CHARACTERIZATION OF *PHYTOPHTHORA CAPSICI* LEONIAN RESISTANCE IN SOME PEPPER GENOTYPES BY PRINCIPAL COMPONENT ANALYSIS

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Abstract. In the study, resistance analyses were performed on pepper lines and genotypes (60 genotypes) of mainly common local genotypes found in the gene pool by classical testing methods. Classical tests were first applied in seedling stage. Second inoculation (the last) was carried out (in the stage of fruit retention) on resistant genotypes determined in the first inoculation. Varieties resistant to *phytophthora capsici* (CM 334 and partially resistant P1, P2 and P4) were also included in the study. Peroxidase and catalase enzyme contents of plant materials have been determined. Scale of 0-5 was applied to inoculated plants. Five pepper properties were measured and used as original variables. The first two principal components accounted for 74% of total variance. Score plots of the first two principal components were used to map genotypes according to their morphological properties. Some relationships between genotypes and their morphological traits were obtained. The results revealed that genotypes of P1, 13 (Urfa), 25 (UKST), 38 (UI), 48 (UKDT), 57 (ANKSB) were partial resistant genotypes while CM334 was fully resistant. To conclude, principal component analysis was shown to be a useful tool for mapping the pepper genotypes in terms of *phytophthora capsici* resistance.
Keywords: *pepper, biotic stress, resist, peroxidase, catalase, PCA*

Introduction

Pepper is one of the species of genus *Capsicum* in the family *Solanaceae*. The genus *Capsicum* is divided into twenty-five different species (Baral and Bosland, 2002). *Capsicum* originates from Mexico, South Peru and Bolivia. Almost all *Capsicum* species are diploid with 12 chromosome pairs (Moscone et al., 1996). *Capsicum annuum*, *Capsicum frutescens*, *Capsicum chinense*, *Capsicum baccatum* and *Capsicum pubescens* were cultured (Pickersgill, 1997). These species are the most important species in terms of economic and nutritional status (Djian-Caporalino et al., 2006).

Phytophthora capsici Leonian (*P. capsici*), is one of the most important soil born plant pathogens in the world and first was found by Fernandez-Pavia and his friends; of the 26 isolates, only 7 were pathogenic on the cucumber. While 24 of them were virulent in pepper, no stable condition was observed in tomato (Pavia et al., 2004). *Phytophthora capsici* causes damages on tissues of root, fruit and leaves of various vegetables (Manohara and Rizal, 2002). Phytophthora species secrete a large number of effectors during the infection of host plants (Stam et al., 2013). Bell pepper succumbed to disease in particularly humid and humid conditions. Only some partially resistant pepper cultivars are commercially available (Candole et al., 2010; Gisbert et al., 2010). Also *Phytophthora blight* caused by *Phytophthora capsici* is a very important disease in vegetable production (crop losses exceeding 50%) on worldwide and selective use of

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fungicides is the main part of disease management programs (Jackson et al., 2012; Sanogo et al., 2012; Yin et al., 2012). *Phytophthora capsici* is an oomycete that causes reductions in the production of capsicum (*Capsicum annuum* L.) worldwide. Nowadays, there are no chilles varieties that are resistant to this pathogen and the fungicides used for its struggle increase the resistance of disease races and increase the environmental damage (Garcia-Rodriguez et al., 2010).

Identification of initial symptoms of disease is very difficult and usually cannot be detected by the producers. Yellowing, fading and falling of leaves on the upper part of pepper plants are the most important and obvious signs of the disease (Ton et al., 2005). When these symptoms were observed, the infection has already reached to the advanced stage and caused root rot. *P. capsici* is very difficult to be controlled and causes a marked decline in pepper yields at pepper producer countries. For example, in Indonesia, root rot reduces 30 to 40% pepper yield in some gardens while in others it may cause 100% loss of yield (Nam, 2012). In Malaysia, more than 95% of pepper production areas have been similarly infected with *P. capsici*, resulting in a 5 to 10% reduction in productivity (Kueh, 1990). Yield loss in India was around 30% (Anandaraj, 2000). Successful management with *P. capsici* cannot be made due to the insufficient biologic information on *P. capsici*. According to the majority of scientists, A1 and A2 are the pathogen reproduction types (Ton, 2005; Hausbeck and Lamour, 2004; Ristaino and Johnston, 1999).

Plant enzymes are involved in defense mechanisms of plants against diseases caused by pathogens. Plant enzymes include antioxidants, such as peroxidase that enhance the cell structure and contribute to the formation of inhibitors in defense and catalyze the formation of lignin and other oxidative phenols. Studies conducted with plants such as pepper, tomato and wheat revealed a significant relationship between enzymes and protection against pathogens (Mohammadi and Kazemi, 2002). Catalase (CAT) enzyme is found in vertebrates, invertebrates, plants, fungi and all aerobic organisms (Bergmeyer and Grassl, 1982). The CAT enzyme has the highest conversion rate among all enzymes (Koç and Üstüm, 2016). The CAT enzyme is inhibited at high light intensity and by H_2O_2 which occur at high concentrations in stressed plant cells (Fridovich, 1986). Peroxidase (POX) is a part of the enzyme complex in the superoxide dismutase and CAT containing plant cell that regulates the level of harmful oxygen radicals formed under unfavorable external conditions. The POX enzyme has been isolated and characterized many times and the amino acid sequence of the enzyme has been revealed (Koç, 2011). The CAT and POX enzymes take part in purification of ROS (cell reactive oxygen species) from toxins. The enzymes disintegrate H_2O_2 and regulate the concentration of H_2O_2 in the cells. The CAT enzyme and different types of POX enzymes catalyze the disintegration of H_2O_2 (Chang et al., 1984). Since the catalase enzyme is not involved in chloroplasts, the breakdown of H_2O_2 in chloroplasts takes place by specific peroxidases (Asada, 1992). Principal component analysis (PCA) is a multivariate analysis technique and is also known as eigenvector analysis. The main objective of a PCA is to reduce the dimensionality of a set of data. This is particularly advantageous if a set of data with many variables lies, in reality, close to a two-dimensional subspace. In this case the data can be plotted with respect to these two dimensions, thus giving a straight forward visual representation of what the data look like, instead of appearing as a large mass of numbers to be digested (Jolliffe, 2002). The PCA is usually applied in environmental and agricultural studies (Panishkan et al., 2012; Yılmaz et al., 2017). The PCA was used to cluster patterns of 60 pepper

