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RESEARCH PAPER

Screening Some Bean Genotypes for Resistance to *Macrophomina phaseolina* in Türkiye

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Abstract

Macrophomina phaseolina (Tassi) Goid. is a fungal pathogen causes significant economic losses, including root and hypocotyl rot, root collar and stem blight, which are common in bean production areas. In this study, 24 bean genotype developed by the Black Sea Agricultural Research Institute and *M. phaseolina* isolate M1 isolated from bean cultivation areas were used.
This research was carried out with pot experiments in climatic chamber conditions to determine the reactions of some bean (*Phaseolus vulgaris* L.) genotypes against *M. phaseolina*, which causes charcoal rot disease in beans. Among the bean genotypes evaluated within the scope of the study, *KMF-11-30* and *ARSLAN* genotypes were found to be tolerant, while the *20-BBarBVD-10* genotype was found to be resistant.

INTRODUCTION

Bean, which is an important plant, an indispensable legume in human nutrition due to its high protein and carbohydrate content. 29 million tons of beans were produced in 33 million hectares of land, with an average yield of 87 kg per hectare in 2019 according to FAO (Faostat, 2020). Fresh bean production in Türkiye in 2020 was 547,349 tons and dry bean production was 279,518 tons (Anonymus, 2020). Agricultural products used in the production of food and beverages, which have national value for our country, have a special importance in the domestic market for Türkiye (Canpolat and Maden 2020). Bean production is widespread in almost all geographical regions of our country.

There are many disease factors that cause significant economic losses in terms of yield and quality in bean plants in the world (Hall, 1994). One of the important fungal diseases affecting bean production is charcoal rot disease caused by *Macrophomina phaseolina* (Tassi) Goid. *M. phaseolina* is a plant pathogenic fungus belonging to the Botryosphaeriaceae family that causes the collapse of many plant species, seedling blight, crown rot, stem rot, charcoal rot, basal stem rot and root rot. *M. phaseolina*, one of the most harmful seed and soil-borne pathogens, is a fungal pathogen that infects more than 100 plant species (Babu *et al.* 2007). Hosts include peanuts, cabbage, peppers, chickpeas, soybeans, sunflowers, sweet potatoes, alfalfa, sesame, potatoes, sorghum, wheat and maize. It has been reported that the disease agent commonly causes disease in sesame, soybean, sunflower, cotton, melon, beans, tobacco, tomato, potato, apricot and cucumber in Türkiye (Maden and İren, 1984; Arca and Yıldız, 1990; Tezcan and Yıldız, 1991; Kınay and Yıldız, 1994; Baran and Kurt, 2001; Sağır *et al.*, 2009; Pekgöz and Tok, 2018; Tok, 2019; Anonymous, 2020; Lavkor and Onat, 2021). *M. phaseolina* is a common fungus in bean production areas in Türkiye and causes significant economic losses by causing root collar, root, stem and hypocotyl rot. *M. phaseolina* usually affects the roots and then causes shortening of the internodes on the stem which inhibits the transport of water and nutrients from the soil to the upper parts of the plants, causing symptoms of wilting, stunting and chlorosis in infected plants, beside in severe infections, diseased plants may die (Khan, 2007). Root rot pathogens also damage the primary root while killing the small lateral roots of plants such as beans, peanuts, soybeans and asparagus (Pandey, 2020).

It is reported that charcoal rot disease causes crop losses of up to 100% (Dhingra and Sinclair, 1978; Hagedorn, 1994; Ghosh *et al.*, 2018). It has been reported that at high temperatures (30–35°C) and low soil moisture (below 60%), the disease causes significant yield losses in soybean and sorghum crops (Kaur *et.al.*, 2012). 100% yield loss was recorded in the pre-emergence period in peanut cultivars when suitable conditions were established for the disease (Sharma and Bhowmik, 1986). Yield reductions of 5% to 50% have been reported for beans, depending on environmental conditions and genotype susceptibility. It has been observed that artificially inoculated bean plants cause a 60% decrease in grain yield compared to bean plants grown on naturally contaminated soils. It is also stated that agricultural practices such as crop rotation are not suitable for reducing the severity of *M. phaseolina* due to the wide host range and the inoculum to remain viable in the soil for many years, and it would be more appropriate to use a resistant cultivar for disease control (Zanella *et al.*, 2020).

