Biochemical basis of anthracnose [*Elsinoe ampelina* de Bary) Shear] Tolerance in Grape

अंगूर में ऐंथ्रेक्नोज़ [एल्सिनोय एम्पेलीना (डी बैरी) शीयर] का जैव–रासायनिक आधार

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Biochemical Basis of Anthracnose [*Elsinoe ampelina* de Bary) Shear] Tolerance in Grape

By

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This is to certify that the thesis entitled **"Biochemical basis of anthracnose [*Elsinoe ampelina* de Bary) Shear] Tolerance in Grape"** submitted to the Faculty of Post Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfilment of requirements of degree of **Master of Science in Horticulture,** embodies the results of a *bona fide* research work carried out by **Ms. Suman Beniwal, Roll No. 20179** under my guidance and supervision. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that any help or source of information, as has been availed for this work, has been duly acknowledged.

Date: 29.06.2013 (S.K. Singh)

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Dedicated to My Parents and Teachers

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ACRONYMS

PPO	Polyphenol oxidase
POD	Peroxidase
PAL	Phenylalanine ammonia lyase
MDA	Malondialdehyde
mg	milligram
g	gram
FW	Fresh weight
µg	Microgram
CV	Coeffecient of varience
DSI	Disease severity index
^{O}C	Degree Celsius
RH	Relative Humidity
ANOVA	Analysis of Variance
DI	Degree of Freedom
CD	Critical difference
SD	Standard deviation
h/hrs	hour/hours
min	Minute(s)
ml	Millilitres

CHAPTER 1 Introduction

Grape (*Vitis* sp.) is among the oldest plants (90-95 million years) on the earth, existing almost at the time when dinosaurs flourished as evidenced by the recent discoveries from western Kazakhstan (Shanmugavelu, 2003). It is believed that grape cultivation originated near Caspian Sea in Russia that spread westward to Europe and America and eastward towards Iran and Afghanistan. In India, grapes were introduced into the northern parts from Iran and Afghanistan by Muslim invaders in 12th century. Later it spread to south India in 1338. Grape is an important fruit crop grown all over the world. Grape cultivation in India has been commercially taken up under a wide range of soil and climatic conditions. While there are three distinct regions, *viz*., temperate (Jammu & Kasmir and Himachal Pradesh), sub-tropical (Punjab, Delhi, Haryana, Rajasthan, western Uttar Pradesh and Mizoram) and tropical (Maharashtra, Karnataka, Tamilnadu and Andhra Pradesh), nearly 85% of the cultivated area falls in the tropical region. At present the total area under grapes in India is estimated to be 1,11,000 hectare with an annual production of 2.22 million tonnes, which is 1.6% of total fruit production. Among the different states, Maharashtra stands first occupying the largest area 86 thousand hectare with production of 1.80 million tonnes (NHB database, 2011).

Its production and productivity is greatly affected by occurrence of several diseases, *viz*., powdery mildew, anthracnose, downy mildew, *Cercospora* leaf spot etc. Of these diseases, anthracnose of grape commonly referred as 'bird's eye spot' is an important disease of after downy mildew and powdery mildew under sub-tropical regions. The anthracnose of grape is caused by an ascomycete, *Elsinoe amplina* (de Bary) Shear. de Bary (1874) described the pathogen as *Sphaceloma ampelinum*, the imperfect stage and this anamorphic name was used for long time. In 1929, Shear named the fungus *Elsinoe ampelina* (de Bary) Shear with *Gloeosporium ampelophagum* (Pass.) Sacc, as the anamorphic state of the pathogen. The disease was first referred by Pliny in the Italy in the 1st Century of the Christian era and later reported by Burrill during 1886 from USA. In India, the disease was first recorded in 1903 near Pune (Butler, 1905). The disease is widely prevalent in Maharashtra, Karnataka, Punjab, Haryana, Andhra Pradesh, Uttar Pradesh and Tamil Nadu. The annual losses due to anthracnose are estimated to be 15-30%. The disease affects all the aerial parts in the green stage; mostly on the new shoots and fruits. On the leaves, small circular to irregular, 1-5 mm in dia., dark brown spots appear, which later turn

grey in centre and dark brown at the margin. The central necrotic tissue often falls off leaving a shot hole appearance. In severe cases, complete drying of leaves is also noticed. On shoots and tendrils, small isolated dark brown spots develop which elongate to form elliptical, slightly sunken lesion. Later the central area of the lesion develops into ashy grey colour bordered by darker rim. The affected shoot may be restricted in growth as well as shedding of flower bud takes place due to infection. On berry typical bird's eye spot symptom appear having violet to greyish centre and dark brown margins. Majority of our established cultivars are highly susceptible to anthracnose. The losses due to diseases are high and for their management may require sprays of several fungicides, which account for 30% of the cost of production. In general, the disease is controlled by fungicides but in fruit crop it is somewhat difficult and hazardous for human health, if residues are beyond permitted levels, make it difficult to export for European countries. Moreover, their application needs special attention. Hence, use of resistant varieties in cultivation and breeding can be the safest and eco-friendly measure to tackle the problem. Thus, the available germplasm must be thoroughly studied, screened and compared as sources of resistance. The analysis of biochemical parameters and screening of germplasm would be useful to grape breeders. Hence, keeping the above points in view, the present investigation was undertaken with the following objectives;

Objectives:

- Field screening of grape genotypes for reaction to anthracnose incidence.
- To find out biochemical basis of tolerance to anthracnose in grape genotypes.

