aldesuquy *et al* 2015). Under stress conditions (biotic or abiotic), the production of reactive oxygen species (ROS) increases and causes plant oxidative stress (Latef and Chaoxing 2014). In fact, ROS damages cellular membranes in the processes of lipid peroxidation and are also able to cause harmful effects on DNA, proteins, and chlorophyll (Mittova *et al* 2003). Plants produce several major antioxidant enzymes, peroxidase and total phenols which have an essential role in singlet oxygen and scavenging ROS from the cytosol, mitochondria, and chloroplasts in the cell (Lu and Finkel 2008). Peroxidase play an active role in cell wall toughening and production of toxic secondary metabolites and its simultaneous oxidant and antioxidant properties make it an important factor in the defense response of plants to a variety of stresses (Idrees *et al* 2011). The peroxidase activity in uninfected and infected leaves and the wayerone acid biosynthesis in infected faba bean plants were successfully used to

ascertain the resistance and susceptibility of faba bean cultivars to *B. fabae*, that is, these compounds are preliminary markers for the resistance of faba bean to the chocolate spot disease caused by *B. fabae* (Nawar and Kuti 2003). Photosynthetic pigments content are linked to the plant health, so increasing them will increase the plant's ability to resist pathogens (Saber *et al* 2009). Plant phenolics increase the rigidity of plant cell wall acting as molecular bridge between cell wall components (Ozyigit 2008). Also the amino acid proline proved to act as a potent scavenger for preventing the induction of programmed cell death by Reactive Oxygen Species (ROS) (Ashry and Mohamed 2011). Proline also has important role in osmotic balance in the cell cytoplasm which leads to reduced absorption of water by the pathogen (Uiddin *et al* 2012).

The extensive applications of fungicides to protect plants against this disease had a harmful effect such as an appearance of new fungicide-resistant pathogenic strains, contamination of the environment and modification of the balance of the beneficial microorganisms (Tola *et al* 2016). The strategies used to control chocolate spot disease on faba bean include crop rotation, reduced planting density, timely fungicide applications, use of clean seeds, and host-plant resistance (Stoddard *et al* 2010). Genetic resistance is a key part of any integrated disease management approach to prevent yield loss caused by chocolate spot disease (Temesgen *et al* 2015 and Maalouf *et al* 2019). Breeding program efficiency depends largely on the availability of useful genetic variation, the selection procedure and the heritability of traits (El-Emam 2005). He added that, the presence of sufficient genetic variability within and between populations lead to success of selection. Although genetic resistance to chocolate spot disease generally provides partial protection, the use of resistant cultivars remains the major means to reduce yield losses (Rhaiem *et al* 2002) since it is less cost effective and environmentally safe than the use of chemicals (Silleroa *et al* 2000 and Torres *et al* 2006). Consequently, faba bean yields and disease resistance are affected significantly by weather and soil conditions as mentioned by (Podlesny *et al* 2017). Moreover (Waly *et al* 2019) reported that the resistant genotypes may produce high yield under low

and moderate infection of foliar diseases but in the high infections, yield will be decreased in the resistant and susceptible genotypes together.

Therefore, this study aimed to discover new genotypes resistant to chocolate spots disease for using them in breeding programs.

MATERIALS AND METHODS

Plant material

Eight new faba bean genotypes (GB6, GB11, GB12, GB13, GB16, GB17 GB18 and GB20) were provided by the National Gene Bank, Agriculture Research Center and six faba bean cultivars (Giza716, Sakha 1, Sakha 4, Misr 1, Giza 843, and Giza 40) obtained from Legume Dept. Field Crops Research Institute, Agricultural Research center, Giza, Egypt were used in this study (Table 1).

Table 1. The pedigree of the faba bean genotypes used in this study.

No.	Genotypes	Pedigree
1	GB 6	Landrace collected from New valley
2	GB 11	Landrace collected from New valley
3	GB 12	Landrace collected from Ismailia
4	GB 13	Landrace collected from Ismailia
5	GB 16	Landrace collected from Qena
6	GB 17	Landrace collected from Qena
7	GB 18	Landrace collected from Qena
8	GB 20	Landrace collected from Assiut
9	Sakha 1	Giza 716 X 620/283/85
10	Sakha 4	Sakha 1 X Improved Giza 3
11	Giza 716	Crossing between (416/842/83 X 503/453/83)
12	Giza 843	(561/2076/85 Skh X 461/485/83)
13	Misr 1	(G3 X 123A /45/76) X (62/1570/66 X G2) X Romi X Habashi
14	Giza 40	An individual plant selection from Rebaya 40

Experiment design

Greenhouse experiment

This experiment was conducted in pots under greenhouse conditions to study the reactions of eight faba bean genotypes and six faba bean cultivars to chocolate spot disease caused by *Botrytis fabae*. Pots (25 cm diameter) filled up with sterilized soil before planting and four pots were used for each treatment. Five seeds from each genotype were sown in each pot at 15th of November 2023. The different genotypes of faba bean plants (45 days old) were sprayed with spore suspension of *B. fabae* isolated from Nubaria location (*B. f*N) at the rate of $(2.5 \times 10^5 \text{ spore/ml})$. All pots were kept in the greenhouse for 48h at 20°C under high relative humidity. The inoculated plants were examined for chocolate spot disease severity and the data were recorded 2, 4 and 6 days after inoculation.

Chocolate spot disease severity

Chocolate spot symptoms on the foliage were recorded at regular intervals using a 0-9 scale. The response of the genotypes was expressed as the disease severity (D.S) values according to Ding *et al* (1993). Six resistance levels were used as follow: (HR) (highly resistant), DS ranging between 0 and 2.0 %, (R) (resistant); D.S =2.1–15.0% MR (moderately resistant), D.S =15.1–40.0%, MS (moderately susceptible); D.S =40.1–60.0% S (susceptible), D.S =60.1–80.0% HS (highly susceptible), D.S =80.1–100%. The plants were divided into the previous categories and the results were recorded after 2, 4 and 6 days of artificial inoculation in the form of percentage of disease severity (D.S) according to the following formula:

$$\text{Disease severity}\% = \epsilon \frac{n \times v}{9 N} \times 100$$

(n) = Number of plants in each category.

(v) = Numerical values of symptoms category.

(N) = Total number of plants.

(9) = Maximum numerical values of symptom categories

Physiological activities under greenhouse conditions

Samples were taken after 6 days from infection (51 days from sowing) to determine chlorophyll (a, b and total), Total Phenols, Peroxidase activity, proline content, shoot dry weight and leaf area index by the same methods as described in the field experiments.

