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FIELD REACTION AND BIOCHEMICAL RESPONSE OF GRAPE GENOTYPES TO ANTHRACNOSE INCIDENCE UNDER SUB-TROPICAL CONDITIONS

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ABSTRACT
Thirty two genotypes, consists of 12 parents and 20 hybrids were taken for study. Leaves were sampled during April (healthy phase) and first week of August (peak disease occurrence) for estimation of different biochemical parameters, namely, total phenols, chlorophyll, carotenoids, sugar, peroxidase (POD), polyphenol oxidase (PPO) and phenylalanine ammonia lyase (PAL) activities in year 2012 and 2013. Standard disease scoring technique (0-9) was followed to estimate disease severity index (DSI) during the peak disease incidence in field and in artificial inoculation of leaves. Results showed that *Vitis parviflora* was immune (DSI= 0), Male Hybrid was extremely resistant (DSI=2.40), Pusa Navrang was found to resistant (DSI=14.54). Four genotypes were moderately resistant, while 10 were moderately susceptible, eight were susceptible, five were highly susceptible and Perlette, Hybrid 70-56 were rated as extremely susceptible (DSI =>85) to the disease. The biochemical parameters showed distinct trend, *i.e.*, the resistant genotypes had higher phenols, coupled with higher POD, PPO and PAL activities for both healthy and diseased leaves. Negative correlations were reported among DSI and biochemical parameters like total phenols ($r = -0.879^{**}$), POD ($r = -0.744^{**}$), PPO ($r = -0.792^{**}$) and PAL ($r = -0.884^{**}$). Positive correlations were reported among DSI and stomata count ($r = 0.828^{**}$) and total sugar ($r = 0.679^{**}$).

KEY WORDS: Grape, anthracnose, biochemicals, Disease Severity Index (DSI).

INTRODUCTION

Anthracnose or "Bird's eye spot" of grape (*Vitis vinifera* L.) caused by *Elsinoe ampelina* (de Bary) Shear, is widespread and the most destructive disease in the vineyards. In India the disease has become a potential threat to grape cultivation. In north India, it appears every year from July to September with peak damage during August and reduces the quality and quantity of produce. In south India, the disease prevails from March to October with peak damage during May-July. Deshmukh (2006) reported that annually 15-30 percent losses due to anthracnose of grape occur in Maharashtra state. The disease affects all the aerial parts in the green stage; mostly on the new shoots and fruits. On leaves small circular to irregular, 1-5 mm dia. in size, dark brown spots appear which later turn gray in the centre and dark brown at the margins. On berries, typical bird's eye spot symptoms appear having violet to greyish centre and dark brown margins. This disease is mainly controlled by fungicide treatments that increase economic costs and negatively affect the environment. It is observed that many a times the viticulturists have to spray the vineyards 15-20 times; simply to keep the vine's vegetative parts and fruits free from this disease. Furthermore, fungal strains are developing resistance to some commonly used fungicides (Savocchia *et al*., 2004). Although the most commonly cultivated species, *V. vinifera,* has proved to lack resistance to anthracnose, the degree of susceptibility varies with the cultivar and the environmental conditions (Peros *et al*., 2006). Thus, the possibility of selecting less-susceptible, high-quality cultivars is an alternative management strategy of great importance. Defensive enzymes and phenolics are among the most influential and widely distributed products in the plants. Biochemical parameters, *viz*. PAL, PPO, POD activities, phenols and sugars were reported in plants treated with various biotic and abiotic inducers. Phenylalanine ammonia lyase (PAL) is the key enzyme catalyzing the biosynthesis of phenolics and lignin from the aromatic amino acid phenylalanine (Cartea *et al.*, 2010). The involvement of phenols in plant disease resistance is based on their cytotoxicity, which is associated with their oxidation products. It has been claimed that the first stage of the defense mechanism of plants involves rapid accumulation of phenols at the infection site, which function to slow down the growth of the pathogens. Many researchers found a correlation between increased host resistance and high phenolic compound content (Sathisha *et al*., 2008). The present investigation focused on screening of grape genotypes and study of biochemical changes in resistant genotypes of grape during healthy and pathogen infection stage.

