# EVOLUTION OF COMMON BEANS COLLECTED FROM LAKE VAN BASIN FOR THEIR RESISTANCE TO THE COMMON BACTERIAL BLIGHT (*XANTHOMONAS AXONOPODIS* PV. *PHASEOLI*)

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**Abstract.** The present study determined the reactions of common bean landraces grown in Lake Van Basin of Turkey against common bacterial blight disease (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*). For this purpose 83 bean landraces collected from the basin and two resistant (HR-45, HR-67) and one susceptible (Dresden) lines were evaluated for their reaction to *Xap*. The experiments were conducted in randomized experimental design with three replications in a growth chamber having 23 ± 2 °C temperature and 16 h light-8 h dark period. Bean seeds were sown in the pots having 2:1 mixture of peat:perlite and *Xap* inoculated by spraying when the seedlings reached two trifoliate leaves stage. Disease severity was assessed three weeks after Xph inoculation using 1-5 scale. In light of the findings, it was detected that there was a variation with regard to tolerance to the disease among common bean landraces. While fourteen landraces were assigned as resistant against CBB, 49 landraces were found to be moderately susceptible to CBB. The present study demonstrated the existence of resistance sources against CBB within Lake Van Basin bean landraces that could potentially be used for breeding resistant cultivars.
**Keywords:** *disease, Phaseolus vulgaris L., landraces, Xap, artificial inoculation*

## Introduction

Common bean is among the most widely grown species in the world and have been grown widespread in Turkey due to economical and nutritional aspects. It is originated in South and Central America (including Mexica, Guatemala, Colombia, and Peru) and has been cultivated since 5000 BC. Common bean has a wide distribution area from sea level to 3000 m altitude (Şalk et al., 2008; Koutsika-Sotiriou and Traka-Mavrona, 2008). While China is the first fresh bean producer in world, Turkey ranks fourth with 651.094 tons meeting about 2.75% of the world production (FAO, 2016). There has been a large variability in common bean having a widespread distribution in Turkey (Erdinc et al., 2013, 2017a, b). Common bean ranks third in the world in terms of importance in *Fabaceae* (Blair et al., 2009). It reported that besides common bean supplies 30% need of protein, it helps to fight many diseases because of the antioxidant


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compounds it contains. Additionally common bean has been used cosmetic and paint industry (Singh et al., 2007).

Many abiotic and biotic stress factors have caused problems in common bean. Common bacterial blight (CBB) is one of the major seed-borne diseases which give rise to yield and quality losses and is caused by *Xanthomonas axonopodis* pv. *phaseoli* (*Xap*). Pathogen shows its detrimental effects in tropical, subtropical and temperate regions (Vandemark et al., 2008). Seed is a very significant factor in the spread of Xph because the viability of the pathogen can be maintained 30 years on the seed (Dursun et al., 2002; Shi et al., 2011a), sometimes leads to yield losses exceeding 40% (Vandemark et al., 2008). There is no satisfactory chemical control for CBB and usage of the resistant cultivars is one of the most effective and environmentalist approaches (Opio et al., 1996; Park et al., 1999).

It is indicated that CBB is a common threat today on all continents where grown common bean. In Turkey, it was determined first time by Sönmezalp in 1966. *Xap* was also identified in Nigde, Kayseri and Yozgat provinces and in some areas of East Anatolia (Bozkurt, 2009).

The climate of the Lake Van Basin differs from the cold Eastern Turkey where it is located. The basin has a microclimate which allows for production vegetable including common bean etc. (Erdinc et al., 2008). The province of Van is located between 37°55′ and 39°24′ north longitude and 42°05′ and 44°22′ east latitude and at an altitude of 1720 m above sea level. It has a continental climate. Comparatively rich genetic diversity was found among the Lake Van Basin common bean population by phenotypic and molecular markers (Ekincialp, 2012).

Many researchers reported that the resistance to *Xap* was managed by a dominant gene (Drijfhout and Blok, 1987; Silva et al., 1989; Urrea et al., 1999; Zapata et al., 2011). Resistance to CBB in common bean has been reported as a quantitative trait with low to medium heritability (Silva et al., 1989), conditioned by 1-5 genes with additive gene action (Tar'an et al., 2001). Therefore, screening of genetic resources of the region and providing the resistant or tolerant landraces in breeding programs by determining reactions against the disease of genetic material is important in development of resistant cultivars.