Percentage of chocolate spot infection and disease severity (under field conditions)

The appearance of the first chocolate spot symptoms was recorded 45 days after sowing. Infected plants were calculated based on visible symptoms. The disease severity of chocolate spot disease was recorded 45, 75 and 90 days after sowing using 0-9 scale as described by Ding *et al* (1993).

The source of pathogen isolate

The virulent isolate of *Botrytis fabae* with accession No. (OR528633) in gene bank isolated from Al -Nubaria distraction (El-Blasy 2014) (*B. f* N) were used. *B. fabae* isolate was identified by Legume and Forage Diseases Dep. Plant Pathology Research Institute, A.R.C., Giza, Egypt. It was grown onto faba bean leaf extract agar medium (FBLA) which consists of 250g faba bean leaves, 30g sucrose, 20g sodium chloride and 20g agar in one liter of distilled water in order to obtain high number of spores according to the method described by Leach and Moore (1966). The prepared medium was autoclaved at 1Lb/inch for 15 minutes and poured before solidification into sterilized Petri dishes. The Plates were inoculated with equal discs (5mm) of virulent isolate and incubated at 20±2°C for 12 days (Last and Hamley 1956) under alternating light and darkness regime (12-h/12-h) to enhance spore production. Ten plates were used after the elapse of the incubation period, the plates were flooded with 10 ml of sterilized distilled water added to the culture. Fine brush was used to facilitate spores fragment to suspend into the added water. The suspension was filtered through three layers of cheesecloth to remove the mycelial residues. Number of spores/ml was counted in the collected spore suspension by using a Spencer haemocytometer slide to about (2.5×10⁵) spore/ml.

Field experiments

One field experiment was conducted under natural infection during two growing successive seasons of 2021/2022 and 2022/2023 at Etay El-Baroud, Agric., Res., Station in Beheira governorate where the history of chocolate spot disease is known in this the field. Genotypes were arranged in a randomized complete block design with three replicates. The experimental unit included two ridges 60 cm apart, and 3 meters long occupying an area of 5.4 m². Seeds of the studied faba bean genotypes were planted on 18th and 22th of November 2021 and 2022 seasons, respectively, on both sides of the ridges in hills distanced 25 cm with two seeds per hill. All normal agricultural practices were performed. The meteorological data were obtained from Central Laboratory for Agricultural Climate (CLAC) during growth periods of faba bean (November- April) (Table 2).

Table 2. Monthly maximum and minimum air temperatures, relative humidity% and solar radiation at Etay El-Baroud, Agric., Res., Station in 2021/2022 and 2022/2023 seasons.

Month	2021/2022					2022/2023				
	Relative humidity %	Temperature °C			Solar radiation (MJ/m ² /day)	Relative humidity %	Temperature °C			Solar radiation (MJ/m ² /day)
		Min	Max	Mean			Min	Max	Mean	
November	72.13	11.06	24.29	17.67	75.27	69.56	11.53	23.88	17.71	105.16
December	80.23	10.40	21.60	16.00	70.67	81.83	10.26	22.07	16.17	77.13
January	80.61	8.36	17.62	12.99	97.33	77.03	9.74	16.85	13.30	74.28
February	83.59	10.17	22.12	16.15	94.06	82.19	11.82	23.02	17.42	93.11
March	79.96	13.70	26.15	19.93	96.28	81.04	12.71	25.63	19.17	96.42
April	71.78	11.47	28.66	19.51	212.57	64.05	11.75	27.47	19.19	235.40
Average	78.05	10.86	23.41	17.04	107.70	75.95	11.30	23.15	17.16	113.58

Studied traits

1- Percentage of chocolate spot infection and disease severity (under field conditions)

The appearance of the first chocolate spot symptoms was recorded 45 days after sowing. Infected plants were calculated based on visible symptoms. The disease severity of chocolate spot disease was recorded 45, 75 and 90 days after sowing using 0-9 scale as described by Ding *et al* (1993).

2- Chemical analysis

Photosynthetic pigments

Total chlorophyll content in fresh leaves (mg/g fresh weight) 70 days after sowing was determined and calculated according to Moran (1982).

Total phenolics compounds

At 70 days from sowing, total phenolics were determined in fresh shoot using the Folin-Ciocalteu reagent according to Malik and Singh (1980). Total phenol content was determined as mg catechol/ 100 g fresh weight.

Proline content of leaves

Proline in leaves was determined according to Bates *et al* (1973). The results were calculated in mg / g dry weight.

Antioxidant enzymes activity of leaves

Enzymes activity of peroxidase and polyphenol oxidase (mg/g f. wf.) were determined by employing the method mentioned by Sadasivam and Manickam (1996) using spectrophotometer model UV-Vis spectronic 601.

Growth traits

1- Shoot dry weight / plant (g)

Dry weight of plant shoot was estimated as the average of five plants chosen randomly at 60 and 90 days after sowing.

2- Leaf area (LA) /plant (cm²)

The area of 20 disks ($20 \times 3.14 \times (0.5)^2$) = 15.70 cm² was calculated and the leaf area was determined according to Watson (1952) as the following formula:

LA = 15.70 x dry weight of leaves per plant/dry weight of leaves disks;

Leaf area index (LAI) = unit leaf area per plant/unit ground area occupied by plant

3-Crop growth rate (CGR) (60-90) DAS (g/m²/day)

The following formula was used to determine CGR according to **Watson (1952)**

$CGR = 1/\text{planting area} [(W_2 - W_1) / (T_2 - T_1)]$ Where: $W_2 - W_1$ = differences in dry matter accumulation between two successive samples in grams. $T_2 - T_1$ = the number of days between two successive samples in day.

4- Yield and yield component characters

At harvest, ten plants were handled individually from each replicate and the following characters i.e number of branches plant⁻¹, number of pods plant⁻¹, number of seeds plant⁻¹, seed yield plant⁻¹ (g) and 100-seed weight (g) were recorded. Also, seed yields were recorded per plot and converted to seed yield (ardab/fad).

Statistical analysis

The collected data were statistically analyzed by ANOVA using MSTATC statistical package. Means were compared using the least significant difference (L.S.D) test at 0.05 level of probability as published by Gomez and Gomez (1984). Dendrogram was generated using UPGMA method in PAST 3.20 software (Hammer *et al* 2001).

