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Field reaction and metabolic alterations in grape (*Vitis vinifera* L.) varieties infested with anthracnose

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ABSTRACT

The present study was conducted to evaluate the biochemical response of five selected grape varieties (H 516, Pusa Navrang, Flame Seedless, Beauty Seedless and Perlette) to anthracnose incidence under field conditions. The selected grape varieties were screened for their reaction during the peak disease incidence according to a standard disease scoring technique (0–5) to estimate percent disease index (PDI). Results showed that H 516 was resistant (PDI = 0.66), Pusa Navrang (PDI = 27.66) and Flame Seedless (PDI = 36.33) rated as moderately resistant varieties, whereas varieties Beauty Seedless (PDI = 61.66) and Perlette (PDI = 73.00) are rated as susceptible to the disease. The level of different biochemical constituents were examined in healthy and anthracnose infected leaves for their possible association with disease resistance. The infected leaves of resistant variety (H 516) showed higher levels of total soluble sugars (8.04 mg/g DW), total soluble proteins (1.05 mg/g DW), free amino acids (0.107 mg/g DW), total phenols (5.64 mg/g DW), proline (52.01 μg/g FW), α-tocopherol (2.39 mg/g DW, peroxidase activity (3.51 ΔA/min/g FW) and polyphenol oxidase activity (0.0224 ΔA/min/g FW) from their healthy counterparts and these were significantly different from the other evaluated varieties. MDA content was significantly higher in the most susceptible variety Perlette. A decrease in the content of chlorophyll, carotenoids and ascorbic acid was recorded in infected leaves as compared to their healthy counterparts. Highest Pearson correlation coefficient was reported between PDI and chlorophyll b ($r = -0.886^{**}$) followed by phenols ($r = -0.846^{**}$).

1. Introduction

Grape (*Vitis vinifera* L.) is an important fruit crop cultivated all over the world. It is amongst the oldest existing flora on the mother earth (Shanmugavelu, 2003). Its global importance is attributed to its multiple uses viz., wine production, table purpose, raisin making etc. (Ghosh et al., 2008). Grape is a main source for vitamin A, C, B_6, B_9 in addition to potassium, calcium, iron, phosphorus, magnesium and selenium. The antioxidants such as flavonoids and resveratrol reduce damage caused by free radicals and help to suspend the process of senescence. India is among the world's top ten countries in grape production. The area under grape cultivation in India is 136,000 ha with production of 2,683,000 tonnes (Anonymous, 2016). In Northern India, grape is cultivated in states like Punjab, Haryana, Uttar Pradesh and Himachal Pradesh. In Punjab, the area under cultivation is 297 ha with a production of 8493 metric tonnes (Anonymous, 2016). The grape production is adversely affected by biotic agents, among these anthracnose or 'Bird's eye spot' caused by fungal pathogen *Elsinoe ampelina* (de Bary) Shear poses serious threat under different climatic zones (Suhag and Grover, 1972). Anthracnose of grapes causes severe damage to viticulture industry of Punjab (Chandermohan et al., 2002). The disease attacks all the aerial parts of the vine, such as berries, leaves, tendrils and petioles. The symptoms that appear on the leaves as numerous circular spots coalesce and appear as lesions around the edges. They become sunken and reddish-black. The necrotic regions or centers drop out and produce shot-hole type appearance (Michael and Erincik, 2008). Grape anthracnose reduces the quality and quantity of the berry production. It causes the death of grapes and economic losses (Singhrot et al., 1982). Evidence suggests that using fungicides can reduce damages (Thind et al., 1997), but their frequent use may be hazardous for human population and the environment (Poolsawat et al., 2012). Thus, the attention is now on use of resistant cultivars. Therefore, to reduce the damage caused by this disease, the selection of resistant genetic resources to anthracnose requires more time. When the pathogen attacks the host, the plant shows different mechanisms under this biotic stress such as, accumulation of secondary metabolites,

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particularly phenols and defense proteins. Biochemical indices such as polyphenol oxidase, peroxidase activities, phenols and sugars were reported to be associated with anthracnose resistance (Gurjar et al., 2015). Therefore the present study was conducted to evaluate five grape cultivars; Perlette, Flame Seedless, Pusa Navrang, Punjab MACS Purple and Beauty Seedless as resistant, tolerant or susceptible to anthracnose caused by *Elsinoe ampelina* (de Bary) Shear; and to identify biochemical indices conferring resistance to this malady.

2. Material and methods

The present investigation was carried out in the Fruit Research Farm of Department of Fruit Science and in the laboratories of Department of Botany, Punjab Agricultural University, Ludhiana during the year 2016–17. Vines from each of the five grape varieties, viz; Flame Seedless, Perlette, Pusa Navrang, H 516 and Beauty Seedless grown in the Fruit Research farm, Department of Fruit Science, PAU Ludhiana were selected.

