Bitki Koruma Bülteni / Plant Protection Bulletin

http://dergipark.gov.tr/bitkorb

Original article

Reactions of some common bean cultivars grown in Turkey against some isolates of angular leaf spot disease, caused by *Pseudocercospora griseola* (Sacc.) Crous & U. Braun

Ülkemizde yetiştirilen bazı fasulye çeşitlerinin *Pseudocercospora griseola* (Sacc.) Crous & U. Braun'dan kaynaklanan Fasulye köşeli yaprak lekesi hastalığına karşı reaksiyonları

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ARTICLE INFO

Article history:

DOI: 10.16955/bitkorb.630968

Received : 08.10.2019

Accepted : 18.01.2020

Keywords:

angular leaf spot, common bean, reaction

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ABSTRACT

Vegetable bean production as mid-season crops in greenhouses has increased in two provinces, Bartın and Zonguldak, in the Western Black Sea region. Green bean is sown in about half of the total area of 510.000 da in this region. Angular leaf spot disease caused by *Pseudocercospora griseol*a has been a serious disease for the last five years and it is so widespread in the region, showing almost 100% incidence. Since the beans are used for fresh consumption and are harvested continuously, fungicide use is restricted due to unwanted residues. Because of the farmers in the region widely use two local cultivars susceptible to the disease, providing resistant cultivars for farmers was the aim of this study. Although the causal agent has great pathogenic variation, determination of pathotypes of the *P. griseola* was not succeed since the three of the differential cultivars did not produced seed in two tested regions of Turkey. For this reason seventeen common bean cultivars in Turkey were selected and their reactions against ten randomly selected the most aggressive isolates of *P. griseola* were used for screening resistance. Two bush type bean cultivars, Bourgondia and Yalova 17, out of nine showed resistant reaction against all of ten isolates while three climbing types, Fabio, Burayşe and Selvi, out of eight were found resistant against all of the isolates of *P. griseola*. All of these resistant cultivars have good culinary characteristics.

INTRODUCTION

Common bean cultivation in greenhouses has been developed rapidly over the past 25 years, and it is now grown

on 109.000 ha and produced approximately 632.301 tons in Turkey (TÜİK 2014).

Angular leaf spot (ALS) of common bean (*Phaseolus vulgaris* L.), caused by the fungus *Pseudocercospora griseola* Sacc. Crous & U. Braun is a serious disease on greenhouse grown beans in Western Black Sea region, Turkey which is the largest producer and consumer of common bean. Also because of our dinner's national beans, bean is a special plant for our country. ALS causes severe yield losses depending on the susceptibility of the cultivars and environmental conditions. The disease affects pods and foliage, and is particularly destructive in warm, humid areas (Saettler 1991). The name of the disease comes from the small greyish brown lesions which are angular in shape. The lesions coalesce, turn brown, and cause the yellowing of leaves which fall off prematurely. Lesions may also appear on pods and stems. The fungus usually sporulates on the lower surface of the leaf by producing dark grey to black synnemata. The disease also affects the quality and marketability of pods and seeds across bean-production areas of the world (Pastor-Corrales et al. 1998). If infected seeds are sown, disease symptoms may appear on the primary leaves. In developing and adult plants, usually the older leaves of the plant canopy show more severe symptoms, spreading to other parts, including pods and seeds (Mora et al. 1985).

Mora (1985) and Santos-Filho et al. (1978) observed that inoculations made between 30 and 45 days after sowing caused maximum yield losses in Brazil. The latter authors also noted that inoculations made 60 day after planting reduced seed weight, while inoculations made 75 days after had no effect on seed yield and weight. Although ALS may cause severe leaf defoliation Cole (1966) and Dhingra and Kushalappa (1980) found no correlation between disease severity on pods and percentage of seeds infected with the pathogen. The fungus always grows at the hilum. Thus, a seed became infected with *Pseudocercospora griseola* (Pg) only when it was attached to the pod suture directly beneath a lesion (Pastor-Corrales et al. 1998). Losses can be as high as 80% under favourable environmental condition (Schwartz et al. 1981).