RESULTS AND DISCUSSION

1- The reaction of new different faba bean genotypes and cultivated varieties to infection by chocolate spot disease using the virulent isolate of *B. fabae*

1-1. Under greenhouse conditions

Data presented in Table (3) show the reaction of different eight new faba bean genotypes and six faba bean cultivars to chocolate spot disease, using *B. fabae* (Nubaria isolate) (*B.f* N). Significant variations among the tested genotypes to the disease were found. Generally, it was found that the disease severity of infection increased with advance in plant age, irrespective of different of either new genotypes or cultivars.

Giza 40 cultivar was susceptible to chocolate spot disease and its severity of infection was 44.60, 65.60 and 77.36% after 2, 4 and 6 days from inoculation, respectively. Four new faba bean genotypes (GB16, GB17, GB18 and GB20) and two faba bean cultivars (Sakha 1 and Giza716) showed a resistance against chocolate spot. The means of disease severity were 13.74, 12.91, 12.29, 11.87, 14.66 and 14.16 %, respectively. These results can be due to the genetic variance among genotypes (El-Emam 2005).

Table 3. Reactions of different faba bean cultivars and some genotypes infected by *B. fabae* (*B. f N*) caused chocolate spot disease under greenhouse conditions.

Genotypes	Disease severity (%)				Response
	2 days	4 days	6 days	Mean	
GB6	13.75	17.50	20.62	17.29	MR
GB11	35.61	45.95	51.25	44.27	MS
GB12	32.67	42.65	55.48	43.60	MS
GB13	15.62	20.00	23.12	19.79	MR
GB16	8.75	14.37	18.12	13.74	R
GB17	9.37	13.12	16.25	12.91	R
GB18	8.75	12.50	15.62	12.29	R
GB20	8.12	12.50	15.00	11.87	R
Saka 1	13.12	14.62	16.75	14.66	R
Saka 4	14.37	16.87	20.62	17.28	MR
Giza 716	11.25	14.37	16.87	14.16	R
Giza 843	16.65	21.56	23.29	20.50	MR
Misir 1	15.23	17.90	22.67	18.60	MR
Giza 40	44.60	65.60	77.36	62.52	S
C.V %	3.44	2.67	1.93		
L.S.D 0.05	0.884	0.912	0.098		

MR= moderately resistant, MS= moderately susceptible, R=resistant, S= susceptible

Nevertheless, five faba bean genotypes (GB6, GB13, Sakha 4, Giza 843 and Misr 1) recorded the moderately resistance with means between 17.29 to 20.50%. Another faba bean genotypes (GB11 and GB12) were moderately susceptible after 2, 4 and 6 days with means of 44.27 and 43.60 %, respectively.

Data shown in Table (4), clearly revealed the significant effect of the resistant category of the tested faba bean genotypes infected with (*Botrytis fabae* N) under greenhouse conditions on chlorophyll a, chlorophyll b, total chlorophyll, total phenol, peroxidase activity and proline. Results showed that genotypes; GB17 and GB20 had the highest values with chlorophyll a being (3.63 and 3.49), chlorophyll b (1.35 and 1.46) and total chlorophyll (4.97 and 4.95). On the other hand, Saka4 and Giza 716 had the highest values of chlorophyll a, chlorophyll b and total chlorophyll, (3.18, 1.37 and 4.55) and (3.53, 1.60 and 5.13), respectively. In contrast, the cultivar Giza 40 and genotype GB 11 recorded the lowest value in all photosynthetic pigments (2.27, 1.56 and 3.83) and (2.40, 1.61 and 4.01), respectively.

Total amounts of phenol and proline in different new genotypes and common cultivars increased after inoculation with (*Botrytis fabae* N) in resistant and moderate resistant genotypes compared with moderate susceptible genotypes. Data in the same table revealed that GB17, GB20, Sakha 4 and Giza 716 greatly increased the concentration of the total phenols up to 50.02, 49.20, 46.08 and 46.79 mg/g fresh weight while; proline amount was 0.93, 0.98, 0.93 and 0.94 mg/g fresh weight, respectively. However, other moderate resistant genotypes showed intermediate amount of total phenol and proline amount ranging from 39.71 to 42.49 and 0.87 to 0.90 mg/g fresh weight compared with moderate susceptible Giza 40 which recorded the least amount of total phenols and proline 35.50 and 0.83 mg/g fresh weight, respectively. These findings are in accordance with those mentioned by Nawar and Kuti (2003), El-Blasy (2014), El-Abssi *et al* (2019) and Marwa and El-Bakery (2020). The increase of these pigments and antioxidant enzymes helps the plant tissues to be more resistant against pathogens and increase lignin production.

Table 4. Mean performance of physiological characters for some faba bean genotypes infected with of *B. fabae* (*B. f* N) under greenhouse conditions.

Genotypes	Chl a mg/gm fresh weight	Chl b mg/gm fresh weight	Total chl. mg/gm fresh weight	Total Phenols mg/g/f. wt
Moderate resistant (MR)				
GB 6	3.07	1.41	4.48	39.71
GB 13	3.97	1.58	5.56	41.48
Giza843	2.35	1.37	3.72	42.49
Misr 1	2.80	1.58	4.38	41.16
Resistant (R)				
GB 16	3.44	1.46	4.90	46.92
GB 17	3.63	1.35	4.97	50.02
GB 18	3.48	1.45	4.93	42.99
GB 20	3.49	1.46	4.95	49.20
Sakha 1	2.73	1.48	4.21	42.60
Sakha 4	3.18	1.37	4.55	46.08
Giza716	3.53	1.60	5.13	46.79
Moderate susceptible (MS)				
GB 11	2.40	1.61	4.01	35.00
GB 12	2.73	1.44	4.16	34.77
Giza 40	2.27	1.56	3.83	31.50
C.V. %	4.59	7.74	4.57	9.61
L.S.D. 0.05	0.24	N.S	0.35	6.80
Geno.	Peroxidase mg/g/f.wt	Proline mg/g/f.wt	shoot dry weight plant ⁻¹ (g) at 70 days	LAI
Moderate resistant (MR)				
GB 6	11.99	0.90	3.41	0.84
GB 13	11.14	0.87	3.00	0.81
Giza843	11.11	0.90	3.09	0.78
Misr 1	11.07	0.88	3.04	0.75
Resistant (R)				
GB 16	12.37	0.92	3.65	0.88
GB 17	13.47	0.93	4.95	1.08
GB 18	12.56	0.93	3.90	0.90
GB 20	13.47	0.98	4.02	0.95
Sakha 1	12.39	0.93	3.86	0.90
Sakha 4	12.28	0.93	3.55	1.01
Giza716	12.78	0.94	3.79	1.08
Moderate susceptible (MS)				
GB 11	8.46	0.85	2.35	0.73
GB 12	9.70	0.85	2.69	0.74
Giza 40	8.11	0.83	2.13	0.54
C.V.%	6.81	4.95	4.38	10.64
L.S.D. 0.05	1.30	0.07	0.25	0.16