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genotypes based on their morphological properties. The aims of this study were to study classification of pepper genotypes according to their morphological and chemical properties by using PCA.

Materials and methods

Materials

Fifty-nine pepper lines in the pepper gene pool of the Siirt University/Turkey, Department of Horticulture and CM334 (Criollos de Morelos 334) resistant to *P. capsici* were used in the experiment (*Table 1*). Fungal material was obtained from Van Yüzüncü Yıl University/Turkey, Faculty of Agriculture, Plant Protection Department. The isolate used is the most aggressive isolate of the strain.

Table 1. *Materials used in the study, fruit shape, hotness and resistance to P. capsici status*

Genotype/ variety No	Shape of fruit	Hotness	Resistance to *P. capsici*	Genotype/ variety No	Shape of fruit	Hotness	Resistance to *P. capsici*
1	B	Not hot	VS	31	C	Hot	S
2	B	Slightly hot	VS	32	SCG	Hot	S
3	C	Hot	PR	33	SCG	Slightly hot	S
4	SCG	Hot	S	34	C	Slightly hot	S
5	C	Hot	S	35	C	Slightly hot	S
6	SCG	Slightly hot	S	36	C	Hot	S
7	SCG	Slightly hot	S	37	C	Slightly hot	S
8	SCG	Hot	S	38	SCG	Hot	PR
9	C	Hot	S	39	SCG	Slightly hot	S
10*	C	Hot	R	40	C	Hot	S
11	C	Hot	S	41	SCG	Slightly hot	S
12	B	Not hot	VS	42	C	Hot	S
13	C	Hot	PR	43	B	Not hot	VS
14	C	Hot	S	44	C	Hot	S
15	SCG	Slightly hot	S	45	B	Not hot	VS
16	SCG	Hot	S	46	C	Hot	S
17	SCG	Not hot	S	47	C	Hot	S
18	C	Hot	S	48	C	Hot	PR
19	C	Hot	S	49	SCG	Slightly hot	S
20	SCG	Hot	S	50	C	Slightly hot	S
21	B	Not hot	VS	51	C	Slightly hot	S
22	SCG	Not hot	S	52	C	Hot	S
23	SCG	Slightly hot	S	53	C	Slightly hot	S
24	C	Slightly hot	S	54	C	Hot	S
25	C	Hot	PR	55	C	Hot	S
26	C	Hot	S	56	SCG	Hot	S
27	SCG	Slightly hot	S	57	C	Hot	PR
28	C	Hot	S	58	C	Hot	S
29	C	Slightly hot	S	59	C	Slightly hot	S
30	B	Not hot	VS	60	C	Slightly hot	S

SCG: Semi Capia-Green; **B:** Bell Pepper, **C:** Capia; **S:** Sensitive; **VS:** Very Sensitive; **PR:** Partially Resistant; **R**: Resistant
*Control genotypes: CM334 (Criollos de Morelos 334)

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Methods

Experimental design and treatments

Field studies were carried out in research and application fields of the Department of Horticulture, Faculty of Agriculture, Siirt University, Turkey. The effects of *P. capsici* on pepper varieties/lines have been investigated in two stages. At the first stage, resistance or sensitivity status of varieties/lines were determined at seedling period. After that, the resistant varieties/lines were selected and the effect of pathogen on plants was investigated in field conditions following the seedling period.

Prior to planting, the pepper seeds were placed in 2% sodium hydroxide solution for 5 min, subjected to surface disinfection, and washed twice using sterile distilled water. Peat was used as seedling growing medium. The experiment was designed with 3 replicates and each replicate included 10 plants.

The seedlings at 3-4 leaf stage were infected with spore solution containing zoospores of *P. capsica*. Seven-day cultures of *P. capsica* (in 9 cm petri dishes) grown in an incubator purely under dark at 25 °C within Potato Dextrose Agar medium were used for this purpose.

Fungi incubation

The petri dishes were left under the light for 2 days to allow sporulation. Twenty mL of sterile distilled water was added in each petri dish and incubated in the refrigerator at 4 °C for 40 min and then kept at room temperature for 30 min. Thus, zoosporogenesis of the fungus has been promoted. The zoospores of *P. capsici* were collected by filtration through two layers of cheesecloth. The zoospores of the fungus were then adjusted with hemocytometer at a concentration of 2×10^6 zoospor ml^{-1}.