Pathogenic variation of the fungus has been reported in several countries (Buruchara 1983, Correa-Victoria 1987, Monda 2001) and its control, especially by growing resistant cultivars is complicated by this variation of the fungus.

Based on morphological and molecular markers, two gene pools of origin for common bean have been defined namely; the Andean and Middle American. The Andean isolates are pathogenic to large seeded beans while the Middle Americans are pathogenic to both small and large seeded ones (Pastor-Corrales et al. 1998). Nonetheless, Pg pathotype structure might also be associated with the compatibility of cultivated bean genotypes. Whereas most large seeded commercial varieties in Uganda are susceptible to ALS (Namayanja et al. 2006), cultivars such as AND 277, G5686 and Mexico 54 have been identified as valuable sources of resistance (Aggarwal et al. 2004, Nietsche et al. 2000, Pastor-Corrales et al. 1998). A number of studies have demonstrated that the level of variability among and within Pg populations is considerably high, even though the sexual form of the fungus has not been found (Liebenberg and Pretorius 1997). Studies demonstrated variation in pathogenicity of fungal isolates as 53, 13 and 50 different pathotypes described among 54, 30 and 112 isolates that were obtained from Africa, Brazil and Central America, respectively (Busogoro et al. 1999, Mahuku et al. 2004, Nietsche et al. 2000). Though ALS has been determined to occur in Turkey (Ozan 2009, Vural and Soylu 2012), the genetic structure, pathogenicity, disease incidence and severity, and molecular characterisation of the fungus remains unknown and probably as a consequence of this there is hardly any bred commercial cultivar, either tolerant or resistant.

In recent years, this disease has been one of the most important bean production constraints in greenhouses in Western Black Sea region, Turkey. In 2005-2015, severe outbreaks of ALS of common beans caused by Pg was observed in greenhouses in Bartın, Zonguldak and Karabük, Turkey (Ozan 2009, Ozan and Maden 2010). The purpose of this study was to screen the resistance of common bean cultivars grown extensively in Turkey against ten isolates of the fungus obtained from various places.

MATERIALS AND METHODS

Sampling and isolations

Totally 118 common bean leaf samples having typical ALS symptoms; being 85, 32 and 1 from Bartın, Zonguldak and Karabük provinces of Turkey. The samples were collected from 100, 82 and 41 da greenhouse cultivation areas of the three provinces which have 326, 185 and 59 da greenhouse cultivation area, respectively.

The information about the growth size and pattern, the seed size and colour of the bean cultivars and the location where they were collected was recorded. For induction of fungal sporulation, tissues from infected bean leaves were placed

on petri dishes, lined with moistened filter paper, and were incubated at room temperature for 3 to 4 days. Conidia from sporulated ALS lesions were picked up with a tiny piece of agar placed on the tip of a sterilized dissecting needle, streaked onto V-8 juice agar, and then incubated for 24 h at 24 °C. Individual germinated conidia were then transferred to V-8 juice agar to obtain monosporic cultures for each Pg isolate. Each monosporic isolate obtained from a different common bean genotype, was cultured on V-8 juice agar at 24 °C and kept on V-8 juice agar at 4 °C for long-term preservation.

Determination of the reactions of some bean cultivars

Inoculum preparation

Ten single spores Pg isolates obtained from bean growing districts of Bartın, Zonguldak and Karabük provinces were used for the experiments. Inoculum for greenhouse screenings was obtained from a 2 week-old culture of each monosporic Pg isolate grown on petri dishes containing V8- juice agar. Spores for inoculation were prepared from these plates by adding sterile distilled water and scraping the surface of the plate with a spatula to dislodge the conidia. Spore concentration in the inoculums was calculated using a haemocytometer and adjusted to a final concentration of 2 x 10^4 conidia/ml using sterile distilled water (Pastor-Corrales et al. 1998).