There is no doubt that, total chlorophyll content in plant leaves taken as a good parameter reflecting the plant health. There is a positive relationship between net photosynthesis process and carbohydrate content which formed in plant organs. Carbohydrates in plant includes structurally polysaccharides such as pectin, which acts as a barrier in cell wall against pathogen invasion (Hamideh *et al* 2013). On the other side, Lamba *et al* (2008) explained that the accumulation rapidly of phenols compound at the pathogen infection site is considered the first step of defense mechanism in plants. The role of phenols is due to its action as antioxidant, antimicrobial and photoreceptor which lead to restricting or slowing pathogen growth. Moreover, results obtained in the current study show an increase in peroxidase activity in infected faba bean plants. Maximum increase in peroxidase activity after inoculation with *B. fabae*, cleared in resistant and moderate susceptible genotypes which means that each target pathogen should be studied individually to gain maximum possible control of certain pathogen.

Another explanation was achieved by Tarrad *et al* (1993) who reported that increase in peroxidase activity; enhance lignification in response to chocolate spot infection which may restrict the fungal penetration. Peroxidases protect plants against pathogen stress (Chowdhury 2003). These findings indicate a positive relationship between resistance and peroxidase activity. Peroxidase also produces free radicals and hydrogen peroxide which are toxic to many microorganisms.

The accumulation of total free amino acids especially proline amino acid is one of the most frequently reported modifications induced by biotic and abiotic stress conditions. Therefore, proline content is one of the stress resistance mechanisms in plants. Also, proline act as a storage compound for energy and reduced carbon and nitrogen needs as well as displayed as antioxidant agent which acts an important role of plant protection. Where the metabolism of proline in the plants that is: proline glutamate Krebs cycle (TCA) CO₂.

Moreover, proline protecting the photosynthetic apparatus and displaying an antioxidant activity (Ashraf *et al* 2008). In addition,

proline has a clear role on cell osmosis and radical detoxification (Taie *et al* 2013).

Generally, the activation of peroxidases key enzymes in the phenylpropanoid and isoflavonoid pathways may play a crucial role of resistance to pathogenic attack in plants through, the rapid accumulation of phenols at the infection site, which function to slow the growth rate of the pathogen and the synthesis of molecules related to pathogen stress (Baraka *et al* 2008; Mahmoud *et al* 2012 and El-Blasy 2014).

1-2. Under field conditions

Table 5 showed that faba bean cultivars and new genotypes had a significant variation in response to chocolate spot natural infection under field conditions in both seasons after 45, 60, 75 and 90 days from sowing suggesting a stable resistance over the time of the experiment. Meantime, in the first season (2021/2022) four new genotypes (GB16, GB17, GB18 and GB 20) as well as three faba bean cultivars (Sakha 1, Sakha 4 and Giza 716) recorded the lowest percentage mean values of disease severity (5.22, 4.11, 5.95, 4.00, 7.00, 5.44 and 8.33 %). Whereas, GB11, GB12 and Giza 40 showed to be moderate susceptible to *B. fabae* which, had the highest mean values of infection 32.00, 30.53 and 33.29%, respectively compared to other tested genotypes which were moderate resistant to fungal infection. For the second season (2022/2023), the results were almost similar where, GB16, GB17, GB18 and GB 20 as well as three faba bean cultivars (Sakha 1, Sakha 4 and Giza 716) ranked as the highly significant resistant genotypes while, GB11, GB12 and Giza 40 showed to be moderate susceptible. The other tested cultivars ranked as a moderate resistance to chocolate spot infection. This response reaction was shown over the duration of the experiment. The obtained results combined across both seasons confirmed the stability of resistance and/or susceptibility of the examined cultivars and new genotypes.

The obtained results showed slight differences in faba bean cultivars and new genotypes response to chocolate spot disease between greenhouse (Table 3) and field experiments (Table 5). For example, Sakha 4 which recorded (MR) under greenhouse turned to be (R) under field condition.

Table 5. Reactions of different faba bean cultivars and some genotypes infected by chocolate spot disease under field conditions at Etay –El-Bord Res. Station in both seasons 2021/2022 and 2022/2023.

Genotype	After 45 days		After 75 days		After 90 days		Mean		Response	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
GB6	15.83	14.73	18.33	15.73	21.54	20.74	18.57	17.07	MR	MR
GB11	18.83	17.57	32.95	29.23	44.22	41.87	32.00	29.56	MS	MS
GB12	19.50	18.47	29.00	27.97	43.09	40.32	30.53	24.92	MS	MS
GB13	14.32	14.13	16.92	15.77	18.68	17.80	16.64	15.90	MR	MR
GB16	3.33	1.53	5.00	2.74	7.33	5.30	5.22	3.00	R	R
GB17	1.67	1.50	4.17	2.40	6.50	4.03	4.11	2.76	R	R
GB18	2.50	1.83	6.67	2.50	8.67	6.80	5.95	3.71	R	R
GB20	2.50	2.27	4.17	2.97	5.33	4.67	4.00	3.30	R	R
Sakha 1	5.00	2.32	8.33	5.10	11.67	8.03	7.00	4.73	R	R
Sakha 4	3.67	1.90	5.00	3.20	12.33	9.10	5.44	5.00	R	R
Giza 716	1.17	2.90	5.83	4.50	9.33	7.60	8.33	5.15	R	R
Giza 843	15.10	14.15	17.17	16.58	19.33	17.87	18.54	17.52	MR	MR
Misr 1	16.52	15.85	18.95	16.75	20.16	19.95	17.20	16.20	MR	MR
Giza 40	20.02	18.57	34.17	30.70	45.67	42.95	33.29	30.74	MS	MS
C.V.%	3.91	7.43	3.52	4.41	2.99	3.13	----	----	----	----
L.S.D 0.05	0.65	1.13	0.86	0.92	0.98	0.92	----	----	----	----

MR= moderately resistant, MS= moderately susceptible, R=resistant, S= susceptible

On the other hand, Giza 40 which scored (S) in greenhouse turned to be (MS) in the field and other cultivars and genotypes showed stability in disease severity in greenhouse and field conditions, respectively.