2.1. Percent disease index

The disease symptoms were noted and the selected grape varieties were screened for resistance and susceptibility against anthracnose on the basis of disease incidence. Three vines of each variety were selected for the evaluation of disease incidence on leaves. Data was recorded in 10 units per vine. The degree of severity was measured on the basis of visual observations using 0–5 scale based on the percent area of leaf affected (0, 1–10, 11–25, 26–50, 51–75 and above 75%). The percent disease index (PDI) was calculated as per the formula given by Sridhar and Sohi (1970).

$$\text{PDI} = \frac{\text{Sum of all numerical values}}{\text{Total number of leaves x Maximum disease scale}} \times 100$$

Resistance level of each variety was rated on the basis of PDI, i.e. the cultivar with the highest PDI was considered as an extremely susceptible variety and in opposite, the cultivar with the lowest PDI were identified as a resistant variety. Disease resistance levels of different varieties were classified according to the Table 1.

2.2. Biochemical estimation

Different biochemical parameters such as chlorophyll a, chlorophyll b, total chlorophyll, total soluble sugars, total soluble proteins, free amino acids, non-enzymatic antioxidants (total phenols, proline, MDA content, Ascorbic acid, α-tocopherol and carotenoids) and enzymatic antioxidants (peroxidase and polyphenol oxidase) were analyzed in infected and non-infected leaves of the selected five varieties. Chlorophyll a, chlorophyll b, total chlorophyll content in leaves was estimated as per the method given by Hiscox and Israelstam (1979). The carotenoid content was determined according to the method described by Kirk and Allen (1965). Quantity of total soluble sugars was estimated by method of Dubois et al. (1956). Total soluble proteins were estimated by the method given by Lowry et al. (1951). Free amino acid content was determined according to the method described by Lee and Takahashi (1966). The total phenolic content was determined according to the method of Swain and Hills (1959). Ascorbic acid content (Vitamin C) and proline determination was done according to the methods of Roe and Oesterling (1943) and Bates et al. (1973) respectively. Lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA) using Thiobarbituric acid (TBA) method described by Heath and Packer (1968). α-Tocopherol content was determined according to Asthir et al. (2009).

Peroxidase (PO) (EC 1.11.1.7) and Polyphenol oxidase (PPO) (EC 1.10.3.2) activity was assayed by the method of Thomas et al. (1981) and Zauberman et al. (1991) respectively.

Table 1
Disease resistance levels.

Percent Disease Index (PDI)	Reaction
0	Immune
0.1-25	Resistant
26-50	Moderately Resistant
51-75	Susceptible
76-100	Extremely Susceptible

2.3. Agrometeorological data

The data of agrometrological parameters such as maximum and minimum temperature, relative humidity, rainfall, evaporation and sunshine hours for the year 2016–17 was attained from School of Agricultural Meteorology, PAU (Appendix A).

2.4. Statistical analysis

The data percent disease index, physiological and biochemical traits were analyzed statistically using Tukey's HSD test. Differences were considered statistically significant at the levels ($p < 0.05$) using statistical analysis software SAS (Version 9.3 for Windows).

3. Results

3.1. Percent disease index

In the present study, screening of grape varieties based on field reaction to anthracnose was taken up in the year 2016–17. The average temperature and relative humidity was recorded respectively 30.4 °C and 85% during the onset of anthracnose infection (Appendix A). As shown in Table 2, among the five varieties, H 516 was recorded as resistant (PDI = 0.66), Pusa Navrang (PDI = 27.66) and Flame Seedless (PDI = 36.33) were rated as moderately resistant to disease. Beauty Seedless (PDI = 61.66) and Perlette (PDI = 73.66) were found susceptible to the disease.

3.2. Biochemical analysis

Analysis of biochemical and enzymatic activity of five selected varieties showed some interesting results.

3.2.1. Chlorophylls

There was a reduction in contents of chlorophyll a, chlorophyll b and total chlorophyll in infected leaves compared with non-infected leaves in all varieties (Table 3). The maximum chlorophyll a (1.36 mg/g FW), chlorophyll b (1.69 mg/g FW) and total chlorophyll (3.04 mg/g FW) has been observed in the non-infected leaves of the resistant grape variety i.e. H 516. The minimum chlorophyll a (0.84 mg/g FW), chlorophyll b (0.86 mg/g FW) and total chlorophyll (1.68 mg/g FW) has been recorded in the non-infected Perlette leaves which scored maximum PDI. Similar linear relation in the chlorophyll a, chlorophyll b and total chlorophyll has been observed for the infected leaves of the

Table 2
Screening of grape varieties against anthracnose (Percent Disease Index in leaves).

VARIETY	Percent Disease Index (PDI)	DISEASE REACTION
H 516	0.66	Resistant
Pusa Navrang	27.66	Moderately Resistant
Flame seedless	36.33	Moderately Resistant
Beauty seedless	61.66	Susceptible
Perlette	73.00	Susceptible
Mean	39.86	–
LSD(P < 0.05) = 7.512		