Three ml of this solution was taken and inoculated to the surrounding of seedling roots to be inoculated with pathogen. After the first inoculation, the surviving seedlings were inoculated second time as indicated in the first stage. Peroxidase and catalase enzymes of samples taken from the sensitive varieties/lines in the uninoculated control group were also determined.

Enzyme activity assay

Catalase and peroxidase enzyme activities were determined by using Thermo Scientific UV-VIS instrument at Siirt University Science, Technology Application and Research Center. The plant leaf samples taken in accordance with the cold chain rules were broken into pieces with homogenate buffer for 5 min within a homogenizer and centrifuged at 15000 rpm for one hour at + 4°C. Both enzyme activities were measured in 100 µl homogenate of the substrate added to the buffer solution.

Catalase enzyme activity was determined using the method outlined by Jebara et al. (2005). Enzyme activity and enzyme calculation formula are given in *Table 2*. The measurement was performed at 240 nm in a quartz cuvette with 3 repetitions following the calibration of the instrument. The EU / ml values were obtained by calculation.

The activity measurement of peroxidase enzyme was determined spectrophotometrically according to the procedure applied by Şişecioğlu et al. (2010). This procedure is based on the oxidation of guaiacol chromogenic substrate by H_2O_2 and the monitoring the increase in absorbance resulted from colored compound at 470 nm (Şişecioğlu et al., 2010). The enzyme activity and enzyme calculation formula are

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given in *Table 3*. After device calibration, the measurement was made at 470 nm in a quartz cuvette with 3 repetitions. The EU/ml values were determined by calculation.

Table 2. *Catalase enzyme mechanism and enzyme calculation formula*

Catalase: $2H_2O_2 \longrightarrow 2H_2O + O_2$ Calculation of Catalase: EU/ml = 3.45 (pure) Catalase/min (V_E) 3.45 = 240 nm which corresponds to degradation of 3.45 µm dead caused by hydrogen peroxide produced in 3 ml of reaction for absorbance decline from 0.45 to 0.40. df = dilution factor min = time required to decrease absorbance at 240 nm from 0.45 to 0.40. V_E = The amount of enzyme used

Table 3. *Peroxidase enzyme mechanism and enzyme calculation formula*

Peroxidase mechanism: 2Guaiacol + $2H_2O_2 \longrightarrow$ 2oxide guaiacol + $2H_2O$ Calculation of Peroxidase: EU/ml = (Peroxidase, absorbance difference occurs in 1 min) * df / 1*(0.01) df: dilution factor 1: per enzyme / increase in 1 min 0.01: amount of enzyme homogenant used Unit/mg protein: (Unit / ml enzyme) / (mg protein / ml enzyme)

0-5 scale

Disease severity of plants was determined using 0-5 scale after 4, 8, 12 and 16 days of pathogen inoculation. In this evaluation; 0 = no sign of disease; 1 = brown lesions began to appear on lightly faded leaves and body; 2 = disease indication in 30-50% of the plant; 3 = disease symptoms at 50-70% of the plant; 4 = disease symptoms at 70-90% of the plant; 5 = dead plant (Sunwoo et al., 1996; Ozgonen and Erkilic, 2007).

Principal component analysis

The first principal component (PC), y_1 is a linear combination of x_1, x_2, …, x_p (*Eq. 1*):

$$y_1 = a_{11}x_1 + a_{12}x_2 + \cdots + a_{1p}x_p = \sum_{i=1}^{p} a_{1i}x_i \quad \text{(Eq.1)}$$

such that the variance of y_1 is maximized given the constraint that the sum of squared (SS) weights is (*Eq. 2*):

$$\sum_{i=1}^{p} a_{1i}^2 = 1 \quad \text{(Eq.2)}$$

The random variables x_i, can be either deviation from mean scores or standardized scores. If the variance of y_1 is maximized, then so is the SS correlations of y_1 with the original variables x_1, x_2, …, x_p (*Eq. 3*):

$$\sum_{i=1}^{p} r_{y_i x_i}^2 \quad \text{(Eq.3)}$$

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PCA finds the optimal weight vector $(a_{11}, a_{12}, \ldots, a_{1p})$ and the associated variance of y_i which is usually denoted by λ_i. The second PC, y_2, involves finding a second weight vector $(a_{21}, a_{22}, \ldots, a_{2p})$ such that the variance of (*Eq. 4*)

$$y_2 = a_{21}x_1 + a_{22}x_2 + \cdots + a_{2p}x_p = \sum_{i=1}^{p} a_{2i}x_i \quad \text{(Eq.4)}$$

is maximized subject to the constraints that it is uncorrelated with the first PC and (*Eq. 5*)

$$\sum_{i=1}^{p} a_{2i}^2 = 1 \quad \text{(Eq.5)}$$

the results in y2 having the next largest SS correlations with the original variables. The first two PCs together have the highest possible SS multiple correlations (Dunteman, 1989).

Statistical analysis was performed using MINITAB 14 Software.

Results and discussion

Enzymatic component of antioxidative enzymes are important for the formation of antioxidative reactions in plants and in obtaining cell homeostasis for defense system. Antioxidative enzymes are being frequently used in recent transgenic studies to increase the resistance of plants to different stressors. The fruit shape and hotness of peppers may also be an indicative of resistance. The resistance of capia, half-capia-pointed pepper or bell peppers was different. The capia peppers showed resistance to *phytopthora capsici*, while the genotypes or varieties with semi capia-pointed peppers were included in the partially resistant or sensitive or even very sensitive group. The bell pepper genotypes and varieties did not show any resistance to the disease and were found as sensitive or even very sensitive.