This finding can be explained in the light of the fact that field results often do not correspond completely with greenhouse results since the environment is less controlled and possibly natural infection could introduce unwanted pathogen(s) or other factors. This is in agreement with results obtained by Tivoli *et al* (1986).

However, plants in pots growing under greenhouse conditions may be at certain points in their life cycle react more strongly to infection than they would naturally in the field (Tivoli *et al* 1986 and El-Badawy *et al* 2012). In contrast, a field trial mimics a natural infection and take place more gradually and more slowly than under the controlled conditions of the greenhouse, revealing more clearly the overall resistance of the plant and the interaction of the pathogen with different plant organs at different stages of disease progression. On the other hand, in the field experiment, phenotypic and metabolic characters express themselves in a quantitative manner reflecting the numerous infection and defense mechanisms involved in successive phases of the disease (Rapilly 1991). This contrast with previous finding that variation in resistance in different faba bean cultivars to the four prevalent races in the Mediterranean region particularly in Egypt, Italy, Morocco and Tunisia, these genotypes constitute an interesting genetic source for future breeding programs develop chocolate spot-resistant cultivars. Thus, genetic resistance against pathogens is highly race-specific. These findings are in harmony with those of Yitayih and Azmeraw (2018) and Marwa and El-Bakery (2020) who evaluated productivity of fifteen faba bean genotypes under natural infections with chocolate spot and rust diseases. The results showed that faba bean genotypes Santamora, L2, Giza 843, Sakha 4 and Sakha 3 showed higher resistance to rust and chocolate spot diseases. This was positively reflected on seed yield in both seasons.

The behavior of the life cycle of *Botrytis fabae* was different from region to another, where it is associated with temperature and

moisture conditions. Chocolate spot disease can rapidly develop under optimal environmental conditions. Data show that the severity of chocolate spot disease differed according to differences in climatic conditions between the first and second seasons. It seems that the spreading of chocolate spot diseases was faster in the first season than the second one under El-Beheira governorate conditions. These data show that severity of the diseases was lower in the second season than the first one. Moreover, the severity of the diseases was lower in February than March in both seasons. These results could be due to high temperature and low humidity that furnished suitable environmental conditions to enhance pathogen spreading. It is known that increased temperature and decreased relative humidity are the two climatic factors that increase foliar diseases (Harrison 1988). Also, low solar radiation has positive effects on pathogen activity where ultraviolet radiation in sunlight is the major factor affecting the germination of fungal propagules (Rotem and Aust 1991) through damaging DNA and proteins. Accordingly, it seems that temperature above 25°C and below 7-8°C controlled chocolate spot spreading under Beheira governorate conditions. Consequently, it is expected that chocolate spot pathogen would be unable to traverse the leaf surface to penetrate the plant by stability of air temperature and relative humidity and solar radiation from a month to another. Also, chocolate spot is favored by warm and humid conditions that extend for four to five days. Moreover, Yitayih and Azmeraw (2018) mentioned that chocolate spot disease results in heavy premature defoliation and under warm moist conditions crop lodging may occur.

2-Yield and yield components criteria

Mean performance of yield and yield components characters were recorded in Table (6). The collected data showed that there was a remarkable significant difference at probability 5% between all genotypes under study for these characters in both seasons; this indicating that expected genetic gain from selection for these traits could be fast in these genetic materials.

Table 6. Means of yield and yield component characters for some faba bean genotypes infected naturally with *B. fabae* at Etay El-Baroud Res. Station in both seasons 2021/2022 and 2022/2023.

Genotype	No. of branches plant ⁻¹		No. of pods plant ⁻¹		No. of seeds plant ⁻¹	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Moderate resistant (MR)						
GB 6	1.89	2.21	9.57	10.76	23.13	34.53
GB 13	2.00	2.80	10.57	14.00	23.57	34.70
Giza 843	2.11	2.87	11.27	13.95	26.50	34.72
Misr 1	1.67	1.94	12.20	11.18	28.30	31.27
Resistant (R)						
GB 16	1.33	2.93	9.10	13.73	31.30	45.48
GB 17	1.22	3.22	9.07	14.07	28.83	49.67
GB 18	1.34	2.19	8.10	14.36	23.93	34.70
GB 20	1.11	2.47	7.33	12.83	29.00	38.53
Sakha 1	2.22	2.36	12.33	12.13	29.17	33.70
Sakha 4	1.56	2.15	13.93	14.79	34.30	34.79
Giza 716	2.56	2.27	13.10	10.26	25.40	27.87
Moderate susceptible (MS)						
GB 11	2.22	1.92	10.97	10.33	19.93	29.79
GB 12	1.56	2.73	9.93	15.60	20.90	29.87
Giza 40	1.56	2.22	6.77	9.13	12.20	25.34
C.V %	24.36	12.71	10.08	12.89	4.97	7.16
L.S.D 0.05	0.74	0.52	1.74	2.74	2.12	4.16
Genotype	Seed yield plant ⁻¹ (g)		100-seed weight (g)		Seed yield ardab fad ⁻¹	
	1 st season	2 nd season	1 st season	2 nd season	1 st season	2 nd season
Moderate resistant (MR)						
GB 6	20.113	29.899	86.914	86.730	9.045	11.574
GB 13	21.785	31.705	92.379	92.477	9.725	12.273
Giza 843	24.783	31.535	93.484	90.840	10.476	12.207
Misr 1	26.550	28.689	93.757	92.737	10.194	11.106
Resistant (R)						
GB 16	29.744	42.447	93.337	93.998	12.707	15.431
GB 17	27.316	47.029	94.690	94.600	12.330	16.538
GB 18	23.206	33.634	96.927	96.784	11.727	13.019
GB 20	28.007	37.184	96.498	96.567	12.638	14.394
Sakha 1	27.427	31.005	94.004	92.148	11.950	12.002
Sakha 4	36.441	36.962	106.242	106.236	13.457	14.308
Giza 716	23.702	26.669	95.034	96.096	10.869	10.323
Moderate susceptible (MS)						
GB 11	18.489	27.324	92.722	91.956	8.257	10.577
GB 12	19.943	28.813	95.471	96.486	9.106	11.531
Giza 40	10.103	20.798	82.732	81.932	6.522	8.051
C.V %	5.80	8.91	1.64	11.02	4.96	9.23
L.S.D 0.05	2.35	4.85	2.59	17.30	0.89	1.91