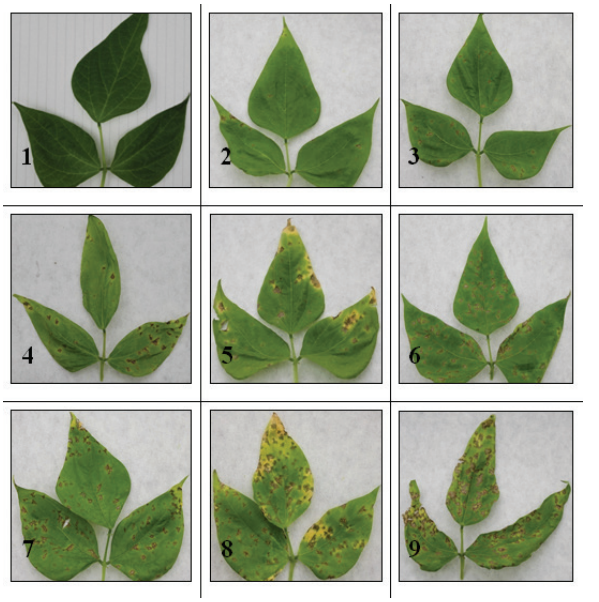

Figure 1. The CIAT 1-9 scale used for the evaluation of the reactions of bean cultivars against Pg

Inoculation and determination of reactions

Seventeen bean cultivars, nine bush type and 8 climbing type, were used for resistance screening (Table 1). Reactions of all the seventeen cultivars were tested against ten monospore isolates of Pg. Bean seeds were sown in pots with 1.5 kg of soil, manure and sand in a ratio of 2:1:1 and five seeds/pot. Ten days after sowing, plants were thinned to three/pot. The

Table 1. Common bean cultivars used for disease screening, their growth habits, seed shape and seed colour

Bush types		Pole or climbing types	
Name of the cultivar	Seed shape and colour	Name of the cultivar	Seed shape and colour
1. Karaayşe	Kidney shape, black	1. Perla	Spherical, white
2. Yalova 17	Kidney shape, white	2. Sofya	Elliptical, white
3. Bourgondia	Kidney shape, white	3. Özayşe	Spherical, white
4. Nadide	Ovoid, white	4. Fabio	Kidney shape, white
5. Java	Oblong, white	5. Helda	Kidney shape, white
6. Strike	Kidney shape, white	6. BT Burayşe	Kidney shape, white
7. Volare	Ovoid, brown	7. Selvi	Kidney shape, white
8. Gina	Ovoid, white	8. Sarıkız	Spherical, white
9. Magnum	Kidney shape, brown		

Table 2. The CIAT 1-9 scale used for the evaluation of the reactions of bean cultivars against Pg

Scale value	Infection description	Reaction type
1	Plants with no visible disease symptoms	R
3	Plants with 1-15% of the leaf area with lesions	R
5	Plants with 16-40% of the leaf area with lesions	MR
7	Plants with 41-70% of the leaf area with lesions	MS
9	Plants with 71-100% of the leaf area with lesions, frequently associated with early loss of the leaves and plant death	S

experiment was laid out randomised complete block design with three replications. Inoculation was done when plants were in the V3 stage (Schoonhoven and Pastor-Corrales 1987). The conidial suspension was sprayed both onto the upper and lower surfaces of leaves of the selected cultivars until wash off. After inoculation, plants were placed into the growth chamber set to 20-22 °C, and 95-100% relative humidity. After 4 days, the inoculated plants were transferred into the greenhouse having 24-28 °C with a 12-h-photoperiod. Disease evaluations on individual plants were made using a CIAT 1-9 scale (Schoonhoven and Pastor-Corrales 1987) for 18 days, at an internal of three days, described as Figure 1 and Table 2.

RESULTS

Characteristics of the cultivars grown in the region and analysed for resistance

In the research, two local cultivars, Şeker Ayşe and Barbunya were grown in greenhouses while the cultivar Gina which was also tested for resistance was found grown in the field at one location. No disease was observed on this variety in the field. The cultivars which were screened for resistance (Table 1) were selected among the bean cultivars mostly grown in Turkey.