The highest peroxidase activity among genotypes investigated was obtained with line 10 (0.0744), and followed by genotypes 3 (0.0568), 38 (0.0540), 57 (0.0533) and 11 (0.0482), respectively. The genotype 10 showed a similar profile (2.9571429) in terms of catalase activity and followed by genotypes 8 (1,5525), 56 (1,5525), 9 (1,514,6341) and 11 (1,444186), respectively (*Tables 4* and *5*). Similar to our findings, Lamour et al. (2012) also showed that CM 334 genotype had resistance to all *P. capsici* isolates.

Seven of the 60 genotypes (genotypes 3, 10, 13, 25, 38, 48 and 57) survived after the first inoculation. Significant disease symptoms were observed in other 53 genotypes and these genotypes died in a short time after the inoculation. Different genotypes resistant to *P. capsici* may be developed when early symptoms are not ignored (Black et al., 1991; Ortega et al., 1995; Pochard et al., 1983). Similar to the findings of Kim et al. (1989), resistance to *P. capsici* increased during 12-leaf period. All other genotypes except 10 genotypes died after the second inoculation. The highest peroxidase enzyme activity was obtained with the seven genotypes survived following the first inoculation (*Tables 4* and *5*). The seedlings were not significantly affected from *P. capsici* at the beginning of their developments. However, when seedlings reached to 4 true leaf stage, the initial symptoms started to appear, and the resistance mechanism was different for the different parts of plants (Bosland and Lindsey, 1991; Sy et al., 2005). The peroxidase enzyme activity was low in very sensitive genotypes, while enzyme activity

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was very high in very resistant genotype (genotype 10-CM 334) and partially resistant genotypes (3, 13, 25, 38, 48 and 57).

Table 4. *Peroxidase activities of the genotypes used in the study*

GNP	Peroxidase (EU/ml)	Std deviation	GNP	Peroxidase (EU/ml)	Std deviation
10	0.074	0.002	51	0.025	0.007
3	0.057	0.001	14	0.022	0.001
38	0.054	0.009	46	0.022	0.004
57	0.053	0.014	42	0.021	0.011
11	0.048	0.001	39	0.021	0.009
13	0.048	0.000	6	0.018	0.001
25	0.048	0.004	29	0.018	0.000
48	0.047	0.007	32	0.017	0.006
34	0.044	0.008	41	0.017	0.005
59	0.043	0.001	18	0.016	0.000
26	0.043	0.025	19	0.015	0.002
28	0.043	0.003	23	0.015	0.001
35	0.041	0.003	33	0.014	0.007
5	0.041	0.001	16	0.013	0.001
54	0.041	0.004	49	0.012	0.003
37	0.041	0.011	43	0.012	0.003
36	0.041	0.008	15	0.011	0.001
58	0.041	0.002	17	0.011	0.000
24	0.040	0.006	22	0.011	0.004
31	0.040	0.009	4	0.011	0.000
55	0.038	0.002	20	0.010	0.000
47	0.038	0.028	27	0.009	0.002
9	0.036	0.002	7	0.009	0.000
40	0.036	0.007	8	0.008	0.001
52	0.033	0.002	30	0.007	0.001
60	0.032	0.002	12	0.006	0.001
50	0.030	0.006	2	0.006	0.000
53	0.030	0.006	45	0.005	0.001
56	0.028	0.008	1	0.005	0.001
44	0.027	0.006	21	0.003	0.000

Although catalase enzyme content was different in some genotypes, similar results were obtained for peroxidase. The peroxidase (POX) content of genotypes that sensitive in the early stage of inoculation had generally high but catalase content significantly varied among these genotypes. The POX enzyme content can be used as a determinant because POX is an oxide-reductive enzyme which stimulates oxidation and suberization of phenols participating in cell wall polysaccharide processes, lignification of host plant cells during defense and provides a reaction against pathogenic substances (Ray et al., 1998). The POX content is very high in stable plant tissues (Breusegem et al., 2001; Lin and Kao, 2001). Lignin accumulation and phenolic compounds have been correlated with the disease. Similar results were also obtained in wheat-*Fusarium graminearum* (Mohammadi and Kazemi, 2002) and cucumber-*Pythium aphanidermatum* (Chen et al., 2000) treatments. The POX is believed to participate in cell wall metabolism (Welinder,

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1993) and production of anti-microbial compounds (Kobayashi et al., 1994) that play a role in healing of wounds. The POX content was tended to increase in resistant variety/line/genotypes (Jung et al., 2006).