Number of branches plant⁻¹ for the studied cultivars ranged from 1.56 to 2.56 in the first season and from 1.94 to 2.87 in the second season, Giza 716 exceeded all cultivars for No. of branches plant⁻¹ in the first season this result is in accordance with Amina *et al* (2020). On the other hand, all new genotypes recorded low number of branches plant⁻¹ in both seasons, except GB 16 and GB17 in the second season. These genotypes recorded 2.33 and 12.44 % increase as comparing to check cultivar Giza 843 which had the highest value in the same season. Sakha 4 cultivar had the highest mean value for No. of pods, No. of seeds, seed yield plant⁻¹, 100-seed weight (g) and seed yield ardab fad⁻¹, While Giza 40 recorded the lowest mean value for the pervious traits. These results are in agreement with those obtained by Marwa and El-Bakery (2020), who reported that Giza 40 and Atona genotypes gave lower seed yield per plant than the other genotypes probably due to their susceptibility to chocolate spot disease that had adverse effects on their productivity.

The climatic change had a great effect on yield component traits in second season comparing to first season as shown in Table (2). With regard to second season, the moderate resistant new genotype GB13 had 0.53% increase in seed yield plant⁻¹ and seed yield ardab fad⁻¹ as comparing to moderate resistant genotype (Giza 843). The new resistant genotypes (GB16, GB17 and GB20) possessed high mean value for No. of seeds, seed yield plant⁻¹ (g) and seed yield ardab fad⁻¹ as comparing to cultivar Sakha 4. This may due to the high infection of *B. fabae* in first season as shown in Table (5). They had an increase in No. branches plant⁻¹ in the second season, this increase was (24.15, 36.44 and 4.66%, respectively as comparing to the best resistant cultivar Sakha 1, they also recorded an increase in seed yield ardab fad⁻¹ (6.33, 3.18 and 5.76 %) at the first season. In the same time, they had an increase in No. of seeds, seed yield plant⁻¹ (g) and seed yield ardab fad⁻¹ as comparing to the best cultivar Sakha 4. This increase was 30.72, 42.77 and 10.75% for the first character and 14.84, 27.24 and 0.60% in the second character, while for the third character it was 7.85, 15.59 and 0.60% for GB 16, GB 17 and GB 20, respectively in the second season.

The two moderate susceptible new genotypes (GB 11 and GB 12) showed an increase in mean value for all yield components in both seasons as comparing to the moderate susceptible cultivar (Giza 40).

2- Growth and chemical composition traits under field conditions

2-1. Chemical composition

Table (7) shows that the genotypes of faba beans had significant effects on chl. a, b, a+b, total phenol, peroxidase activity and proline content in both seasons. The resistance of new genotypes (GB 17 and GB 20) were surpassed by compared to values from resistant parental genotypes (Sakha 1, Sakha 4 and Giza 716) in Chl a, b and a+b, while Giza 40 scored the lowest values in both seasons.

In general, there were large variation between all genotypes under study for Chl. a, b and a+b in both seasons. Chl a content ranged from 5.75 to 10.63 and from 7.01 to 9.44 in both seasons, respectively, and Chl. b content ranged values from 1.84 to 4.32 and from 2.76 to 3.97 in both seasons, respectively. Also, Chl. a+b ranged from 7.59 to 14.95 in the first season and from 9.95 to 13.41 in the second season. The differences in chlorophyll content may be due to the variations in genetic background among genotypes, which were translated into the expression and activity of the chlorophyll as enzyme. Reduction in chlorophyll content is due to pathogen influence on chloroplast structural modification by the fungus, such as dilation of the whole chloroplast, separation of grana accumulation of starch granules, which have a direct bearing on the photosynthetic capacity of chloroplast (Raghavendra *et al* 2007). These results are in agreement with the findings reported by Amina *et al* (2020), Waly *et al* (2019) and Marwa and El-Bakery (2020).

As for total phenols, peroxidase activity and proline results presented in Table (7) indicated that there was a significant positive effect between the disease severity of chocolate spots and total phenols, peroxidase activity and proline in both seasons. Whereas increasing total phenols, peroxidase activity and proline decreased disease severity. Data mentioned in Table 7 show that Giza 716 had the highest total phenols followed by GB 20 and GB17 in the first season followed by Giza 716 and Sakha 4 in the second season.

Table 7. Mean performance of physiological characters for some faba bean genotypes infected naturally with chocolate spot disease at Etay El-Baroud Res. Station in both seasons 2021/2022 and 22/2023.

Genotype	Chla mg/gm fresh weight		Chlb mg/gm fresh weight		T. chl. mg/gm fresh weight		Total Phenols mg/g/f.wt.		Peroxidase mg/g/f.wt.	
	First	Second	First	Second	First	Second	First	Second	First	Second
Moderate resistant (MR)										
GB 6	6.49	7.57	2.18	3.25	8.67	10.82	26.11	23.30	10.90	11.18
GB 13	6.89	7.95	2.32	3.34	9.21	11.29	28.63	23.63	11.45	11.65
Giza843	6.97	7.19	2.49	2.76	9.46	9.95	27.91	27.62	10.87	11.01
Misr 1	6.61	7.09	2.23	3.25	8.84	10.34	27.00	27.35	10.82	11.04
Resistant (R)										
GB 16	9.29	8.73	3.01	3.76	12.30	12.49	30.88	29.20	11.74	13.15
GB 17	10.21	9.44	4.18	3.97	14.39	13.41	31.01	30.34	12.13	13.58
GB 18	9.10	8.18	2.74	3.53	11.84	11.71	30.69	24.35	11.46	11.73
GB 20	10.63	8.69	4.32	3.56	14.95	12.25	31.27	27.07	13.43	12.06
Sakha 1	8.60	7.47	2.53	2.96	11.13	10.43	29.73	29.35	10.93	11.19
Sakha 4	9.07	8.20	2.59	3.36	11.66	11.56	30.57	32.56	11.27	12.37
Giza716	9.49	7.95	3.00	3.32	12.49	11.27	34.87	35.60	11.55	11.20
Moderate susceptible (MS)										
GB 11	5.93	7.27	2.03	3.04	7.96	10.31	26.66	22.45	10.52	10.60
GB 12	6.11	7.43	2.06	3.12	8.17	10.55	25.56	18.91	10.63	10.94
Giza 40	5.75	7.01	1.84	3.00	7.59	10.01	24.30	18.63	10.58	9.82
C.V.%	8.39	9.28	16.92	9.04	8.23	9.17	7.35	11.88	5.12	4.40
L.S.D 0.05	0.54	0.59	0.37	0.24	1.47	1.73	3.58	5.28	0.47	0.41

Table 7. Cont.