Disease incidence and severity in the study area

Angular leaf spot disease was observed almost in all the greenhouses. The disease affected mainly the leaves and caused angular leaf spots at the beginning of disease onset, then the spots coalesced and formed leaf blight, and eventually leaf shed. It also affected pods where the spots are rounded and watery at first then dry and dark in colour (Figure 2). The severity of ALS varied in spring and autumn growing seasons, being in the ratios of 74.50-80.35% and 88.60-92.88% in Bartın and Zonguldak provinces respectively.

Figure 2. Symptoms of angular leaf spot disease, a) on the leaves, b) on the pods

Reactions of bush type bean cultivars

Reactions and disease severity (%) rate of 10 bush type bean cultivars against 10 isolates of Pg are summarized in Table 3 and 4. Half of the bush type bean cultivars; Java, Strike, Volare, Gina and Magnum, all foreign originated cultivars, showed susceptible reactions against all of the Pg isolates. The cultivars Karaayşe and Nadide were also susceptible against most of the isolates, the former being susceptible against 8, the latter against 6 isolates. Reactions of the bush type bean cultivars did not differ so much among the isolates, in other words all the 10 isolates produced almost similar reactions on the cultivars.

Reactions of climbing type bean cultivars

Three climbing type bean cultivars; Sarıkız, Sofya and Helda, all were susceptible, while three cultivars; Fabia, Burayşe and Selvi were resistant against all of the ten isolates of *P.g.* f. *griseola*. The cultivars; Perla and Özayşe gave intermediate reaction (Table 5 and Table 6).

Reactions of the climbing type bean cultivars also did not differ so much among the isolates. In general, climbing type bean cultivars seemed more resistant to ALS. The reactions of the climbing type bean cultivars were not different among the

Table 3. Reactions of 9 bush type bean cultivars against *Pseudocercospora griseola f. griseola* isolates

Bean Cultivars (Bush type)	*Pseudocercospora griseola.* f. *griseola* isolates* / Reaction type									
	51	27 A	11 A	62A	112	49	85	79	82	100
Java	S	S	S	S	S	S	S	S	S	S
Strike	S	S	S	S	S	S	S	S	S	S
Volare	S	S	S	S	S	S	S	S	S	S
Gina	S	S	S	S	S	S	S	S	S	S
Magnum	S	S	S	S	S	S	S	S	S	S
Karaayşe	S	S	S	S	S	S	MS	S	MS	S
Nadide	S	MS	MS	MS	MS	S	S	S	S	S
Bourgondia	MR	MS	MS	MR	MR	MR	R	R	R	R
Yalova 17	MR	MR	MR	R	R	R	R	R	R	R

*The origin of the *Pseudocercospora griseola*. f. *griseola* isolates were as; 51 Gökçebey-Bakacakkadı, 27A Devrek-Alduvaklar, 11A Devrek-Çaydeğirmeni, 62A Gökçebey-Kadoğlu, 112 Çaycuma-Dereköseler, 49 Gökçebey-Bakacakkadı, 85 Bartın-Hacıhatipoğlu, 79 Bartın-Dolaşıklar, 82 Bartın-Eysanlar, 100 Karabük-Merkez.

Table 4. Disease severity rate (%) of 9 bush type bean cultivars against *Pseudocercospora griseola* f. *griseola* isolates

Bean Cultivars (Bush type)	*Pseudocercospora griseola.* f. *griseola* isolates* / Disease severity (%)									
	51	27 A	11 A	62A	112	49	85	79	82	100
Java	89.50	97.00	97.75	88.00	88.50	91.00	94.75	93.00	96.00	84.75
Strike	89.00	96.75	96.75	88.25	91.25	76.75	95.50	83.50	78.50	85.75
Volare	95.25	98.00	96.20	97.00	80.75	94.00	95.00	95.00	84.75	88.25
Gina	96.00	96.00	100	90.25	94.00	79.25	96.00	96.00	100	100
Magnum	95.50	97.00	100	92.50	97.75	96.00	97.75	99.00	100	98.00
Karaayşe	91.00	76.50	76.25	80.50	79.25	82.25	63.75	73.75	56.75	95.00
Nadide	84.50	63.00	64.50	70.25	59.75	73.75	73.50	83.75	76.25	75.25
Bourgondia	30.25	51.25	52.50	28.75	37.25	25.25	14.00	11.00	11.00	11.00
Yalova 17	21.00	20.00	20.50	11.00	11.00	11.00	11.00	11.00	11.00	11.00