Table 5. *Catalase activities of the genotypes used in the study*

GNP	Catalase (EU/ml)	Std deviation	GNP	Catalase (EU/ml)	Std deviation
10	2.96	0.02	43	0.64	0.04
8	1.55	0.05	2	0.62	0.12
56	1.55	0.06	59	0.62	0.07
9	1.51	0.03	52	0.61	0.21
11	1.44	0.03	36	0.60	0.04
13	1.11	0.07	51	0.60	0.02
15	1.11	0.03	19	0.60	0.04
7	1.07	0.08	55	0.60	0.09
3	0.99	0.06	58	0.58	0.07
6	0.99	0.05	26	0.58	0.06
33	0.96	0.03	28	0.58	0.03
29	0.93	0.08	39	0.57	0.05
34	0.91	0.06	30	0.56	0.04
60	0.89	0.13	35	0.54	0.06
38	0.87	0.07	21	0.52	0.04
25	0.84	0.03	48	0.50	0.14
37	0.83	0.04	45	0.50	0.02
41	0.83	0.10	24	0.49	0.05
57	0.83	0.04	40	0.48	0.07
22	0.82	0.03	31	0.47	0.05
53	0.81	0.05	49	0.46	0.04
16	0.78	0.01	44	0.45	0.04
27	0.71	0.03	1	0.43	0.10
47	0.69	0.03	42	0.40	0.24
50	0.69	0.03	5	0.35	0.15
20	0.68	0.13	46	0.34	0.08
17	0.68	0.03	32	0.28	0.07
18	0.68	0.02	12	0.20	0.12
54	0.66	0.02	4	0.13	0.00
14	0.65	0.06	23	0.04	0.08

The scale of 0-5 was used to identify the disease (*Table 6*). Zero stands for no disease symptoms and 5 represents the highest symptoms of disease. The disease was very vigorous and continuous in genotypes with a 5-scale value. Distinction of symptoms required great attention in genotypes received scores between 0 and 5. Some of the genotypes (3, 25 and 38) received high values at the initial assessment whereas their scores were lower at later observations and included in resistant genotypes. Sixteen days after the inoculation, the differences between susceptible and resistant and partially resistant genotypes started to be apparent. The enzyme (peroxidase and catalase) contents were also compatible with the resistance of genotypes. Some genotypes were classified within the sensitive group at initial assessments, whereas they scored lower in the later evaluations, meaning less disease symptoms (i.e. Gayoso et al., 2004; Ray et

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al., 1998; Ponmurugan et al., 2007). Resistance or sensitivity did not follow a linear trend in some genotypes. Genotypes of 28, 35 and 45 were finally determined as susceptible, and despite the deaths at the first inoculation they were classified among the genotypes that responded as if the disease symptoms had improved. The genotypes 3, 25 and 38 received higher scale values at the beginning and followed by lower values at the subsequent observations. The genotype 10 had a 0-scale value in every observation and it was identified as the most resistant plant material without any signs of disease. The genotype 33 showed initial disease symptoms even at the fourth observation following the first inoculation. The enzyme activity content of sensitive genotypes with no stabilized score values do not have also sufficient values for enzyme activity. Inconsistent differences in the scale values may be due to enzyme activity, and this may also a sign of morphological clarification and genetical impurities.

Table 6. *Evaluation of pepper lines and genotypes in first inoculation according to 0-5 scale*

***Capsicuum* spp. genotypes**	**DAYS**				***Capsicuum* spp. genotypes**	**DAYS**			
	4	8	12	16		4	8	12	16
1	0	4	5	5	31	0	2	1	5
2	0	4	5	5	32	0	3	3	5
3	0	0	1	1	33	0	3	4	5
4	0	2	2	5	34	0	2	1	5
5	0	1	1	5	35	0	2	1	5
6	0	1	2	5	36	0	2	1	5
7	0	1	3	5	37	0	2	1	5
8	0	1	3	5	38	0	1	1	3
9	0	1	2	5	39	0	2	3	5
10	0	0	0	0	40	0	2	2	5
11	0	0	1	5	41	0	2	4	5
12	0	3	5	5	42	0	2	3	5
13	0	0	1	2	43	0	1	4	5
14	0	1	3	5	44	0	2	2	5
15	0	1	3	5	45	0	3	5	5
16	0	1	4	5	46	0	2	4	5
17	0	1	4	5	47	0	1	2	5
18	0	3	4	5	48	0	1	1	3
19	0	1	4	5	49	0	2	4	5
20	0	2	3	5	50	0	2	4	5
21	0	1	5	5	51	0	2	3	5
22	0	3	5	5	52	0	1	4	5
23	0	3	4	5	53	0	1	3	5
24	0	2	2	5	54	0	1	3	5
25	0	1	1	2	55	0	1	2	5
26	0	2	1	5	56	0	1	3	5
27	0	2	5	5	57	0	0	1	3
28	0	3	2	5	58	0	1	2	5
29	0	2	3	5	59	0	1	2	5
30	0	3	4	5	60	0	1	3	5

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Significant correlation between POX activity and content of phenolic compound of plants with different families in which pepper is included has been reported (Candole et al., 2012). The peroxidase and similar enzymes trigger the formation and enhancement of the phenolics in the plant cell wall during defense against pathogens (Gayoso et al., 2004).

In some studies, approximately one week after the infection of disease, the increase in peroxidase, as well as phenol contents especially in the stem and leaves of plant, was reported and even the increase reached to the maximum level (Gayoso et al., 2004). The CM334 is a worldwide known resistant genotype (partial wild) against *P. capsica* and has been defined as a resistant genotype in our study based on both peroxidase and catalase contents. The peroxidase activity of resistant CM334 genotype was higher compared to the sensitive genotypes.

Inadequate enzyme activity in sensitive varieties may have caused these genotypes defeating against the disease. Living in a certain period of time for sensitive genotypes despite the disease may be attributed to the consumption rate of phenolic substances. Ponmurugan et al. (2007), it is predicted that it may be aimed at producing some metabolites which may cause the metabolism of the pathogens to be distributed and balanced. The overproduction of ROS (free radicals) and the consumption of antioxidant defenses may cause disruption of oxidant–antioxidant balance and occurrence of oxidative stress in the cells (Koç and Üstün, 2016).