Genotype	Proline mg/g/f.wt		Shoot dry weight plant ⁻¹ (g) at 70 days		Shoot dry weight plant ⁻¹ (g) at 90 days		CGR gm/Plant/day		LAI	
	First	Second	First	Second	First	Second	First	Second	First	Second
Moderate resistant (MR)										
GB 6	0.80	0.81	8.19	11.41	25.53	33.65	0.85	1.10	2.85	3.58
GB 13	0.84	0.83	9.23	11.65	31.52	36.85	1.00	1.38	2.92	3.70
Giza843	0.83	0.83	9.03	10.96	28.20	35.62	1.10	1.23	2.92	4.11
Misr 1	0.81	0.86	8.70	9.82	26.86	35.42	0.66	1.20	2.86	4.06
Resistant (R)										
GB 16	0.84	0.86	9.86	13.05	36.06	43.90	1.36	1.54	3.24	4.00
GB 17	0.90	0.96	10.69	15.97	37.56	44.94	1.69	1.58	3.42	4.51
GB 18	0.85	0.87	9.33	11.94	31.95	41.74	1.34	1.45	3.04	3.72
GB 20	0.93	0.99	10.25	12.64	34.40	42.21	1.21	1.53	3.45	3.80
Sakha 1	0.85	0.87	9.81	11.53	30.94	36.40	1.11	1.33	2.98	4.16
Sakha 4	0.87	0.91	11.28	12.97	39.37	48.90	1.71	1.80	3.06	4.82
Giza716	0.88	0.90	10.32	11.98	36.22	39.91	1.25	1.40	3.72	4.80
Moderate susceptible (MS)										
GB 11	0.77	0.80	7.46	9.26	24.23	32.69	0.74	1.04	2.32	3.23
GB 12	0.75	0.71	7.48	10.24	25.26	33.32	0.84	1.05	2.79	3.54
Giza 40	0.71	0.68	7.56	9.60	25.99	29.56	0.89	1.00	2.54	3.44
C.V. %	4.41	10.37	7.30	11.75	10.56	14.18	17.64	19.89	15.69	7.95
L.S.D 0.05	0.03	0.07	1.13	2.29	2.67	4.43	0.16	0.21	0.41	0.25

On the other side, GB20 had the maximum value in terms of enzyme peroxidase activity in the first season, while GB17 achieved the highest value of peroxidase activity in the second season.

In the same trend, GB20 recorded the maximum value for proline, followed by GB 17 in both seasons. However, the moderately susceptible genotypes (GB11, GB12 and Giza 40) gave the lowest values in terms of total phenols, peroxidase activity and accumulation of proline. The accumulation of phenolic compounds at the infection sites showed a correlation with the restriction of pathogen development since such compounds are toxic substances to pathogens. Also, resistance may be increased by a change in pH of the plant cell cytoplasm due to the

increase in phenolic acid content, resulting in inhibition of pathogen development (Khaledi *et al* 2015).

In addition, He *et al* (2011) and Rani and Jyothsna (2012) found that the biotic and abiotic stresses led to an increase in the production of reactive oxygen species (ROS), which hurt cells lipids, proteins, nucleic acids and finally stopped the natural metabolism of plants under high density. Plants protect themselves from the cytotoxic effects of these ROS with the help of antioxidant enzymes such as peroxidase induced in plants in response to stress. In this present study, it was observed that under disease conditions that induced the accumulation of proline in faba bean plant leaves, it might have an important role in scavenging and coping with ROS and the stability of cell membranes.

2-2 Growth traits

Regarding the behavior of faba bean genotypes, results in Table (7) show significant differences among faba bean genotypes in shoot dry weight plant⁻¹ at 70 and 90 days, crop growth rate (CGR) and leaf area index (LAI). In the first season, resistant new genotypes (GB 20 and GB 17) gave the maximum values for each of shoot dry weight plant⁻¹ at 70 and 90 days with insignificant differences between them. Whereas GB 17 and Sakha 4 scored the highest values for each of shoot dry weight plant⁻¹ in the same growth period. As for (CGR), it could be observed that GB 17 gave the maximum value of CGR followed by GB 20 in first season but Sakha 4 achieved the highest value followed by GB 17 in second season. Also, resistant parental genotypes Giza 716 had an increase in LAI as comparing to other genotypes under study in both seasons. In contrary, moderate susceptible genotypes (GB11, GB12 and Giza40) scored the lowest values on all growth traits. Chocolate spot disease could reduce photosynthesis by inhibiting photosynthetic activity through the necrosis of plant tissues and by damaging the structural and stomatal closures of the plants. So genetic resistance genotypes to brown spot disease contain a high content of the peroxidase enzyme and proline, which have an important role in protecting plant tissues from damage and maintaining the stability of cell membranes by scavenging and coping with ROS and consequently improving the synthesis of chlorophyll, which leads to a significant

increase in the efficiency of the process of photosynthesis and then an increase in dry matter and seed yield. These results agree with those reported by Amina *et al* (2020).

The combined data of all previous traits under field conditions were used to construct two-way hierarchical cluster analysis using Ward's method in order to monitor the relationship among all genotypes under study depending upon disease severity of chocolate spot infection, yield, yield components, growth and chemical composition traits as well as if there any traits are related to each other. According to this analysis, the genotypes under study in the horizontal cluster analysis were distributed into three clusters. The first cluster contained GB 13, Giza 843, Misr 1 and GB 6. The second cluster included GB 16, Giza 716, GB 18, Sakha 1, GB 20, Sakha 4 and GB 17. The third cluster contained GB 11, GB 12 and Giza 40. The moderate resistant genotypes clustered in first group, resistant genotypes clustered in the second group and moderate susceptible genotypes clustered in the third group. This means that the genetic background played significant role in the inheritance of all traits under study and there is a significant genetic diversity among genotypes in relation to the recorded traits in this study. This result is in agreement with that mentioned by Belal *et al* (2018), who found enrich genetic diversity in faba bean genotypes in relation to heat and drought tolerance traits.