## Material and methods

### *Plant materials and pathogen*

Eighty two landraces of common bean and one runner bean genotype (G30) collected from Lake Van Basin of Turkey (Ekincialp, 2012), two resistant (HR-45 and HR-67) (Yu et al., 2004; Gillard et al., 2009; Shi et al., 2011a, b, 2012) and one susceptible lines (Dresden) (Shi et al., 2011a, 2012) from Dr. Ali Reza NAVABİ was used in the present study (*Table 1*). *Xap* isolate was obtained from Prof. Dr. Hüseyin BASIM (Akdeniz University, Agriculture Faculty, Department of Plant Protection).

### *Pathogenicity assays*

In the first artificial inoculation trial, the eighty-three bean landraces and control lines (HR-45, HR-67 and Dresden) were tested against *Xap*, then based on the results, twelve landraces with control lines were selected for the second trial. Common bean seeds was sown to the pots including 2:1 mixture of peat:perlit in a randomized


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experimental design with three replications in a chamber growth having 23 ± 2 °C temperature and 16 h light-8 h dark period.

***Table 1.*** *Passport information of the genotype used in the study*

| Accession | Location | Grow habit | Accession | Location | Grow habit |
|---|---|---|---|---|---|
| G1 | Van-Merkez | Indeterminate | G46 | Van-Gevaş | Indeterminate |
| G2 | Van-Merkez | Indeterminate | G47 | Van-Gevaş | Indeterminate |
| G3 | Van-Merkez | Indeterminate | G48 | Van-Gevaş | Indeterminate |
| G4 | Van-Merkez | Indeterminate | G49 | Van-Gevaş | Indeterminate |
| G5 | Bitlis-Tatvan | Indeterminate | G-50 | Van-Gevaş | Indeterminate |
| G6 | Bitlis-Tatvan | Indeterminate | G-51 | Van-Gevaş | Indeterminate |
| G7 | Bitlis-Tatvan-Gevar | Indeterminate | G-52 | Van-Gevaş | Indeterminate |
| G8 | Bitlis-Tatvan | Indeterminate | G-53 | Van-Gevaş | Indeterminate |
| G9 | Bitlis-Hizan | Indeterminate | G-55 | Van-Gevaş | Indeterminate |
| G10 | Bitlis-Tatvan-Gevar | Indeterminate | G-57 | Van-Gevaş | Indeterminate |
| G11 | Bitlis-Hizan | Determinate | G-58 | Van-Gevaş | Indeterminate |
| G12 | Bitlis-Tatvan | Indeterminate | G-59 | Van-Gevaş | Determinate |
| G13 | Bitlis-Tatvan | Indeterminate | G-60 | Van-Gevaş | Indeterminate |
| G14 | Bitlis-Tatvan | Indeterminate | G-61 | Van-Gevaş | Indeterminate |
| G15 | Bitlis-Tatvan | Indeterminate | G-62 | Van-Gevaş | Determinate |
| G16 | Bitlis-Tatvan | Indeterminate | G-63 | Bitlis-Adilcevaz | Indeterminate |
| G17 | Bitlis-Tatvan | Indeterminate | G-64 | Bitlis-Adilcevaz | Indeterminate |
| G18 | Van-Erciş-Purmak | Indeterminate | G-65 | Bitlis- Adilcevaz | Indeterminate |
| G19 | Van-Erciş-Çelebibağı | Indeterminate | G-66 | Bitlis- Adilcevaz | Indeterminate |
| G20 | Van-Erciş | Indeterminate | G-67 | Bitlis- Adilcevaz | Indeterminate |
| G21 | Van-Erciş-Tekevler | Indeterminate | G-68 | Bitlis- Adilcevaz | Indeterminate |
| G22 | Van-Erciş-Tekevler | Indeterminate | G-70 | Bitlis- Adilcevaz | Indeterminate |
| G23 | Van-Erciş-Tekevler | Indeterminate | G-71 | Bitlis- Adilcevaz | Determinate |
| G24 | Van-Erciş-Tekevler | Indeterminate | G-72 | Bitlis- Adilcevaz | Indeterminate |
| G25 | Van-Erciş-Tekevler | Indeterminate | G-73 | Bitlis-Adilcevaz | Indeterminate |
| G26 | Van-Erciş | Indeterminate | G-74 | Bitlis-Adilcevaz | Indeterminate |
| G27 | Van-Erciş | Indeterminate | G-75 | Bitlis-Adilcevaz | Indeterminate |
| G28 | Van-Erciş | Indeterminate | G-76 | Bitlis-Adilcevaz | Determinate |
| G29 | Van-Gevaş-G.konak | Determinate | G-77 | Bitlis-Adilcevaz | Determinate |
| G30 | Van-Gevaş (*P. coccineus)* | Indeterminate | G-78 | Bitlis-Adilcevaz | Determinate |
| G31 | Van-Gevaş | Indeterminate | G-90 | Van-Edremit | Indeterminate |
| G32 | Van-Gevaş | Indeterminate | G-91 | Van-Edremit | Indeterminate |
| G33 | Van-Gevaş | Indeterminate | G-92 | Van-Edremit | Indeterminate |
| G34 | Van-Gevaş | Indeterminate | G-93 | Van-Edremit | Indeterminate |
| G35 | Van-Gevaş | Indeterminate | G-94 | Van-Edremit | Indeterminate |
| G36 | Van-Gevaş | Indeterminate | G-95 | Van-Bahçesaray | Determinate |
| G37 | Van-Gevaş | Indeterminate | G-96 | Van-Bahçesaray | Indeterminate |
| G39 | Van-Gevaş | Indeterminate | G-97 | Van-Bahçesaray | Indeterminate |
| G40 | Van-Gevaş | Indeterminate | G-98 | Van-Bahçesaray | Indeterminate |
| G41 | Van-Gevaş | Indeterminate | G-99 | Van-Bahçesaray | Indeterminate |
| G42 | Van-Gevaş | Indeterminate | HR-45 | Canada | Determinate |
| G43 | Van-Gevaş | Indeterminate | HR-67 | Canada | Determinate |
| G44 | Van-Gevaş | Indeterminate | Dresden | Canada | Determinate |