Three morphological (resistance, hotness and fruit shape) and two chemical (peroxidase and catalase) characteristics of pepper genotypes were investigated in this study. The descriptive statistics (mean, standard deviation, minimum, maximum and median) for characteristics were presented in *Table 7*. The relationships among genotypes in terms of characteristics studied were evaluated by principal PCA. The mean and standard deviation values revealed that resistance had the lowest variability and peroxidase and catalase had the highest variability. High coefficient of variability is an indication of the differences in genotypes.

Table 7. *Descriptive statistics of pepper genotypes*

Traits	N of obs.	Mean	Std. dev	Minimum	Median	Maximum	CV (%)
Resistance	60	2.9833	0.5365	1.0000	3.0000	4.0000	17.98
Hotness	60	1.6000	0.7178	1.0000	1.0000	3.0000	44.86
Fruit shape	60	1.5333	0.7003	1.0000	1.0000	3.0000	45.67
Peroxidase	60	0.0274	0.0165	0.0030	0.0260	0.0740	60.22
Catalase	60	0.7389	0.4278	0.0360	0.6435	2.9570	57.90

Each component in the table explains a certain portion of the variability in the data. The higher the proportion, the more variability is explained by the PC. Cumulative proportion is the variability explained by the consecutive PCs. The PCs with the largest eigenvalues were retained to explain the variability. A PC with eigenvalues greater than 1.0 is kept for further analyses. The scree plot enables visual comparison of the eigenvalue sizes (*Fig. 1*). The first two PCs have eigenvalues greater than 1.0 and explain 74% of the variation in the data. The eigenvalues start to form a straight line after the second PC. The amount of variability explained by the two PCs is sufficient hence the first two PCs will be used to explain the variability.

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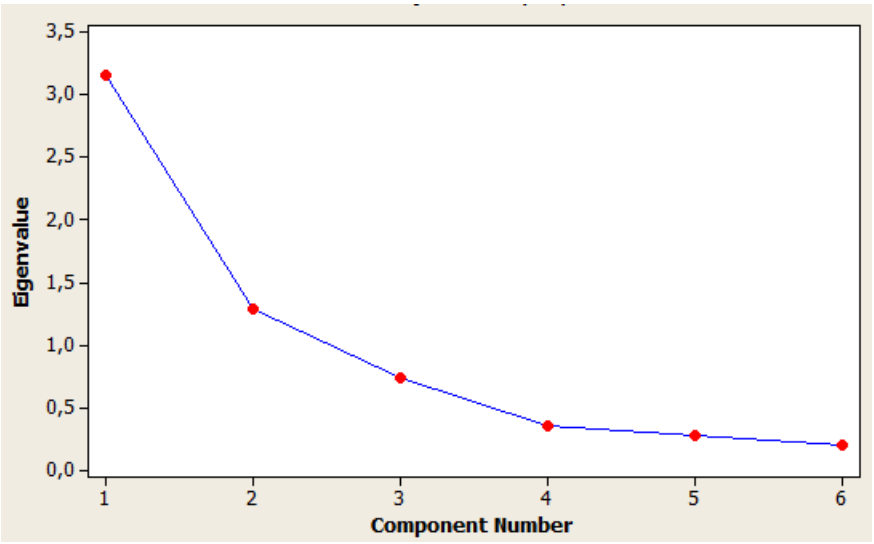

Figure 1. *Scree plot of the eigenvalues*

The PCs are the linear combinations of the original variables that account for the variance in the data. The coefficients indicate the relative weight of each variable in the component. Peroxidase, resistance and fruit shape had the highest influence in PC1 (*Table 8*). The peroxidase and resistance had positive effects in PC1, while fruit shape had negative effect (the effect on ingredient increases as the fruit gets more pointed).

Table 8. *Results of principal component analysis*

Trait	PC1	PC2	PC3	PC4	PC5	PC6
Resistance	0.491860	0.197271	-0.067553	-0.131608	0.491860	0.197271
Hotness	0.440223	-0.051902	0.545261	0.648487	0.440223	-0.051902
Shape of fruit	-0.480311	0.259828	-0.171691	0.208326	-0.480311	0.259828
Genotype	0.116054	-0.718223	-0.580743	0.343676	0.116054	-0.718223
Peroxidase	0.501997	-0.050420	-0.153561	-0.512774	0.501997	-0.050420
Catalase	0.260977	0.610322	-0.554803	0.371071	0.260977	0.610322
Eigenvalues	3.1560	1.2860	0.7356	0.3548	0.2705	0.1972
Proportion %	52.6	21.4	12.3	5.90	4.50	3.30
Cumulative proportion %	52.6	74.0	86.3	92.2	96.7	100.0

The second PC had a large negative association with genotype (71.8%) and positive associations with catalase (61.0%) and fruit shape (25.9%) (*Fig. 2*).