MATERIALS & METHODS

The present study was undertaken on germplasm maintained at Experimental Farm of the Division of Fruits and Horticultural Technology, IARI, New Delhi during 2012 and 2013.Thirty two grape genotypes including 12 parents (Banqui Abyad, Victory, Hur, Cardinal, Beauty Seedless, Bharat Early, Perlette, Pusa Navrang, Pusa Urvashi, Pusa Seedless and Pearl-of-Csaba.) and 20 hybrids (Male Hybrid (BA x Victory), 76-1 (Hur x Card), 70-56 (Hur x BS), 75-32 (BA x Per), Hybrid Seedless (Hur x BE x BS), R_1P_{19} (PoC x BS), R_1P_{21} (PoC x BS), R_1P_{40} (PU x Per), R_1P_{43}(Per x PU), R_1P_{52} (PoC x Per), R_2P_4 (PoC x BE), R_2P_9 (PoC x Per), R_2P_{19} (PoC x BS), R_2P_{20} (PU x Per), R_2P_{32} (PoC x BS), R_3P_{22}, (PoC x BS), R_3P_{26}

(PU x BS), R_3P_{32} (PU x PN), R_4P_{36}, (PoC x BS), R_4P_{37} (PoC x Per) and R_4P_{40} (PoC x BS))were selected for this study. For field screening, evaluation of disease incidence was carried out when severe disease symptoms were noted on the leaves and canes. Five vines of each genotype were randomly selected and around 120 leaves were observed for natural infection and disease severity index was calculated as suggested by Wang *et al.* (1998). Each leaf and cane was graded as: 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 based on the estimated percentage of lesions over the whole leaf and cane area: 0, 0.1-5.0, 5.1-10.0, 10.1-25.0, 25.1-40.0, 41.1-55.0, 55 .1-70.0 and 70.1-85 and > 85.0, respectively and grades were then converted into a disease severity index (DSI) by using following formula.

$$DSI = \frac{[Sum\ of (Grade\ value\ X\ No.\ of\ leaves\ in\ that\ grade)]}{Total\ leaf\ number\ X\ No.\ of\ leaves\ in\ that\ grade}\ X\ 100$$

Resistance level of each genotype was arbitrarily rated in nine categories based on mean DSI value: immune (DSI= 0), extremely resistant (DSI= 0.1-5.0), highly resistant (DSI = 5.1-10.0), resistant (DSI =10.1-25.0), moderately resistant (DSI = 25.1-40.0), moderately susceptible (DSI = 40.1-55.0), susceptible (DSI = 55.1-70.0), highly susceptible (DSI = 70.1-85.0) and extremely susceptible (DSI=>85). In order to verify the results of the aforementioned experiment (natural infection), another experiment was conducted on the genotypes with fungal inoculation of leaves by using culture filtrates from *E. ampelina*. The study used the virulent pathogen strain (EA-1) of *Elsinoe ampelina* Shear, which was isolated from infected grapevine leaves. The *E. ampelina* pathogen was incubated in a Fries medium shake at 140 rpm under 28oC for 21 days then its cell-free culture filtrates (CFCF) were collected from the supernatant centrifuged at 10,000 × g for 5 min and sterilized through a 0.2 µm pore diameter ultra-filtration system. The upper third or fourth leaves from the shoot apex of different grape genotypes were collected and slightly cut with a pencil tip. Thirty µl of culture filtrates diluted to 1:1, 1:2, 1:4 and 1:8 were applied onto the cut portion of the leaves and then incubated in a dark, moist chamber (98% RH) for 3 days at 28ºC. To determine pathogen resistance the area of the necrotic lesion around the wounded leaves was measured after treatment of diluted culture filtrates of *E. ampelina* (Jung *et al.* (2011). Four levels of resistance were determined as follows: highly susceptible (+++, necrotic area >3 mm from wounded spot), susceptible (++, necrosis spreading of 2-3 mm over wounded spot), moderately susceptible (+, necrosis on wounded spot), moderately resistant (±, slight necrosis) and resistant (-, no necrosis).