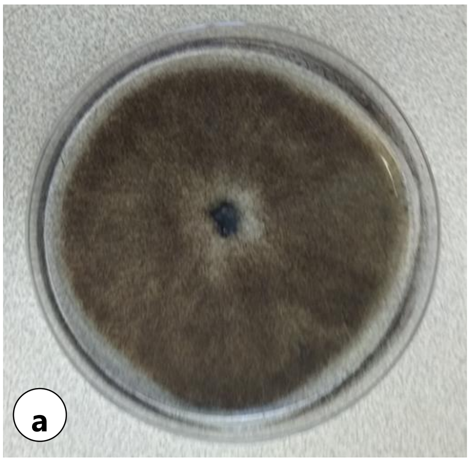
It has been revealed that determining the reactions of bean genotypes developed in bean breeding studies in Türkiye against *M. phaseolina* has great importance in the management of the disease. For this reason, in this study, reactions of bean genotypes developed by KTAE (Black Sea Agricultural Research Institute) against charcoal rot disease caused by *M. phaseolina* and promising gene sources were determined as a basis for breeding.

MATERIALS and METHODS

In the study, *M. phaseolina* isolate (M1, Figure 1a, b) obtained from infected bean plants which is stored in Ankara Plant Protection Central Research Institute culture collection and 24 different bean genotypes developed by KTAE were used.

Inoculum Preparation

The soil inoculation method of Nene and Haware (1980) was modified and used in pot experiments. For the inoculum, a mixture of 45 g



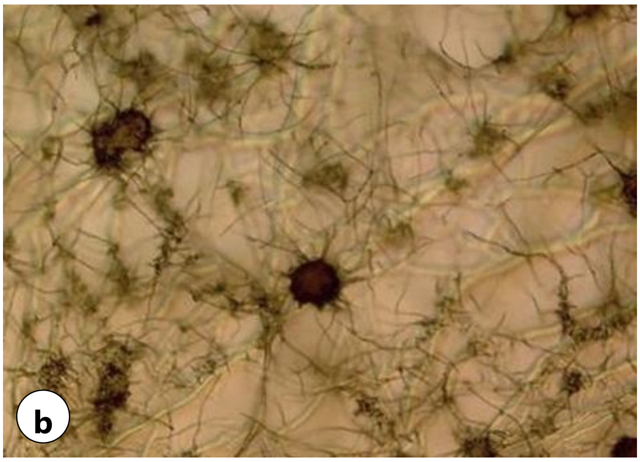


Figure 1. a) Colony morphology of *Macrophomina phaseolina* on PDA, **b)** Sclerotia on PDA.

of sifted sand, 5 g of corn flour and 10 ml of sterile distilled water was prepared, filled in 100 ml bottles and sterilized 2 times at 24-hour intervals, then inoculated with 5 fungal discs taken from 10-day-old fungal cultures developed in PDA (Ozan and Maden, 2004). The bottles prepared in this way were placed in an incubator with 12 hours of light and 12 hours of darkness, and a temperature of 24±1°C (Figure 2a). Fungi cultures grown in bottles for 14 days were mixed into each of the pots containing soil: sand: fertilizer (1:1:0.5, v/v/v) and after light watering, the pots were placed in the climate chamber. It was waited 5-6 days for the inoculum to cover the soil and the seeds were kept in 1% sodium hypochlorite (NaOCl) solution for 3 minutes and surface disinfected. Seeds were sown as 3 in each pot and in 4 replications. After adding 50 g of the cornmeal-sand mixture without inoculum to the control pots, 3 seeds were planted in the same way and left for incubation in the climate chamber (Figure 2b).

Testing of the Breeding Material Against Pathogen

As the most virulent isolate, in this study M1 isolate was multiplied and used in the reaction tests of 24 breeding materials given in table 1 under climatic chamber conditions.

Evaluation of Disease Severity

Evaluations of the pot trials were made after 6 weeks according to the 0-3 scale given in table 2 (Tezcan and Yıldız, 1991). The percentage of disease severity was calculated according to the Townsend-Heuberger formula over scale values (Townsend and Heuberger, 1943).