The proposed research papers to be published are as follows**:**

1. Comparative field reaction of grape (*Vitis* sp.) genotypes to anthracnose [*Elsinoe ampelina* (de Bary) Shear] under sub-tropical conditions.
2. Biochemical analysis of *Vitis* sp. germplasm for resistance to anthracnose [*Elsinoe ampelina* (de Bary) Shear].

CHAPTER 2 Background

Viticulture in general encounters a major threat from three important diseases throughout the world irrespective of variable cultivation pattern and climate. Anthracnose or 'bird's eye spot' of grapes (*Vitis* sp.) caused by *Elsinoe ampelina* (de Bary) Shear, is an economically important disease of grape in the warm and wet tropical and sub-tropical areas of India after downy mildew and powdery mildew. Although worldwide anthracnose is reported to be caused by *Elsinoe ampelina* (de Bary) Shear (anamorph: *Sphaceloma ampelinum* de Bary) (Mirica, 1998), in India, *Colletotrichum gloeosporioides* and *C. acutatum* have also been reported as causal pathogens (Kumar *et al.*, 1994; Chowdappa *et al.*, 2009). This fungus can infect the grapevine at any stage of its life cycle, from seedling to fruit development and reduces the quality and quantity of fruit and weakens the vine. Deshmukh (2006) reported 15-30 percent annual losses in Maharashtra. It is, therefore, a serious threat to grape vineyards. Fungicides have been extensively used to control anthracnose of grape. Bakshi *et al*. (1970) 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. Spray of chemicals cause environmental pollution and leave residues in the soil and produce. Currently, there is a major thrust on residue-free organic grape production. Hence, the use of resistant varieties is the most effective and reliable measure for the control of grape anthracnose. In the following paragraphs, an attempt has been made to review the available literature related to the present investigation and related aspects.

2.1 Occurrence and Distribution

The disease was first reported by Pliny in the Italy in the 1st Century of Christian era and later reported by Burrill during 1886 from USA. This disease was present for centuries in Europe, which was introduced into the United States through cuttings or young vines. It caused 80-100% loss in America during 1950-1951. As high as 60 % losses has also been reported from Russia (Winkler, 1965).

In India, the disease has become a potential threat to grape cultivation and was first recorded in 1903 near Pune (Butlar, 1905). Bedi *et al*. (1969) reported anthracnose from all the grape growing areas of India. In Haryana, 10-15% losses

have been reported. During 1975 the incidence of grape anthracnose was found to the extent of 50% at Parbhani, Maharashtra. It has been also reported from Mysore (Karnataka), Uttar Pradesh and Tamilnadu (Gurme and Kore, 1977). Singhrot *et al*. (1982), while comparing the shift in productivity/ growth characters between diseased and healthy vines recorded drastic reduction in number of leaves, leaf area, and per cent bud sprouting in the affected vines. In north India, it appears every year and is most damaging and widely prevalent and reduces the quality and quantity after pre-monsoon showers. In south India, the disease prevails from March to October with peak damage during May to July. In Karnataka, the drastic reduction in productivity of grape cultivars has been observed every year (Jamadar and Lingaraju, 2011).

2.2 Symptoms

All succulent parts of the plant, including, stems, leaves, petioles, tendrils, young shoots, and berries, can be attacked. Symptoms on young, succulent shoots first appear as numerous small, circular, and reddish spots. Spots then enlarge, become sunken, and produce lesions with gray center and round or angular edges. Dark reddish-brown to violet-black margins eventually surround the lesions. Lesions may coalesce, causing a blighting or killing of the shoot. Anthracnose on petioles appears similar to that on the shoots. Leaf spot become circular with gray centers and brown to black margins with round or angular edges. The necrotic centre of the lesion often drops out, creating a shot-hole appearance (Michael and Erincik, 2008).

Fruit clusters are susceptible to infection any time prior to flowering through veraison. Initially, small, reddish circular spots develop on infected fruit. These spots enlarge to an average diameter of ¼ inch and may become slightly sunken. The centres of the spots turn whitish-grey and become surrounded by narrow reddish-brown to black margins. This distinguishing symptom often resembles a bird's eye, thus the alternative name for the disease (Hartman and Kaiser, 2008).

2.3 Background of Research Area-1

2.3.1 Field screening of grape genotypes

Different genotypes and different plant parts of grapevine behave distinctly with respect to susceptibility for anthracnose as reported by many workers.