Biochemical estimation

Different biochemical parameters such as total phenols, total sugars, polyphenol oxidase, peroxidase, phenylalanine ammonia lyase, total chlorophyll and carotenoids were estimated in healthy leaves and after occurrence of disease under natural field conditions. Total chlorophyll was estimated as per the method given by Hiscox and Israelstam (1979). The total phenols content of leaves was determined according to the method described by Malick and Singh (1982). Quantity of total sugars was estimated by anthrone reagent methods of Hedge and Hofreiter, (1962). Peroxidase (EC 1.11.1.7) and polyphenol oxidase (EC 1.10.3.2) activities were assayed according to the method of Zhang *et al.* (2008) and Matto and Diamond (1963), respectively. Activity of phenylalanine ammonia lyase (EC 4.3.1.24) was assayed as per the method given by Lisker *et al.* (1983). Data for all the parameters were recorded three times and analysis of variance (ANOVA) was carried out in Completely Randomized Design. Pearson's correlation coefficient (r) among DSI and biochemical traits were computed by using statistical analysis system software (SPSS version 2.1)

RESULTS & DISCUSSION

Field and artificial inoculation screening

As evident from Table 1, out of the 32 genotypes, *V. parviflora* rated as immune (DSI = 0.45), Male Hybrid was extremely resistant (DSI = 4.15), Pusa Navrang was resistant (DSI = 21.90), R_2P_4, R_3P_{26}, R_3P_{32}, R_1P_{52} were found moderately resistant (Average DSI = 32.5), Beauty Seedless, Pusa Urvashi, 76-1, R_2P_9, R_2P_{19}, R_3P_{22}, R_1P_{19}, R_1P_{21}, R_4P_{37}, R_4P_{40} were moderately susceptible (Average DSI = 49.15), Bharat Early, Banqui Abyad, Pearl- of-Csaba, Hur, Hybrid Seedless, R_1P_{40}, R_4P_{36}, R_2P_{32} were susceptible (Average DSI = 63.5), Victory, Cardinal, Pusa Seedless, Hybrid 75-32, R_1P_{43} were highly susceptible (DSI = 79.30) and Perlette, Hybrid 70-56 were found extremely susceptible (DSI = >85) to disease. In artificial inoculation all the genotypes showed necrosis symptoms except *V. parviflora* when infected with 1:1 ratio of culture filtrates. The genotypes (Pusa Navrang, Male Hybrid) rated as resistant and extremely resistant under field conditions do not show necrosis with 1:4 ratio culture filtrates. The genotypes rated as susceptible (Pearl-of-Csaba, Banqui Abyad, R_2P_{32}, R_4P_{36}, R_1P_{40}, Pusa Seedless, Hybrid Seedless) and extremely susceptible (H-70-56, Perlette) in vineyard investigation shown necrosis even with 1:8 ratio culture filtrates disease reaction of different grape genotypes against anthracnose has previously been examined by various workers worldwide under different growing conditions (Yanmin *et al*., 2010; Poolsawat *et al*., 2012).

Stomata count

Results revealed that resistant genotypes had less number of stomata as compare to susceptible genotypes. Maximum number of stomata reported in Hybrid Seedless (192.67) followed by Perlette (187.67) and Hybrid 70-56 (187.00) and minimum number in *V. parviflora* (117.33). As pathogen enters through the stomata, it was possible to infer that the lower stomata number in resistant genotypes had limited the infection. Recently, Divya *et al.* (2014) also observed less number of stomata in rust resistant lines in French bean.