There are a number of variants of the biplot idea, but all give a simultaneous display of n observations and p variables on the same two-dimensional diagram. In one of the variants, the plot of observations is identical to a plot with respect to the first two PCs, but the biplot simultaneously gives graphical information about the relationships between variables. The relative positions of variables and observations, which are plotted on the same diagram, can also be interpreted (Jolliffe, 2002). High positive effects of resistance, peroxidase and hotness on PC1 are seen in biplot figure (*Fig. 2*). In other words, the first component focuses on the hotness, peroxidase and catalase

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contents of plants and resistance to phytophthora disease. The second component is more related to the fruit shape of the plant. The relationships among genotypes grouping based on fruit shape, hotness and resistance are illustrated in the score plots (*Figs. 3–5*).

Figures 3 and *4* show that the genotypes form groups according to these morphological characteristics. Based on resistance parameter, genotypes could be grouped absolutely independently (*Fig. 5*).

The first PC accounts 52.4% and the second PC explains 21.6% of total variation. A model based on the first two principal components accounts for 74.00% of total variance.

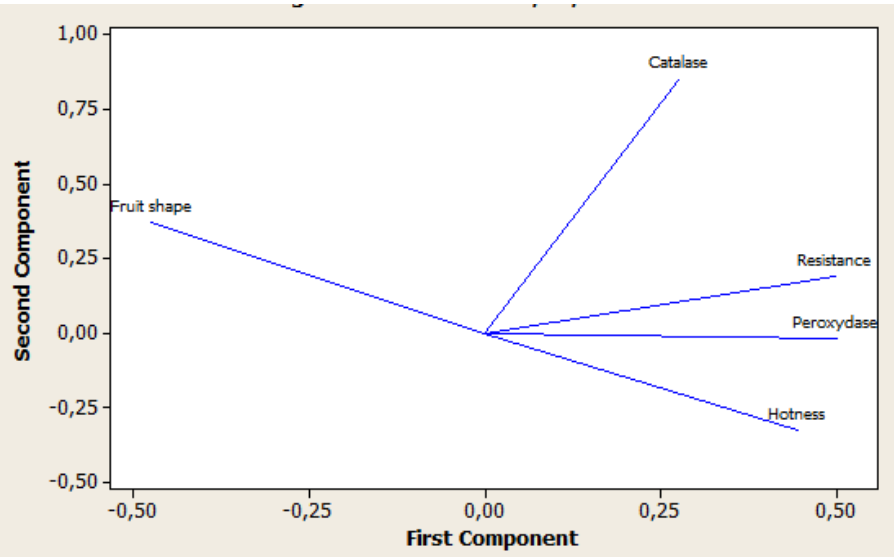

Figure 2. *Biplot analysis of different pepper genotypes*

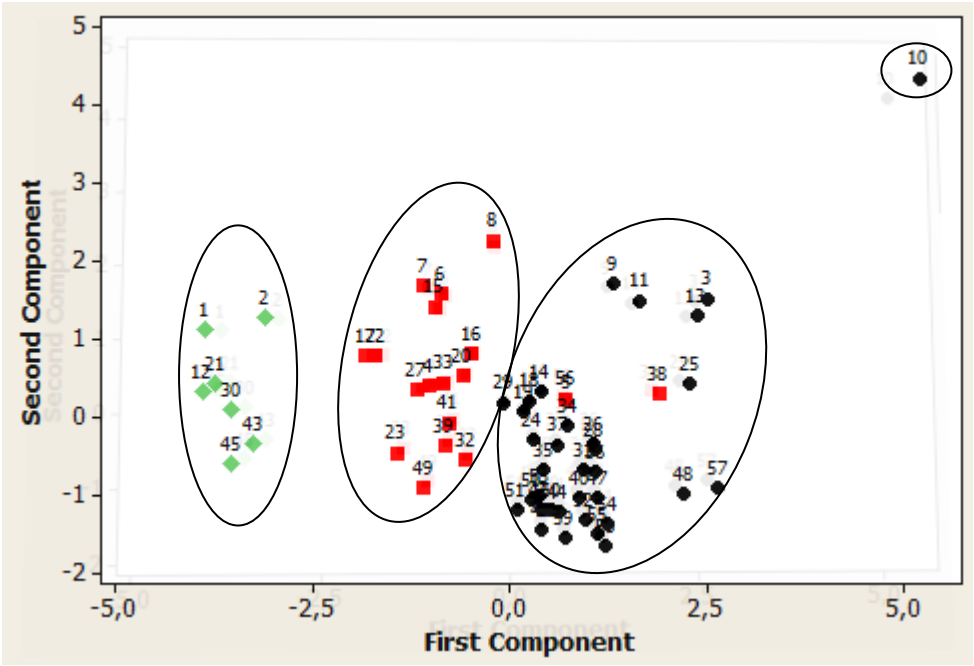

Figure 3. *Score plot of PC1 versus PC2 shows the differentiation of genotypes according to fruit shape (◆ - bell pepper, ■ - semi capia, ● - capia)*