*The origin of the *Pseudocercospora griseola*. f. *griseola* isolates were as; 51 Gökçebey-Bakacakkadı, 27A Devrek-Alduvaklar, 11A Devrek-Çaydeğirmeni, 62A Gökçebey-Kadıoğlu, 112 Çaycuma-Dereköseler, 49 Gökçebey-Bakacakkadı, 85 Bartın-Hacıhatipoğlu, 79 Bartın-Dolaşıklar, 82 Bartın-Eysanlar, 100 Karabük-Merkez.

Table 5. Reactions of 8 climbing type bean cultivars against *Pseudocercospora griseola* f. *griseola* isolates

Bean cultivars (Climbing types)	*Pseudocercospora griseola.* f. *griseola* isolates* / Reaction type									
	51	27 A	11 A	62A	112	49	85	79	82	100
Sarıkız	S	S	S	S	S	S	S	S	S	S
Sofya	S	S	S	S	S	S	S	S	S	S
Helda	S	S	S	S	S	S	S	S	S	S
Özayşe	MS	MS	MS	MS	S	MS	MS	MS	MS	S
Perla	MS	MS	MS	MS	MR	MR	MS	MS	MR	MR
Fabio	R	R	R	R	R	R	R	R	R	R
Burayşe	R	R	R	R	R	R	R	R	R	R
Selvi	R	R	R	R	R	R	R	R	R	R

*The origin of the *Pseudocercospora griseola*. f. *griseola* isolates were as from; 51 Gökçebey-Bakacakkadı, 27A Devrek-Alduvaklar, 11A Devrek-Çaydeğirmeni, 62A Gökçebey-Kadoğlu, 112 Çaycuma-Dereköseler, 49 Gökçebey-Bakacakkadı, 85 Bartın-Hacıhatipoğlu, 79 Bartın-Dolaşıklar, 82 Bartın-Eysanlar, 100 Karabük-Merkez.

Table 6. Disease severity rate (%) of 8 climbing type bean cultivars against *Pseudocercospora griseola* f. *griseola* isolates

Bean cultivars (Climbing types)	*Pseudocercospora griseola.* f. *griseola* isolates* / Disease severity (%)									
	51	27 A	11 A	62A	112	49	85	79	82	100
Sarıkız	74.00	75.00	71.25	73.75	84.50	87.00	86.75	89.00	96.25	85.00
Sofya	96.75	100	97.75	96.00	100	99.00	100	100	100	100
Helda	95.75	96.00	93.50	88.75	73.00	83.75	97.50	81.50	97.50	80.50
Özayşe	63.75	62.00	70.25	61.50	78.00	64.20	65.50	61.75	45.00	73.75
Perla	59.75	67.25	58.00	53.75	33.75	24.50	44.75	46.50	33.25	20.00
Fabio	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Burayşe	11.00	11.00	16.50	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Selvi	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00

*The origin of the *Pseudocercospora griseola*. f. *griseola* isolates were as from; 51 Gökçebey-Bakacakkadı, 27A Devrek-Alduvaklar, 11A Devrek-Çaydeğirmeni, 62A Gökçebey-Kadıoğlu, 112 Çaycuma-Dereköseler, 49 Gökçebey-Bakacakkadı, 85 Bartın-Hacıhatipoğlu, 79 Bartın-Dolaşıklar, 82 Bartın-Eysanlar, 100 Karabük-Merkez.

isolates, in other words all the 10 isolates produced almost similar reactions on the cultivars.