Biochemical parameters

In present study, reduction in the contents of chlorophylls and carotenoids in disease affected leaves in comparison to healthy leaves in all genotypes is in agreement with the

earlier reports of Lobato and Goncalves (2009). They reported 15.2% decrease in chlorophyll and 30.5% decrease in total carotenoids content in susceptible cultivars infected by *Colletotrichum lindemuthianum* causing anthracnose of bean. Disease affected leaves of susceptible genotypes had higher content of total sugars compared to resistant genotypes (Table 2). Hybrid 70-56 (7.27, 8.62 mg/g FW) followed by Perlette (7.03, 8.50 mg/g FW) had highest contents of total sugars, and accordingly both of which showed high degree of susceptibility to disease. Lowest sugar content was found in *V. parviflora* (4.43, 5.32 mg/g FW) which was immune to the disease. Recently, Prakash *et al.* (2011) also reported lower sugar content in fruit rot (*Colletotrichum capsici*) resistant varieties compared to susceptible varieties of chilli. The total phenols content was the highest during healthy and disease infection phases in immune genotype (*V. parviflora*) followed by resistant, moderately resistant, susceptible and the least in extremely susceptible genotypes. The immune genotype, *V. parviflora* recorded the highest total phenols content (4.38, 5.31 mg/g FW) followed by extremely resistant genotype Male Hybrid (4.24, 5.01 mg/g FW) and extremely susceptible genotype Perlette, Hybrid 70-56 the least (2.52, 2.76 mg/g FW). As evident from table 2, the resistant genotypes contain higher total phenols than susceptible genotypes during both healthy and peak disease infection period. Shankar and Jindal (2001) were also reported higher phenol content in anthracnose resistant grape genotypes. Similar observations of high phenolic contents in *Colletotrichum* resistant genotypes of *Capsicum annum* L. was reported by Kaur *et al.* (2011).

Enzymes are known to play decisive role in host parasite interaction. As observed from the table 2, there was wide variation in the enzyme activity among different genotypes. The highest peroxidase (EC 1.11.1.7) activity was observed in *V. Parviflora* (2.17, 2.33 g/min) and Male Hybrid (2.14, 2.29 g/min) followed by Pusa Navrang (1.05, 1.42 g/min), which showed resistant against disease. The lowest activity was reported in Perlette (0.73, 1.37 g/min) followed by Hybrid 70-56 (0.68, 1.42 g/min), which come under extremely susceptible category. Thus, it is concluded that the activity of the enzymes is directly related to resistance in the host. Further the enzyme activity in diseased leaves was higher than that in corresponding healthy leaves. This could be due to the stress created by pathogen establishment. The present investigations are in confirmatory with the investigation of Saharan *et al.* (2000), who reported increased peroxidase activity in response to *Alternaria* blight infection in both resistant and susceptible cluster bean varieties. Paranidharan *et al.* (2009) observed higher peroxidase activity in rice leaf sheaths infected with *Rhizoctonia solani.* Increases in peroxidise activity could be correlated with infection in plants; polymerization of cinnamyl alcohols to lignin is catabolised by peroxidase lignification leading to disease resistance. The highest activity of polyphenol oxidase (EC 1.10.3.2) was observed in *V. parviflora* (61.57, 81.73 g/min) followed by Male Hybrid (54.27, 70.42 g/min) and Pusa Navrang (44.85, 57.29 g/min) in healthy and diseased leaves respectively whereas lowest was observed in Perlette (21.29, 37.70 g/min) and Hybrid 70-56 (20.76, 38.27 g/min) which is significantly different. In the present investigation PPO activity was observed to be higher in infected leaves as comparison to the healthy one and the resistant genotype expressed more PPO activity than the susceptible one. Niranjanraj *et al.* (2006) observed similar results that seedlings of resistant varieties had greater PPO activity than susceptible seedlings of pearl millet. Parihar *et al.* (2012) observed similar results in *Brassica juncea* genotypes during pathogenesis of *Alternaria* blight. The PPO could be enhanced the oxidation of phenolic compounds into the more toxic forms, quinnones, against pathogen (Tyagi *et al.*, 2000). The lowest activity of phenylalanine ammonia lyase(EC 4.3.1.24) was observed in Perlette (3.18, 3.65µmol of transcinnamic acid/ mg protein/ h**)**followed by Hybrid-70-56 (3.17, 3.78µmol of trans cinnamic acid/ mg protein/ h) and Pusa Seedless (3.21, 3.74µmol of trans cinnamic acid/ mg protein/ h) during healthy and disease infection stage. The highest activity of PAL recorded in *V. parviflora* (4.38, 8.23µmol of transcinnamic acid/ mg protein/ h) followed by Male Hybrid (4.36, 7.60µmol of trans cinnamic acid/ mg protein/ h) and Pusa Navrang (3.92, 5.85µmol of trans cinnamic acid/ mg protein/ h) during healthy and disease infection. Similar observations were recorded impervious research, during the plant development, cell differentiation, stress conditions such as irradiation, wounding, nutrient deficiencies, herbicide treatment andviral, fungal and insect attacks (Morelló *et al.*, 2005). Logemann *et al.* (2000) reported that the increase in PAL activity has frequently been mentioned as a defense reaction of plants to pathogen attack. An increase in PAL activity results in increase in concentration of phenolic compounds, which are substrates for oxidative enzymes such as polyphenol oxidase and peroxidase. PAL catalyzed first reaction of phenylproponoid pathway, phenylalanine to t-cinannamic acid, which results accumulation of phenolics and other antimicrobial compounds (Slatnar *et al.*, 2010).