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Xap isolate was inoculated to the bean seedlings with two trifoliate leaves. In this stage, Xph grown on KB medium King B (pepton 20 g/L, gliserol 10 ml/L, $K_2HPO_4$ 1.5 g/L, $MgSO_4./H_2O$ 1.5 g/L, agar 18 g/L) at 28 °C for 48 h. (King and Raney, 1954) and then the grown Xph colonies were suspended in distilled water and adjusted to $10^8$ cfu $ml^{-1}$ (OD = 0.13) (Osdaghi et al., 2009). The bacterial mixture was spread onto 15 days old common bean seedlings with fully expanded trifoliate leaves. The benches with pots were covered with polyethylene sheet for providing moisture to favor development of *CBB*. After 48 h, the covers were opened. Plants were irrigated weekly including Hoagland nutrient solution. In twenty-first days after inoculation, CBB symptoms in the plants were assessed using 1-5 scale (*Table 2*) which was modified for this study and the disease severity was determined with Townsend–Heuberger equation (Townsend and Heuberger, 1943).

The disease severity was calculated using the following formula:

$$Disease\ severity = \frac{\Sigma\ (Rating\ number\ x\ Number\ of\ leaves\ in\ the\ rating)}{Total\ number\ of\ leaves\ x\ Highest\ rating}\ x100$$

***Table 2.*** *The values of 1-5 scale that was used to determine the disease severity of Xap in the bean landraces*

| Scale value | Statement |
|---|---|
| 1 | No visible symptom |
| 2 | Necrosis in 1-5% of leaf or single spots |
| 3 | Necrosis in 6-25% of leaf |
| 4 | Necrosis in 26-50% of leaf |
| 5 | Necrosis in leaf more than 50% or plant death |

As a result of calculating of the scale values, the landraces were assigned as <2: resistant, <3: moderately susceptible, <4: susceptible, <5: extreme susceptible (Dursun et al., 2002; Osdaghi et al., 2009). In the both artificial inoculation trials, the data were analyzed using the statistical software package SPSS. The means were grouped using the Duncan multiple comparison test (Düzgüneş et al., 1987)