In the vertical cluster analysis, disease severity of chocolate spot infection, yield, yield components, growth and chemical composition traits clustered in four main groups. The first group included five traits (number of branches plant⁻¹, CGR, proline, Chl. b and LAI), the second group contained, nine traits (disease severity, Chl a , Seed yield ardab fad⁻¹, peroxidase, Total chl, number of pods plant⁻¹ and shoot dry weight after 70 days) the third one contained four traits (shoot dry weight after 90 days, seed yield plant⁻¹(g), total phenols and number of seeds plant⁻¹) and the last group included only 100- seed weight (g) trait (Figure 1). The most related to each other traits were shoot dry weight at 70 days and Total chl.

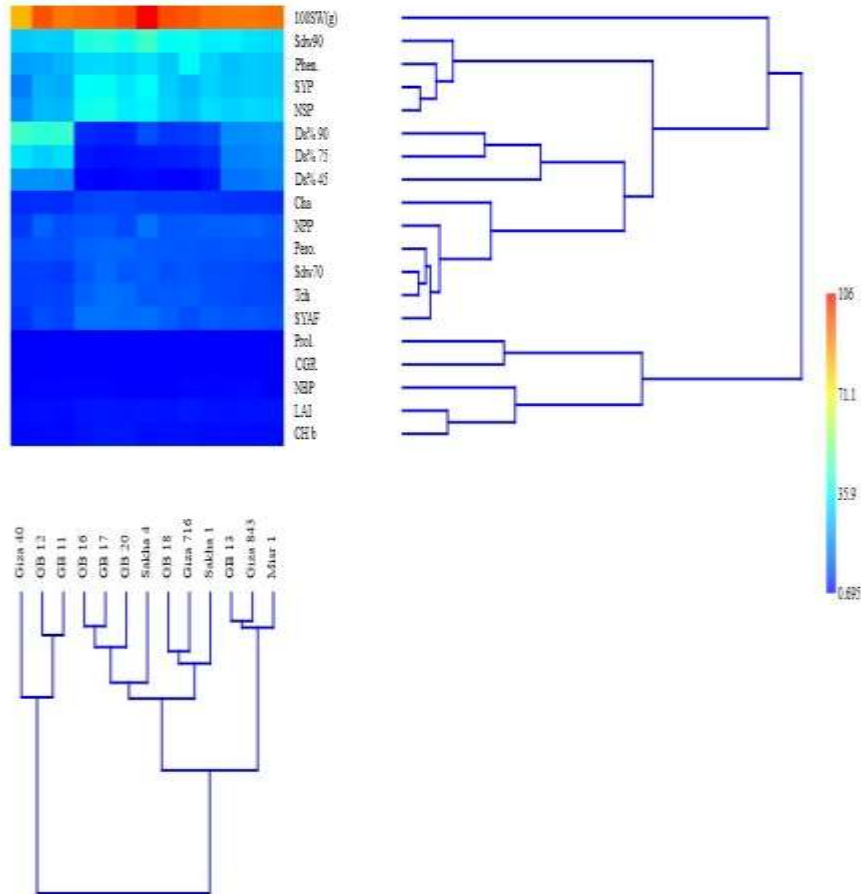


Fig. 1. Two-way hierarchical cluster analysis of new genotypes and some cultivars of faba bean depending upon Chocolate spot disease severity, yield, yield components, growth and chemical composition traits.

CONCLUSION

The findings of our research are quite convincing, and thus the following conclusions can be drawn developing new genotypes with more resistance to chocolate spot disease than local cultivars under both of artificial infection with *Botrytis fabae* virulent isolate (*B. f* N) of *Botrytis fabae in vivo* and under natural infection in the field. The new genotypes (GB 17 and GB 20) and (Sakha 1 and Sakha 4) are the resistant genotypes and have an increase in photosynthetic pigments content (Chlorophyll a, b). There was a positive correlation between decreasing chocolate spot disease severity and the increasing of total phenols, peroxidase activity, proline content and the yield productivity. It is possible to conclude that such resistant genotypes are interesting genetic sources for future breeding programs for developing chocolate spot-resistant cultivars. Therefore, these new genotypes can be used as naturally and safe for the health of humans, animals, and the environment agents to control the chocolate spot disease of faba bean.

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تقييم بعض التراكيب الوراثية من الفول البلدي تحت ظروف الإصابة بمرض

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تم إجراء هذا البحث بمزرعة محطة البحوث الزراعية بإيتاي البارود، محافظة البحيرة خلال المواسم الشتوية ٢٠٢٢/٢٠٢١ و ٢٠٢٣/٢٠٢٢، وذلك لتحديد تراكيب وراثية جديدة مقاومة للتبغ الشيكولاتي أكثر من الأصناف المحلية والربط بين صفات المحصول والصفات الفسيولوجية. تم استخدام ثمانية تراكيب وراثية من الفول البلدي (GB17،GB16،GB13،GB12،GB11،GB6) (GB20،GB18، و ستة أصناف من الفول البلدي (سحا ١، سحا ٤، جيزة ٧١٦، جيزة ٨٤٣، مصر ١، وجيزة ٤٠) في هذه الدراسة. تم تصميم التجربة بنظام القطاعات الكاملة العشوائية في ثلاث مكررات. أظهرت الدراسة أن التراكيب الوراثية الجديدة GB16 و GB17 و GB18 و GB20 مقاومة للتبغ الشيكولاتي تحت ظروف الصوبة والحقل معاً. حققت التراكيب الوراثية المقاومة الجديدة (GB16، GB17، GB20) متوسط قيمة عالية لصفات المحصول و مكوناته في الموسم الثاني فقط. قد يكون ذلك بسبب الإصابة العالية بـ *B. fabae* في الموسم الأول. التركيبين الوراثيين الجديدين نوا صفة المقاومة لمرض التبغ الشيكولاتي (GB17 و GB20) تفوقاً على التراكيب الوراثية الأبوية المقاومة (سحا ١، سحا ٤ والجيزة ٧١٦) في محتواها من كلوروفيل أ، ب، ب+، وكان هناك ارتباط معنوي إيجابي بين شدة الإصابة بمرض التبغ الشيكولاتي والفينولات الكلية ونشاط البيروكسيداز والبرولين في كلا الموسمين. في حين أن زيادة الفينولات الكلية ونشاط البيروكسيداز والبرولين أدت إلى انخفاض في شدة المرض. أظهر التحليل العنقودي الهرمي ثنائي الاتجاه أن صفات شدة المرض، عدد قرون النبات^{-١}، محصول البذور أردب/القدان، كلوروفيل أ، الكلوروفيل الكلي، البيروكسيداز والوزن الجاف للمجموع الخضري عند ٧٠ يوماً هي صفات مرتبطة ببعضها.

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