Correlation studies

The level of resistance or susceptibility of grape genotypes was correlated with abovementioned biochemical parameters. Correlation studies (Table 3) indicated that the activities of PPO and POD were significantly negatively correlated($r = -0.744^{**}$, $r = -0.792^{**}$) with DSI. The disease severity index showed negative correlation with the activity of PAL ($r= -0.884^{**}$). Correlation of enzymatic activities to disease severity index followed trend similar as reported by Zhou *et al.* (2012) while working on *Verticillium* wilt rsistance in eggplant. Results of present investigation also indicated that amongst different enzymatic activities studied, PAL showed the highest correltion coefficient with DSI ($r = -0.884^{**}$), which suggests that this trait would be useful in selecting for anthracnose resistant genotypes.Total phenols showed higher negative correlation ($r= 0.879^{**}$) with disease severity index.

TABLE 1. Disease severity index and disease reaction of grape genotypes in field conditions and in artificial inoculation of leaves

Genotypes	DSI under natural conditions							Ex Situ conditions			
								Culture filtrate concentration			
	Leaf DSI 2012	Leaf DSI 2013	Mean	Cane DSI 2012	Cane DSI 2013	Mean	Disease Reaction	1:1	1:2	1:4	1:8
Pearl-of- Csaba	56.10	58.20	57.15	53.50	50.67	52.08	Susceptible	+++	+++	++	±
Beauty Seedless	41.30	46.20	43.75	38.00	40.78	39.39	Moderately Susceptible	+++	++	±	-
Perlette	88.20	91.00	89.60	84.10	83.45	83.78	Extremely Susceptible	+++	+++	++	++
Banqui Abyad	68.50	68.00	68.25	61.90	56.38	59.14	Susceptible	+++	++	++	±
Pusa Urvashi	46.20	52.40	49.30	46.90	43.12	45.01	Moderately Susceptible	+++	++	±	-
Pusa Navrang	21.30	22.50	21.90	13.40	15.68	14.54	Resistant	+	±	-	-
Pusa Seedless	81.50	78.80	80.15	71.45	74.00	72.72	Highly Susceptible	+++	+++	++	+
Cardinal	73.20	75.50	74.35	65.24	69.67	67.45	Highly Susceptible	+++	+++	++	+
Victory	35.00	38.50	36.75	29.00	27.70	28.35	Moderately Resistant	++	+	±	-
Hur	51.30	48.50	49.90	44.78	48.30	46.14	Moderately Susceptible	+++	++	+	-
Bharat Early	68.50	64.80	66.65	61.00	60.20	60.60	Susceptible	+++	++	++	+
R_2P_{19} (PoC x BS)	48.60	50.50	49.55	40.00	39.50	39.75	Moderately Susceptible	+++	+++	+	±
R_3P_{22} (PoC x BS)	44.20	46.20	45.20	39.50	43.90	41.70	Moderately Susceptible	+++	+++	+	±
R_2P_{32} (PoC x BS)	56.80	63.70	60.25	59.90	56.40	58.15	Susceptible	+++	+++	++	+
R_4P_{36} (PoC x BS)	62.60	59.20	60.90	55.78	58.50	57.14	Susceptible	+++	+++	++	+
R_1P_{19} (PoC x BS)	45.00	48.40	46.70	41.45	42.90	42.17	Moderately Susceptible	+++	+++	+	±
R_1P_{21} (PoC x BS)	48.20	52.00	50.10	44.00	40.80	42.40	Moderately Susceptible	+++	+++	+	±
R_4P_{46} (PoC x BS)	52.50	48.20	50.35	40.50	42.90	41.70	Moderately Susceptible	+++	+++	+	±
R_1P_{52} (PoC x Per)	35.60	38.20	36.90	31.00	32.90	31.95	Moderately Resistant	++	+	±	-
R_2P_9 (PoC x Per)	53.70	52.00	52.85	42.45	38.90	40.67	Moderately Susceptible	+++	+++	+	±
R_4P_{37} (PoC x Per)	58.20	60.50	59.35	50.45	52.70	51.57	Moderately Susceptible	+++	+++	+	+
H-75-32 (BA x Per)	78.40	82.20	80.30	75.20	71.70	73.45	Highly Susceptible	+++	+++	++	+
R_1P_{40} (PU x Per)	62.10	58.00	60.05	55.56	57.00	56.28	Susceptible	+++	+++	++	+
R_1P_{43} (Per x PU)	73.40	74.60	74.00	68.50	71.70	70.10	Highly Susceptible	+++	+++	++	+
R_3P_{26} (PU x BS)	38.70	36.20	37.45	29.00	27.35	28.17	Moderately Resistant	++	+	±	-
R_3P_{32} (PU x PN)	27.40	32.00	29.70	26.00	29.90	27.95	Moderately Resistant	++	+	±	-
76-1 (Hur X Card)	48.20	47.50	47.85	42.90	40.50	41.70	Moderately Susceptible	+++	++	+	±
Male Hybrid (BA x Victory)	4.50	3.80	4.15	2.80	2.00	2.40	Extremely Resistant	±	-	-	-
Hybrid Seedless (Hur x BE x BS)	67.10	68.00	67.55	62.45	60.00	61.22	Susceptible	+++	++	++	+
H-70-56 (Hur x BS)	92.30	89.50	90.90	83.50	85.90	84.70	Extremely Susceptible	+++	+++	+++	++
R2P4 (PoC x BE)	32.80	36.30	34.55	27.90	29.00	28.45	Moderately Resistant	++	+	±	-
V. parviflora	0.40	0.50	0.45	0.00	0.00	0.00	Immune	-	-	-	-

+++; necrosis over 3 mm from wounded spot, ++; necrosis of 2-3 mm over wounded spot, +; necrosis on wounded spot, ±; slight necrosis, -; no necrosis.