## Results and discussion

### *First artificial inoculation trial*

The results of the first artificial inoculation are given in *Table 3*. In the result of the analysis of variance, it was found that the differences between the landraces were significant ($p<0.05$). According to the results obtained, 14 of the landraces were scaled to 1-2 and rated as resistant to CBB and the genotype G99 was the most resistant genotype with a scale value of 1.42. The great majority of landraces (49 landraces) was ranged from 2 to 3 and was found to be moderately susceptible to CBB. It was also determined that 19 landraces were susceptible and their disease severity scales were varied from 3 to 4. The genotype G37 was the most susceptible one with a scale value of 4.32. Two resistant (HR-45 and HR-67) control lines were found to have lower scale values than the studied bean landraces and the susceptible line, Dresden, was found to


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be in the group of sensitive landraces with a score of 3.29 (*Table 3*). When frequency distribution according to disease scale values were examined, 18.60% of the landraces including control lines were found to be resistant, 63.96% of them were moderately susceptible, 16.28% of them were susceptible and 1.17% of them were very susceptible (*Fig. 1*).

***Table 3.*** *The disease severity values of first artificial inoculation in the bean landraces calculated according to Townsend–Heuberger equation*

| Accession # | Scale value | Disease response | Accession # | Scale value | Disease response | Accession # | Scale value | Disease response |
|---|---|---|---|---|---|---|---|---|
| G1 | 1.86 C-J* | R | G30 | 1.73 D-J | R | G63 | 1.84 C-J | R |
| G2 | 2.18 B-J | MS | G31 | 2.69 A-J | MS | G64 | 3.07 A-I | S |
| G3 | 1.75 D-J | R | G32 | 1.00 J | R | G65 | 2.47 A-J | MS |
| G4 | 2.07 B-J | MS | G33 | 2.17 B-J | MS | G66 | 3.25 A-H | S |
| G5 | 1.65 F-J | R | G34 | 1.75 D-J | R | G67 | 2.19 B-J | MS |
| G6 | 2.79 A-J | MS | G35 | 1.82 C-J | R | G68 | 2.58 A-J | MS |
| G7 | 2.36 B-J | MS | G36 | 3.41 A-F | S | G70 | 2.55 A-J | MS |
| G8 | 1.72 D-J | R | G37 | 4.32 A | HS | G71 | 2.27 B-J | MS |
| G9 | 2.16 B-J | MS | G39 | 2.42 B-J | MS | G72 | 3.02 A-I | S |
| G10 | 2.12 B-J | MS | G40 | 3.49 A-F | S | G73 | 3.01 A-I | S |
| G11 | 2.07 B-J | MS | G41 | 2.84 A-J | MS | G74 | 2.38 B-J | MS |
| G12 | 1.69 E-J | R | G42 | 3.10 A-I | S | G75 | 3.90 AB | S |
| G13 | 2.41 B-J | MS | G43 | 2.35 B-J | MS | G76 | 2.38 B-J | MS |
| G14 | 2.28 B-J | MS | G44 | 2.80 A-J | MS | G77 | 3.19 A-I | S |
| G15 | 2.26 B-J | MS | G46 | 2.81 A-J | MS | G78 | 3.01 A-I | S |
| G16 | 2.22 B-J | MS | G47 | 3.01 A-I | S | G90 | 2.29 B-J | MS |
| G17 | 2.45 B-J | MS | G48 | 2.44 B-J | MS | G91 | 3.49 A-F | S |
| G18 | 2.45 B-J | MS | G49 | 2.73 A-J | MS | G92 | 2.48 A-J | MS |
| G19 | 2.62 A-J | MS | G-50 | 2.56 A-J | MS | G93 | 2.40 B-J | MS |
| G20 | 1.95 C-J | R | G-51 | 2.35 B-J | MS | G94 | 3.53 A-E | S |
| G21 | 2.72 A-J | MS | G-52 | 2.91 A-I | MS | G95 | 2.47 A-J | MS |
| G22 | 2.47 A-J | MS | G-53 | 3.89 AB | S | G96 | 3.67 A-C | S |
| G23 | 3.01 A-I | S | G-55 | 3.10 A-I | S | G97 | 2.09 B-J | MS |
| G24 | 3.59 A-D | S | G-57 | 2.87 A-J | MS | G98 | 2.25 B-J | MS |
| G25 | 1.97 C-J | R | G-58 | 3.02 A-I | S | G99 | 1.42 G-J | R |
| G26 | 2.26 B-J | MS | G-59 | 2.49 A-J | MS | HR-45 | 1.40 H-J | R |
| G27 | 1.93 C-J | R | G-60 | 2.29 B-J | MS | HR-67 | 1.34 IJ | R |
| G28 | 2.23 B-J | MS | G-61 | 2.09 B-J | MS | Dresden | 3.29 A-G | S |
| G29 | 2.94 A-I | MS | G-62 | 2.05 B-J | MS | | | |