DISCUSSION

Common bean cultivation in greenhouses, especially for green consumption, in Zonguldak, Bartın and Karabük provinces of Turkey has been practised twice a year, in the spring and autumn at about 1.040 da. The profitability of this crop has been impaired by ALS caused by the fungus Pg especially for autumn growing period. The pathogen is genetically differentiated into two forms; *P. g.* f. *griseola* and

P. g. f. *mesoamericana* (Crous et al. 2006) and molecular analysis of ITS gene sequences of 118 isolates showed that all of isolates were belong to the *P. g.* f. *griseola*.

The most feasible method to control ALS, especially on snap beans is to grow resistant varieties. To achieve this; pathogenic variation, which Pg has many pathotypes, has to be known. Although we tried to determine the pathotypes of Pg, it was not succeed to grow and obtain seeds of three differential cultivars in Turkish climatic conditions. For this reason, ten randomly selected isolates of the pathogen were used to test the reactions of some cultivars, foreign and native origin. Seventeen bean cultivars, 9 being bush type and 8 being climber type, were selected for the tests. Bean cultivars are generally divided into groups based on the growth habits (bush or climber) and seed size and the characteristics of beans are related with these aspects.

Although the reactions of bean cultivars against ten isolates of *P. g.* f. *griseola* showed variation, there were not any differences among the isolates. This shows that the randomly selected isolates of Pg do not have pathogenic variation. To put it clearly, pathotypes of the many isolates of Pg should be determined by using differential genotypes.

Several previous studies have indicated the use of different resistant sources against ALS. For instance, studies on screening common bean genotypes for resistance to ALS have been conducted and some sources of resistance identified. Some of these sources include MAR-1, MAR-2, MAR-3, Mexico 54 and BAT 332 (Buruchara and Bua 1999, Caixeta et al. 2003, Mahuku et al. 2003, Mahuku et al. 2004, Mahuku et al. 2009, Namayanja et al. 2006, Pastor-Corrales et al. 1998). Some of the studies also revealed that Mexico 54 was resistant to most African isolates that have so far been characterized (Namayanja et al. 2006). Out of 163 African isolates, Mexico 54 was resistant to 158 and hence considered as one of the excellent source of resistance to ALS in Africa (Namayanja et al. 2006). However, the resistance in Mexico 54 varies in the number of genes that condition resistance in it (Ddamulira 2016).

Three bean cultivars having Turkish names Burayşe, Selvi, Yalova 17, and two foreign origin varieties, Bourgondia and Fabio, showed resistance to all of the isolates used during the screening test. Therefore, these cultivars may be proposed to the farmers in the region as their horticultural aspects are satisfactory. On the other hand, another approach to reduce disease incidence by sowing even mixtures of resistant and susceptible bean varieties was proposed by Olango et al. (2017) since they found low disease incidence sown by the bulk mixtures. For maintaining the resistance of a variety for a long time and preventing formation of new resistant pathotypes sowing bulk mixtures could be useful.

The difficulty of breeding resistance arises when the disease is inherited by many genes. Although it is not easy to obtain complete resistance to all of the races of ALS disease, breeding programs for resistance eventually help to reduce disease severity in the field. De Faria et al. (2013) evaluated the performance of 40 bean cultivars recommended by various Brazilian research institutions between 1970 and 2013 and found 6.74% grain yield increase during this period. A recent study on QTL markers showed promise for resistance breeding of beans against ALS disease. Oblessuc et al. (2012) found an environmentally stable marker (GATS11b) as a potential tool for marker assisted selection for ALS resistance. Though the pathotypes of *Pseudocercospora griseola* is determined by the reactions formed on the leaves, this might not be suitable when deciding on the resistance of the varieties. Since Borel et al. (2011) found the heritability of pod symptoms was higher than the leaf symptoms which were influenced by environmental factors greatly.

Pathogenic variability of *P. griseola* is very high in some places. For instance, Silva et al. (2008) determined ten pathotypes in Minas Gerais State of Brazil, four of them being first records. Pathogenic variability was also reported in Argentina (Stenglein et al. 2006). The same situation might occur in Turkey.