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TABLE 2. Total chlorophyll, carotenoids, phenol, sugars (mg/g FW), POD, PPO activity (g/min), PAL activity (µmol of trans cinnamic acid/ mg protein/ h) and stomata count (no./mm^2) in healthy **(H)** and diseased **(D)** leaves

Genotypes	Total Chlorophyll		Total Carotenoids		Total Phenol		Total Sugars		POD activity		PPO activity		PAL activity		Stomata
	H	D	H	D	H	D	H	D	H	D	H	D	H	D	Count
Pearl-of- Csaba	2.15	1.49	1.73	1.65	3.22	3.68	6.17	7.69	0.92	1.50	30.82	44.19	3.31	3.95	174.50
Beauty Seedless	2.19	1.25	1.68	1.61	3.28	3.83	6.16	7.45	1.11	1.41	39.66	47.66	3.39	3.86	167.64
Perlette	2.06	1.33	1.73	1.65	2.52	2.76	7.03	8.50	0.73	1.37	21.29	37.70	3.18	4.35	188.16
Banqui Abyad	2.06	1.18	1.74	1.55	3.19	3.68	5.72	5.96	1.02	1.57	30.38	41.95	3.32	3.93	170.17
Pusa Urvashi	2.27	1.52	1.77	1.66	3.88	4.55	5.64	6.77	1.58	1.85	44.85	57.29	3.92	5.85	144.40
Pusa Navrang	2.05	1.42	1.66	1.55	3.29	3.94	5.21	6.59	1.05	1.42	36.23	46.45	3.42	3.74	166.27
Pusa Seedless	2.15	1.28	1.75	1.66	2.75	3.01	7.22	8.64	0.92	1.58	23.00	38.18	3.22	4.13	173.50
Cardinal	2.23	1.43	1.75	1.64	2.81	3.24	6.69	7.78	0.90	1.59	24.14	38.32	3.19	4.19	152.65
Victory	2.10	1.38	1.72	1.63	2.97	3.49	5.64	6.40	1.01	1.52	32.06	43.68	3.32	3.70	171.50
Hur	2.07	1.35	1.75	1.67	2.88	3.31	5.98	7.19	0.96	1.51	28.52	38.19	3.32	3.95	165.51
Bharat Early	2.11	1.29	1.73	1.64	2.63	2.96	6.80	8.34	0.73	1.32	24.75	39.25	3.21	3.74	186.83
R_2P_{19} (PoC x BS)	2.10	1.38	1.74	1.66	3.32	3.71	6.74	7.93	1.08	1.52	38.82	49.53	3.40	4.09	165.77
R_3P_{22} (PoC x BS)	2.37	1.27	1.78	1.68	3.28	3.83	6.10	7.38	1.10	1.54	34.15	44.87	3.38	4.14	161.67
R_2P_{32} (PoC x BS)	2.13	1.36	1.78	1.65	2.94	3.48	5.80	6.94	0.92	1.40	30.93	39.82	3.26	3.97	158.00
R_4P_{36} (PoC x BS)	2.10	1.39	1.75	1.67	2.92	3.28	6.38	7.71	0.91	1.37	32.78	43.76	3.29	4.10	166.72