*There were significant differences among the different letter(s) at $P < 0.05$ level (according to Duncan's multiple comparison test). R: Resistant, MS: Moderately susceptible, S: Susceptible, HS: High susceptible


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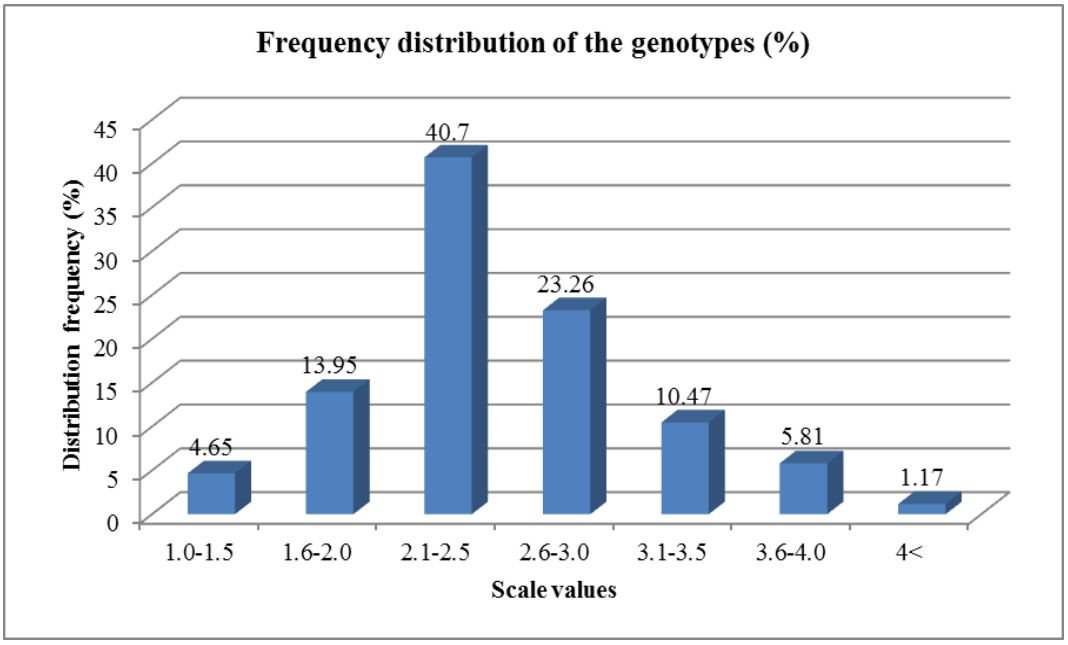


***Figure 1.*** *Distribution frequency (%) of reaction to CBB of the bean landraces according to scale values*

### *Second artificial inoculation trial*

Based on the first artificial inoculation trial, twelve landraces resistant to CBB with sufficient number of seeds (the landraces G12 and G32 were excluded) were artificially re-inoculated. Resistant (HR-45 and HR-67) and sensitive (Dresden) lines were also used for control in the second trial. When the disease severity values obtained at the end of the trial were examined, it was determined that the landraces had a scale value of 1-2 and showed resistance. It was determined that the lowest value of the landraces was 1.27 (in the genotype G27), while the highest value was found in the landraces G35 and G63 with 1.83. It was also found that the HR-45 and HR-67 lines reached lower scale values than the other landraces (scale values of 1.11 and 1.00, respectively). As a result of the analysis of variance, it was found that the differences between the landraces were statistically significant ($p<0.05$). Disease severity reactions of the resistant landraces have generally been observed to be similar to each other in both studies (*Fig. 2*).

Xap, among the most important seed-borne bacterial pathogens in bean (Bastas and Sahin, 2017), is also among the serious bacterial diseases causing yield and quality loses in Turkey (Demir and Gündogdu, 1994; Donmez, 2004). In surveys conducted in the Central Anatolia Region, Xap was reported to be among the most common bacterial pathogens with 11.11% of presence (Bastas and Sahin, 2017).