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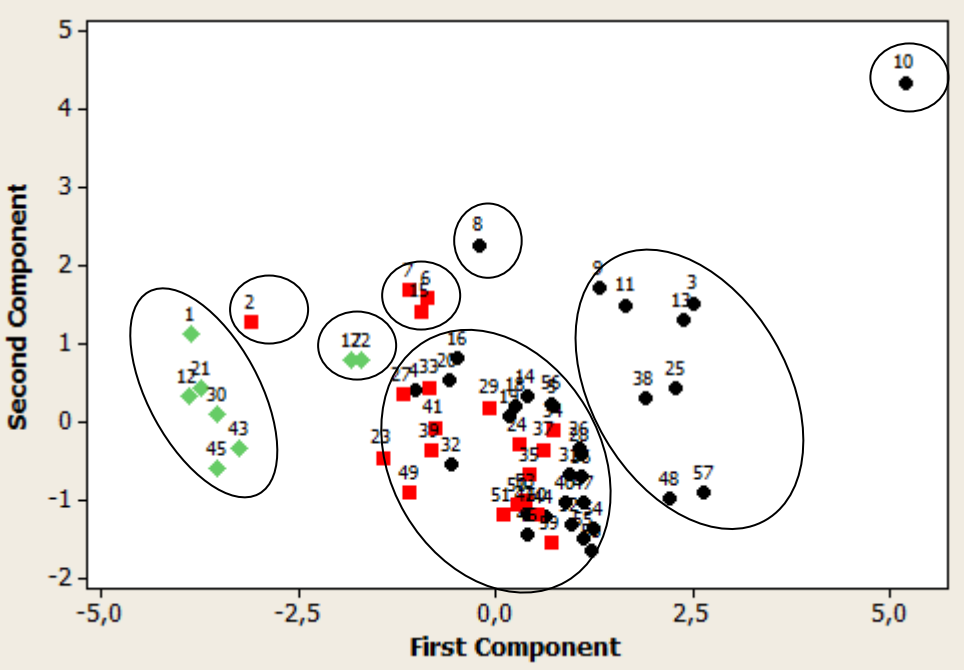

Figure 4. *Score plot of PC1 versus PC2 shows the differentiation of genotypes according to hotness (◆ - not hot, ■ - some hot, ● - hot)*

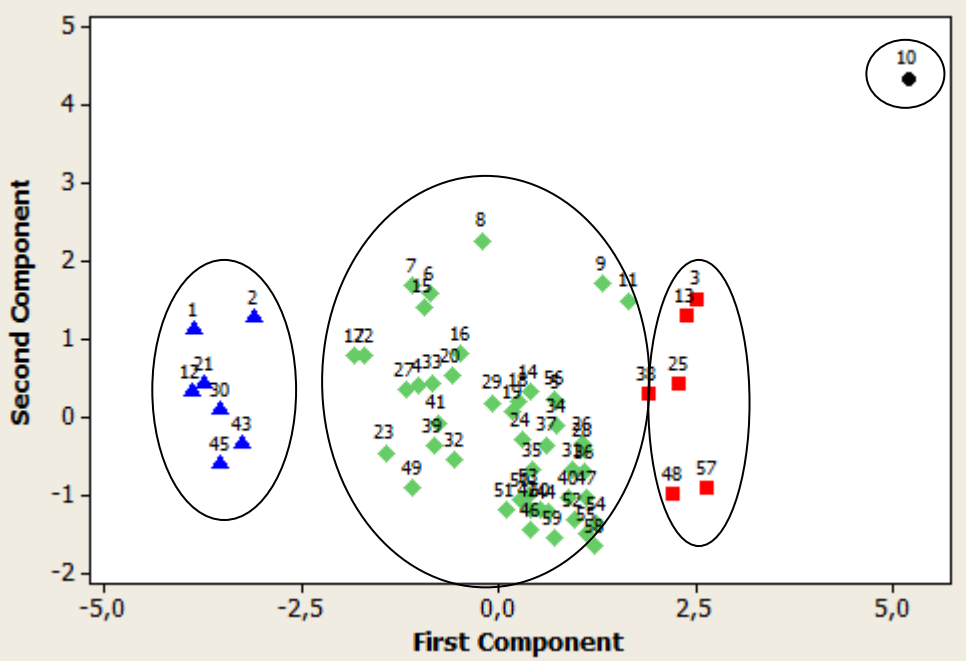

Figure 5. *Score plot of PC1 versus PC2 shows the differentiation of genotypes according to resistance (▲ -very sensitive, ◆ - sensitive, ■ - partially resistant, ● - resistant)*

Most of genotypes died at the first inoculation applying. Few genotypes died in the first two weeks of inoculation. The CM 334 genotype showed resistance in both inoculations and showed no sign of disease as two inoculations. The first inoculation in

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the leaves of the partially endogenous genotypes P1, 13 (Urfa), 25 (UKST), 38 (UID), 48 (UKDT), 57 (ANKSB)] showed weak symptoms. New shoot formation was detected in these genotypes. In the second inoculation, however, these genotypes have also been found at high rates since the first week after the inoculation of the disease indications. Deaths in these genotypes were observed in approximately 2 weeks. The first week after inoculation is important for breeders. As a result of our observations that the genotypes started to show resistance in the first week after inoculation may be breeding material. The yield and fruit qualities of genotypes found to be resistant or very resistant are not available in commercial pepper cultivation. CM 334 genotype has lower fruit quality and yield values. Partly resistant genotypes (deaths in the first two weeks and deaths after second inoculation due to disease) should be used at breeding program because according to Hwang et al. (1996), resistance against *P. capsici* occurs during the last stages of plant development. The genotypes that are resistant (genotype 10 /CM 334) or part-resistant (genotypes 3, 13, 38 and 48) all have the shape of capia fruit. Again, the genotypes listed above have bitter fruits. It was found that the fruits of the fruit-shaped capia and hot were likely to be resistance (*Table 1*). The PCA helped to reveal some relationships between genotypes and morphological properties. The PCA has proved to be a useful approach in characterization of genotypes based on their morphological properties.

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