According to this calculation;

Disease severity (%) = Σ(n x V/Z x N) x 100

n: the number of plants with different disease degrees on the scale;

V: scale value;

Z: highest scale value;

N: total plant quantity observed

In the genotype reaction studies, in determining the reaction types for pot experiments, cultivars with disease severity values between 0-30% were evaluated as resistant, between 30.1-50% as tolerant, and between 50.1-100% as sensitive. The results obtained after the evaluations were subjected to variance analysis and Duncan (P≤0.05) test by using IBM SPSS 22 statistical program to reveal the difference between varieties.

RESULTS and DISCUSSION

Within the scope of the study, the reactions of some bean varieties widely grown in Türkiye against charcoal rot disease were tested by soil inoculation method which has been used by many researchers to determine the reactions of bean genotypes against charcoal rot (Demirci 1997; Baird *et al*., 1996; Tok, 2019).

In the pot experiment of the genotypes tested against *M. phaseolina* isolate, 100% emergence was achieved in the control pots, while different rates were observed in the pots infected with *M. phaseolina*. As a result of the reaction test, statistically significant differences were found between bean varieties. The disease severity value for *M. phaseolina* in all 24 tested cultivars ranged from 16% to 98% (Table 3). In tests, *M. phaseolina* caused preemergence damping, root and crown rot, yellowing and

Figure 2. a) Inoculum of *Macrophomina phaseolina* on Corn meal+sand, **b)** Inoculum with soil in pot.

Table 1. 24 genotypes used in reaction tests in the study.

Row No	Line No	Row No	Line No
1	*KMF-11-24* (Kahramanmaraş)	13	*19-SBarBVD-8* (Eskişehir)
2	*KMF-11-30* (Kahramanmaraş)	14	*19-SBarBVD-9* (Eskişehir)
3	*20-BBarBVD-10* (Eskişehir)	15	*AKMAN 9*
4	*KKF-10-BY-3* (Samsun)	16	*GÖKSUN*
5	*KKF-10-BY-7* (Samsun)	17	*ÖZDEMİR*
6	*KKF-10-BY-9* (Samsun)	18	*KORAY*
7	*TOPÇU*	19	*KARAMAN 201*
8	*GÖYNÜK*	20	*ADABEYAZI*
9	*ÖNCELER 98*	21	*BERRAK*
10	*KORAY*	22	*ARSLAN*
11	*20-SBVD-15* (Eskişehir)	23	*SURURBEY*
12	*20-SBarBVD-7* (Eskişehir)	24	*BATALLI*

Table 2. Disease rating scale for charcoal rot reaction.[a]

Score	Description	Reaction type
1	No infection	I (Immune)
>1- ≤3	Very few small lesion on roots (1.1–2.0 ¼ approximately 5% of root tissue covered with lesions, 2.1–3.0 ¼ approximately 10% of root tissue covered with lesions)	R (Resistant)
>3- ≤5	Lesions on root clear but small, new root free from infection (3.1–4.0 ¼ approximately 17.5% or the root tissue covered with lesions, 4.1–5.0 ¼ approximately 25% of the root tissue covered with lesions)	MR (Moderately Resistant)
>5- ≤6	Lesions on roots are moderate, new roots generally free from infection (5.1–6.0 ¼ approximately 37.5% of the root tissue covered with lesions)	MS (Moderately Susceptible)
>6-≤8	Lesions on roots many, new roots generally free from infection (approximately 6.1–7.0 ¼ 50% of the root tissue covered with lesions, 7.1–8.0 ¼ approximately 62.5% of the root tissue covered with lesions)	S (Susceptible)
>8- 9	Roots infected and completely discolored (8.1–9.0 ¼ approximately 75% infection)	HS (Highly Susceptible)

[a] (Nene *et al.*, 1981; Van Schoonhoven and Pastor-Corrales, 1987; Khan *et al.*, 2017; Pandey *et al.*, 2020)

wilting of leaves. The reaction types of 24 genotypes used in the experiment against *M. phaseolina* were also characterized (Table 3). While some genotypes were categorized as resistant and intermediate resistant, none of the genotypes showed an immune reaction.