R_1P_{19} (PoC x BS)	2.19	1.33	1.77	1.64	3.25	3.70	6.55	7.62	1.08	1.47	39.36	42.47	3.31	3.90	164.12
R_1P_{21} (PoC x BS)	2.13	1.43	1.74	1.65	3.31	3.87	6.52	7.67	1.09	1.39	39.21	45.84	3.37	3.96	156.00
R_4P_{46} (PoC x BS)	2.16	1.46	1.76	1.65	3.27	3.83	6.81	7.89	1.07	1.50	35.14	43.44	3.35	4.10	170.67
R_1P_{52} (PoC x Per)	2.13	1.36	1.73	1.63	3.28	3.88	6.28	7.40	1.04	1.44	37.25	47.77	3.41	3.87	162.68
R_2P_9 (PoC x Per)	2.18	1.43	1.74	1.64	3.59	4.21	6.72	7.57	1.34	1.62	39.64	47.21	3.44	4.02	164.68
R_4P_{37} (PoC x Per)	2.13	1.44	1.73	1.65	2.83	3.29	6.64	7.79	0.96	1.50	25.81	36.87	3.26	3.90	171.32
H-75-32 (BA x Per)	2.19	1.33	1.74	1.65	2.75	3.11	5.89	7.06	0.94	1.58	23.55	38.74	3.27	3.88	178.50
R_1P_{40} (PU x Per)	2.12	1.33	1.76	1.67	2.92	3.31	5.95	7.36	0.89	1.38	30.46	41.89	3.30	3.87	188.00
R_1P_{43} (Per x PU)	2.17	1.39	1.78	1.64	2.72	3.10	6.09	7.52	0.89	1.41	31.66	40.15	3.25	4.04	179.19
R_3P_{26} (PU x BS)	2.15	1.50	1.74	1.65	3.52	4.02	6.26	7.40	1.36	1.69	40.78	47.40	3.38	3.87	169.67
R_3P_{32} (PU x PN)	2.24	1.38	1.76	1.65	3.62	4.09	6.02	7.19	1.39	1.65	44.80	49.27	3.37	3.96	168.66
76-1 (Hur X Card)	2.11	1.30	1.73	1.57	3.25	3.86	5.50	6.71	1.10	1.53	35.93	45.64	3.36	3.67	159.84
Male Hybrid (BA x Victory)	2.16	2.35	1.77	1.73	4.24	5.01	5.11	6.05	2.14	2.29	54.27	70.42	4.36	10.60	128.47
Hybrid Seedless (Hur x BE x BS)	2.21	1.46	1.71	1.64	2.87	3.29	7.16	8.62	0.92	1.43	30.97	41.42	3.26	3.86	193.00
H-70-56 (Hur x BS)	2.19	1.44	1.75	1.65	2.49	2.77	7.27	8.43	0.68	1.42	20.76	38.27	3.17	4.18	188.17
R2P4 (PoC x BE)	2.16	1.46	1.75	1.65	3.61	4.08	6.56	7.48	1.33	1.69	39.39	45.53	3.49	4.24	155.66
V. parviflora	2.29	2.63	1.73	1.57	4.38	5.31	4.43	5.32	2.17	2.33	61.57	81.73	4.38	11.23	116.67
CD at 5%	0.03	0.03	0.05	0.05	0.05	0.06	0.09	0.09	0.07	0.07	5.15	3.57	0.06	0.15	12.15