In current study, the severity of disease in artificial inoculation was evaluated after 21 days in both studies. As a matter of fact, Marquez et al. (2007) reported that even if an aggressive isolate is used, classifications that would have been made by previous evaluations on 14 days might be erroneous.

It has been determined that there is a variation in the reactions of the bean landraces to BCC. As a matter of fact, according to Ekincialp (2012), there is a high genetic variation in the morphological and molecular characterization studies carried out among these landraces. The landraces G30, G37, and G53 which are morphologically and


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genetically most distant from each other, have also been found to differ in their CBB disease reactions, while G30 is resistant and G53 is susceptible and G37 is highly susceptible. It has also been reported that the G30 genotype (*P. coccineus*) is differentiated from other landraces in terms of general habitus and morphological characteristics (number of flower buds in the cluster, seed width, seed length, seed height and seed weight) (Ekincialp, 2012).

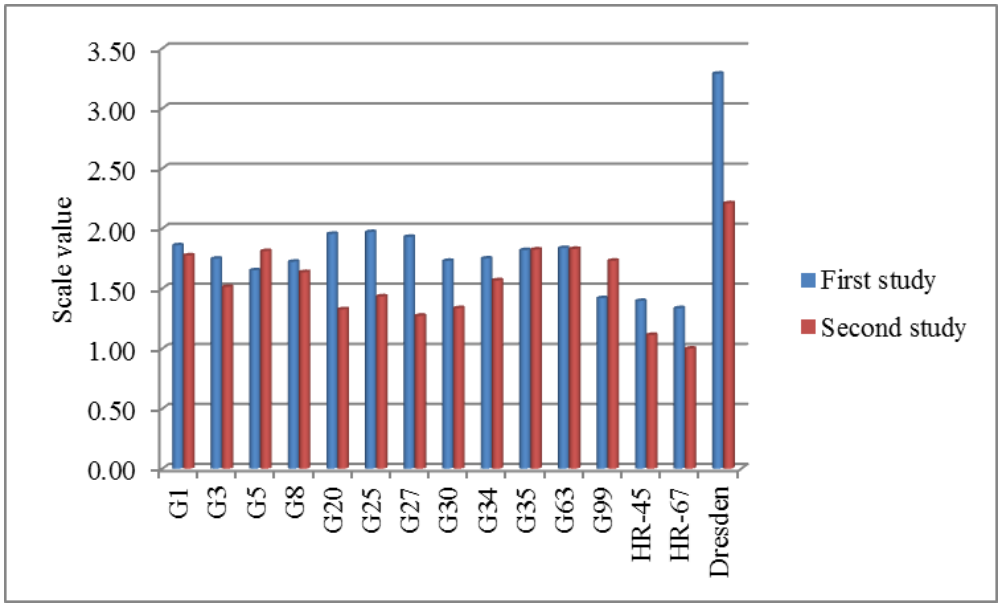


***Figure 2.*** *Reactions of the bean landraces were classified as resistant based on 1-5 scale in artificial inoculation method for CBB in first and second trials*

It was noticed that the landraces G12 and G30 that were resistant to bean anthracnose (Ekincialp and Sensoy, 2018) were also resistant to CBB in the present study. Moreover, all landraces susceptible to CBB have been found to be susceptible to anthracnose disease. It was observed that the G30 genotype belonging to *P. coccineus* was present in a group of resistant landraces in both studies. It is generally stated that *P. coccineus* (runner bean) has similar or higher resistance level as *P. vulgaris*, P. *acutifolius* (tepary bean) shows the highest level of resistance (Drijfhout and Blok, 1987; Singh and Munoz, 1999).

The results of the landraces used for control purposes during pathogenicity tests are shown in *Table 3* and *4*. As seen in the tables, the reactions of these landraces to the disease were determined as resistant (R) in HR-45 and HR-67 sensitive (S) in Dresden. It was also reported in the literature that HR-45 and HR-67 were resistant to *Xap* (Yu et al., 2004; Gillard et al., 2009; Shi et al., 2011 a, b, 2012) and Dresden was susceptible to XAP Shi et al., 2011a, 2012).