This is the first study to determine screening of the reactions of the most commonly grown bean cultivars against ALS disease agent of beans although it was reported previously in Turkey (Anonymous 1991, Ozan 2009, Vural et al. 2007, Vural and Soylu 2012). Studies done in other countries showed that bean cultivars are mostly susceptible to ALS disease especially the big seeded varieties (David et al. 2000, Ddamulira 2016, Leitich 2016, Sartorato 2004, Wagara et al. 2004). It is interesting that most of the selected cultivars grown in Turkey are white seeded and the resistant cultivars belonged to white types, being two kidney shape.

ACKNOWLEDGEMENTS

This study is the PhD thesis of Ankara University Graduate School of Natural and Applied Sciences Department of Plant Protection named ''Determination of the Groups of *Pseudocercospora griseola* Causing Angular Leaf Spot in Protected Beans in West Black Sea Region, Investigations on Prevalence, Searches of Inoculum and Reactions of Some

Bean Cultivation Against This Agent" and this project was supported by Turkish Ministry of Agriculture and Foresty with Project number TAGEM-BS-/10/10-01/02-05 under National Integrated Greenhouse Disease Management Program.

ÖZET

Batı Karadeniz Bölgesi'nde Bartın ve Zonguldak illerinde seralarda ara sezon ürünü olarak fasulye üretimi büyük artış göstermiştir. Taze fasulye, bu bölgedeki toplam 510.000 da alanın yaklaşık yarısında ekilmektedir. *Pseudocercospora griseola*'nın neden olduğu köşeli yaprak lekesi hastalığı son beş yıldır fasulye üretiminde ciddi zararlara neden olmakta ve bölgede neredeyse %100 hastalık çıkısına sebep olmaktadır. Fasulyenin taze olarak tüketilmesi ve sık hasat edilmesi nedeniyle fungisit kullanımı istenmeyen kalıntılar sebebiyle kısıtlanmıştır. Bölgedeki çiftçilerin yaygın olarak hastalığa duyarlı iki yerel çeşidi kullanmaları nedeniyle bu çalışmada çiftçilere dayanıklı çeşitler sağlamak amaçlanmıştır. Hastalık etmeninin patotiplerinin belirlenmesinde kullanılan ırk ayırıcı set içinde yer alan üç fasulye çeşidi Türkiye'nin iki farklı bölgesinde yetiştirilmiş ancak tohum elde edilemediğinden hastalığa neden olan etmenin büyük patojenik varyasyonu olmasına rağmen, *P. griseola*'nın patotipleri belirlenememiştir. Bu nedenle Türkiye'de en çok yetiştirilen on yedi fasulye çeşidi seçilmiş ve tesadüfi olarak seçilen *P. griseola*'nın en agresif 10 izolatı ile dayanıklılık taraması yapılmıştır. Dokuz oturak tipi fasulye çeşidinden ikisi Bourgondia ve Yalova 17, sekiz sırık fasulye çeşidinden ise üçü Fabio, Burayşe ve Selvi tüm *P. griseola* izolatlarına karşı dayanıklı reaksiyon göstermiştir. Bu dayanıklı çeşitlerin hepsi sofralık olarak da iyi özelliklere sahiptir.

Anahtar kelimeler: Köşeli yaprak lekesi, fasulye, çeşit reaksiyonu

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Cite this article: Canpolat, S, Maden, S. (2020). Ülkemizde yetiştirilen bazı fasulye çeşitlerinin *Pseudocercospora griseola* (Sacc.) Crous & U. Braun'dan kaynaklanan Fasulye köşeli yaprak lekesi hastalığına karşı reaksiyonları. Plant Protection Bulletin, 60-2. DOI: 10.16955/bitkorb.630968

Atıf için: Canpolat, S, Maden, S. (2020). Ülkemizde yetiştirilen bazı fasulye çeşitlerinin *Pseudocercospora griseola* (Sacc.) Crous & U. Braun'dan kaynaklanan Fasulye köşeli yaprak lekesi hastalığına karşı reaksiyonları. Bitki Koruma Bülteni, 60-2. DOI: 10.16955/bitkorb.630968