The most resistant genotype against *M. phaseolina* was *20-BBarBVD-10* (Eskişehir), with the lowest disease severity of 16.3%. This genotype was followed by *KMF-11-30* (Kahramanmaraş) which was the most tolerant genotype with 43.8% disease severity, and the 22nd genotype *ARSLAN* with 47.5% disease severity (Figure 3). The genotype that showed the most sensitive reaction was *KMF-11-24* (Kahramanmaraş) (Figure 4). Other bean cultivars

Table 3. Disease severity values (%) and reaction types caused by *M. phaseolina* isolate in 24 bean *genotypes.*

Genotype		Disease severity %±SD[a]	Reaction type
1	*KMF-11-24* (Kahramanmaraş)	98,8±1,3 [A]	Higly Susceptible
2	*KMF-11-30* (Kahramanmaraş)	43,8±19,2 [AC]	**Moderately resistance**
3	*20-BBarBVD-10* (Eskişehir)	16,3±2,4 [BC]	**Resistant**
4	*KKF-10-BY-3* (Samsun)	93,8±6,3 [A]	Higly Susceptible
5	*KKF-10-BY-7* (Samsun)	87,5±7,2 [A]	Higly Susceptible
6	*KKF-10-BY-9* (Samsun)	93,8±6,3 [A]	Higly Susceptible
7	*TOPÇU*	71,3±23,8 [AB]	Susceptible
8	*GÖYNÜK*	69±19,6 [AB]	Susceptible
9	*ÖNCELER 98*	90±5,4 [A]	Higly Susceptible
10	*KORAY*	75±2 [A]	Susceptible
11	20-SBVD-15 (Eskişehir)	72,5±3,2 [AB]	Susceptible
12	20-SBarBVD-7 (Eskişehir)	88,8±6,6 [A]	Higly Susceptible
13	19-SBarBVD-8 (Eskişehir)	81,3±6,6 [A]	Higly Susceptible
14	19-SBarBVD-9 (Eskişehir)	86,3±6,6 [A]	Higly Susceptible
15	*AKMAN 9*	92,5±3,2 [A]	Higly Susceptible
16	*GÖKSUN*	75±2 [A]	Susceptible
17	*ÖZDEMİR*	77,5±7,5 [A]	Susceptible
18	*KORAY*	68,3±4,4 [AB]	Susceptible
19	*KARAMAN 201*	92,5±3,2 [A]	Higly Susceptible
20	*ADABEYAZI*	78,8±3,8 [A]	Susceptible
21	*BERRAK*	63,8±19,6 [AB]	Susceptible
22	*ARSLAN*	47,5±17 [AC]	**Moderately resistance**
23	*SURURBEY*	86,3±6,9 [A]	Higly Susceptible
24	*BATALLI*	83,8±5,2 [A]	Higly Susceptible
25	**Kontrol**	0±0 [C]	

[a] Different letters next to the mean values in the table indicate that the difference in disease severity between the lines is statistically significant (Duncan Multiple Comparison Test, $P<0.05$).

showed varying degrees of disease severity.

In the experiments, cotyledons and lower leaves showed lesions following the emergence of seedlings in pots, and symptoms such as wilting of the leaves, darkening on the stem and subsequent death of the plant were observed 15-20 days after planting. In a study conducted in Van province, 17.5% of the isolates obtained from bean plants were *M. phaseolina* (Temizel and Ertunç, 1992). Beside in another study it is found that 7% of 285 seed samples taken from important bean cultivation areas of Türkiye were contaminated with this pathogen (Maden and İren, 1984). In the study conducted by Demirci (1997), *M. phaseolina* isolates from 17 beans, 9 chickpeas and 1 tomato were obtained in Erzincan and, it was examined that isolates obtained from beans and chickpeas cause wilting and drying in plants.

Demirer's (2017) reported that *M. phaseolina* and *F. oxysporum* were isolated in beans more than other fungi. It was reported