In the present study, a large number of bean landraces were screened and approximately 17% of them was found to be resistant to *Xap* (*Table 3*; *Fig. 3*). Halo blight and common blight screening studies were conducted in beans in different regions of Turkey; it was demonstrated that some landraces were resistance to halo blight (Benlioglu et al., 1994; Dursun et al., 2002) and only one variety was resistant to common blight (Dursun et al., 2002). It was determined that 50% of the landraces used in the mentioned studies were highly susceptible. Osdaghi et al. (2009) determined that


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two of the 30 cultivars/lines were resistant to CBB. On the contrary, Fininsa and Tefera (2006) reported that 117 out of 201 landraces (approximately 58%) in Ethiopia were resistant to CBB.

***Table 4.*** *Disease severity of second artificial inoculation in the bean landraces*

| Accessions # | Scale value |
|---|---|
| G1 | 1.78 B* |
| G3 | 1.51 B-E |
| G5 | 1.81 AB |
| G8 | 1.64 B-D |
| G20 | 1.33 C-F |
| G25 | 1.44 B-E |
| G27 | 1.27 D-F |
| G30 | 1.34 C-F |
| G34 | 1.57 B-D |
| G35 | 1.83 AB |
| G63 | 1.83 AB |
| G99 | 1.73 BC |
| HR-45 | 1.11 EF |
| HR-67 | 1.00 F |
| Dresden | 2.21 A |
| p value | 0.001 |

*There were significant differences among the different letter(s) at P < 0.05 level (according to Duncan's multiple comparison test)

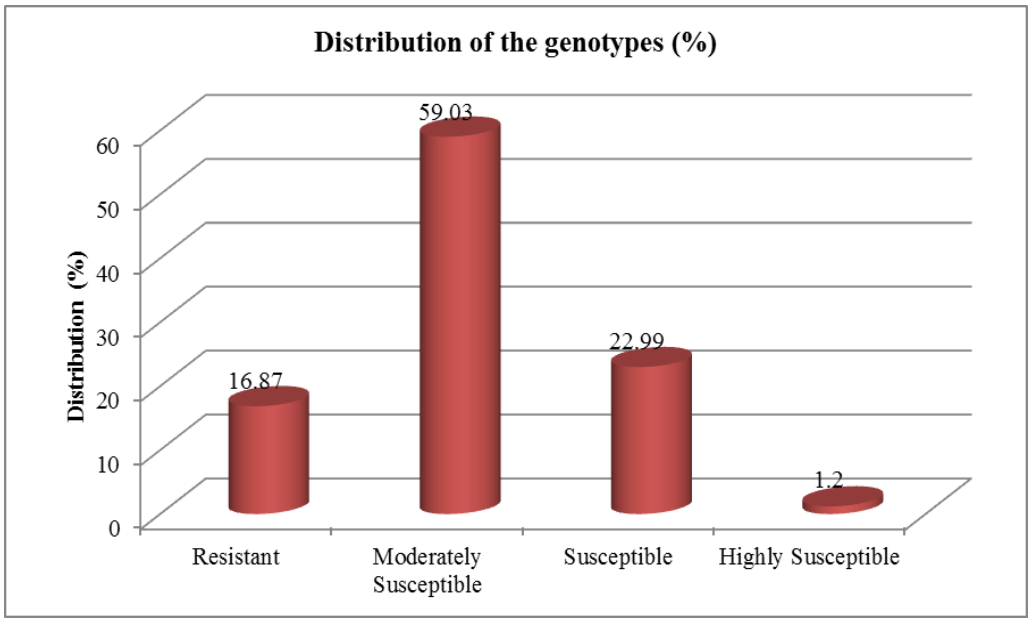


***Figure 3.*** *Distribution of the landraces excluding control lines according to disease response*


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## Conclusions

Turkey is an important country in the production of green beans and meets 3% of the world production. Maintaining or advancing the current position will be through the development of highly productive, tolerant varieties that can meet consumer and producer demands. In the present study, the resistance status of bean landraces collected from Lake Van Basin, which is an important potential for vegetable production in the region, is revealed. From this point of view, it is noticed that there is a source of resistance. Resistant landraces are expected to be used as parents in bean improvement programs in the future. Considering the efforts of countries in recent years to protect their local gene resources, this situation is better understood. This is the first study and report of resistance against CBB in Lake Van Basin bean landraces. The present study demonstrated the existence of resistance sources against CBB within them that could potentially be used for breeding resistant cultivars.

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