TABLE 3. Correlation among Disease severity index (DSI), stomatal count and biochemical parameters

		1	2	3	4	5	6	7	8	9
1	Disease Severity Index	**1.00**	**0.828****	**-0.631****	**0.111**	**-0.879****	**0.679****	**-0.744****	**-0.792****	**-0.884****
2	Stomata Count		1.00	-0.726**	0.042	-0.844**	0.690**	-0.807**	-0.814**	-0.761**
3	Total chlorophyll			1.00	0.065	0.707**	-0.524**	0.874**	0.881**	0.958**
4	Total carotenoids				1.00	-0.111	0.380*	0.073	-0.067	0.095
5	Total phenol					1.00	-0.702**	0.804**	0.901**	0.704**
6	Total sugar						1.00	-0.634**	-0.661**	-0.567**
7	Peroxidase (POD)							1.00	0.873**	0.905**
8	Polyphenol oxidase (PPO)								1.00	0.895**
9	Phenylalanine ammonia lyase (PAL)									1.00

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)

The correlation studies also revealed that total sugars and stomata count were positively correlated ($r = 0.679^{**}$, $r = 0.828^{**}$) with DSI. Dhanumjayarao *et al*. (2006) also reported that total sugars had positive correlation with disease severity (r = 0.743). Divya *et al*. (2014) observed higher positive correlation ($r = 0.856^{**}$) between stomata count and rust severity in French bean. Total chlorophylls ($r = -0.579^{**}$) showed moderate negetive correlation with DSI. The study revealed that PAL, total phenol content and stomata count showed higher correlation with disease severity. Therefore these parameters could be use as markers for identifying genotypes with higher anthracnose tolerance in grape.

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