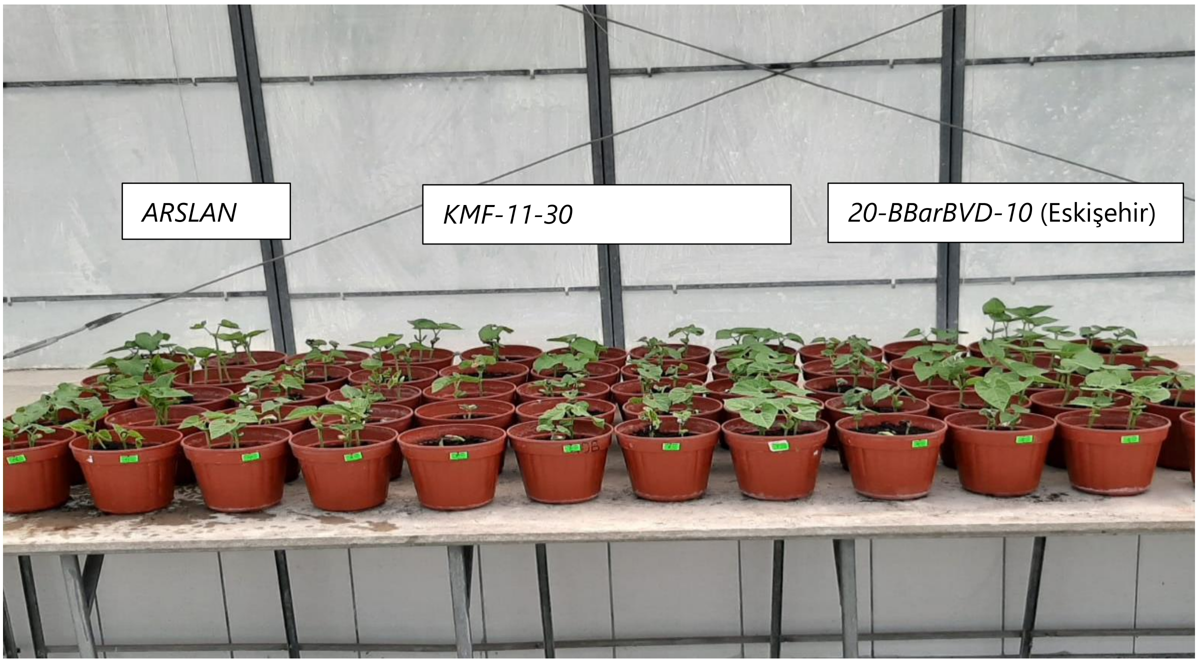


Figure 3. Bean genotypes showing symptoms of charcoal rot and disease reaction in pot.

Figure 4. *KMF-11-24* (Kahramanmaraş) genotypes showing symptoms of charcoal rot and disease higly susceptible reaction in pot.

that *M. phaseolina* and *F. oxysporum* caused significant differences in root and stem length, fresh and dry weight values in bean plants compared to the control plants, and *M. phaseolina* caused quite severe disease in beans. In this study, M1 isolate of *M. phaseolina* caused severe infection in 12 genotypes. Pandey *et al.* (2020) and Chavan *et al.* (2019) reported that although there was no cultivar with high resistance to charcoal rot, some bean genotypes were moderately resistant, and the response of bean genotypes to pathogen isolates was quite different. Only a few lines were found to be moderately resistant in the study. In a similar study, 53 bean lines were tested against *M. phaseolina* both in the field and in the greenhouse by Pastor-Corrales and Abawi (1998). 22 lines are classified as resistant and 15 lines are classified as intermediate resistant.

Although the control of *M. phaseolina* in the bean plant is carried out in the form of cultural practices, fungicide applications and biological control applications in the world, the most effective control method against charcoal rot disease, as in other disease factors, is the use of host plant resistance. Resistant varieties prevent the low yield caused by the disease and can be the best alternative for efficient and environmentally friendly integrated crop management. In the light of the informations from literature, this study has been conducted to it was aimed to determine the reactions of bean genotypes developed in bean breeding studies in Türkiye against chocoal rot disease. After field trials to be carried out in different locations, *20-BBarBVD-10* (Eskişehir), *20-BBarBVD-10* (Eskişehir) and *ARSLAN* genotypes can be recommended to bean growers in areas where the disease is a problem. It has been revealed that among the bean genotypes tested in the study, there is no genotype that can show complete resistance (0% incidence of disease) against *M. phaseolina*. The resistant and intermediate resistant genotypes identified by this study can be used as a source of resistance to develop resistant varieties after successful field trials.

When the results obtained within the scope of the study were evaluated in general; this is the first study conducted on the reactions of the bean breeding material developed in Türkiye against charcoal rot disease. Although there were significant differences between the genotypes tested in the study, a genotype that was completely resistant to the agent could not be determined. Beside, some cultivars are tolerant and moderately resistant to the disease, and the use of these cultivars may be preferred in areas where the disease is intense. In addition, resistance resources in these varieties should be taken into account in breeding studies against the disease. Furthermore, it is thought that the effective application of cultural, chemical and biological control methods against the disease is very important in terms of reducing the economic losses caused by the disease.

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