Singh and Joshi (1971) assessed 14 grape cultivars for resistance to *Gloeosporium ampelophagum*. Cultivars namely, Delight, Gold, Early Muscat and Khalili were most resistant and Chohan Special was the most severly affected, followed by Anab-E-Shahi. Goyal *et al*. (1971) studied incidence of anthracnose on different varieties of grape at Jobner, Rajasthan. Varieties Bharat Early and Hussaini remained free from infection and White Muscat was slightly affected. Bedi *et al.* (1986) studied field reaction of different grapevine cultivars to anthracnose caused by *Sphaceloma ampelinum.* Thirty five cultivars were assessed for percentage of leaf infection, average foliar necrosis; shoot die-back, disease index, and varietal reaction. The cv. Himrod was found highly resistant and eight cultivars resistant to *Sphaceloma ampelinum* [*Elsinoë ampelina*]. The cv. Mukhchalani was highly susceptible, whereas the remaining cultivars were moderately resistant. Patil *et al*. (1990) investigated incidence of anthracnose in 70 *V. vinifera* cultivars, 21 *V. labrusca* cultivars, 3 *V. champimi* cultivars, 1 cultivar each of *V. rotundifolia* and *V. rupestris*, 4 hybrid rootstocks, 14 other wild species of *Vitis* and 66 wild relatives of this genus. The most promising cultivars for inclusion in breeding programmes for anthracnose resistance were *V. vinifera* cv. Phakari, *V. labrusca* cv. Bangalore Blue and Khalili and *V. rotundifolia* cv. James. Of the species from related genera only *Cissus glauca* and *C. repanda* were susceptible; the rest were resistant. Prasad *et al*. (1992) evaluated grape hybrids and found Bangalore Blue and its derivative E21/28 as highly resistance against anthracnose. Shelke *et al*. (1997) screened five varieties, *viz*., Kishmish Charni, Thompson Seedless, Tas-E-Ganesh, Sonaka and Manik Chaman against anthracnose. All the genotypes were found moderately susceptible. Thind *et al.* (1997) evaluated grape cultivars Beauty Seedless, Cardinal, Delight, Himrod, Perlette and Thompson Seedless. Of which Perlette and Beauty Seedless were found most susceptible. Jindal and Shankar (2002) screened 48 genotypes against anthracnose and categorized into resistant, moderately resistant and susceptible. Out of these genotypes H-144 (Cheema Sahebi x Catawba) (per cent disease index of 4.23), *Vitis parviflora* (7.49) and its hybrids were resistant, while Banqui-Abyad x Beauty Seedless 74-10 (75.81) was the most susceptible. There was high correlation of disease index on leaf and canes (0.974) showing that the same set

of genes control disease resistance which express either on leaves or on canes. Yuan *et al*. (2006) evaluated resistance to anthracnose disease in grape germplasm, European grapes, American grapes, and *Vitis* hybrids were tested and they found that 'Black Eye', 'Mario', 'Niunai', 'Rizamat', and 'Rosario Bianco' were sensitive, while 'Campbell Early', 'Niagara', and 'Honey Red' were tolerant to anthracnose. Prasath and Ponnuswami (2008) screened 17 chilli genotype (seven germplasm and 10 hybrids) for anthracnose resistance. Hybrids No. 1, Hyb 2, Hyb 3, Hyb 4 and Hyb 5 were rated as moderately resistance against the disease. More recently, Li and Wan (2008) evaluated 56 accession and 13 wild Chinese *Vitis* sp. for anthracnose and white rot of grape. All the accessions and species show resistance for anthracnose. Two *Vitis vinifera* varieties Caberanet Sauvignon and 'Chardonnay were susceptible to anthracnose and highly susceptible to white rot. Yanminal *et al*. (2010) investigated resistance of five wine grape cultivars to ripe rot caused by *Colletotrichum* sp. by artificial inoculation in laboratory. The results showed that the cultivars showed different resistance abilities and from field investigation and laboratory studies was similar. Merlot and Carbernet Gernischet had the strongest resistance, Cabernet Sauvignon showed strong resistance, while Chardonnay and Syrah were poorly resistant to the disease. Poolsawat *et al*. (2012) carried out field evaluation of grape genotypes for resistance against anthracnose. Resistance evaluations classified 'Wilcox 321', 'NY88.0507.01', 'NY65.0550.04' and 'Illinois 547-1' as resistant lines useful as parents for future breeding programmes. Moreover, one F_1 hybrid 'SUT0404.40' was also found to be resistant.

2.3.2 Environment in Relation to Disease Development

Anthracnose incidence has been reported to be greatly influenced by the epidemiological factors such as rainfall, relative humidity and temperature. Singh and Joshi (1971) related the disease to pre-monsoon showers, which not only increased the relative humidity but also reduced temperature. Rao and Satyanarayana (1991) carried out step down regression analysis with meteorological parameters and disease, which indicated that rainfall (49.9 mm/week) distributed over 3.16 days/wk with 39 h sunshine / week (cloudy weather) favouring anthracnose build up and severity on Anab-E-Shahi grape. Mohan Chander and Thind (1995) made observations that rainfall seemed to influence the disease epidemiology significantly.

The disease was generally more severe during July-August when the rains were abundant. Positive correlation between rainfall and disease was observed. Anthracnose appears to be largely affected by humid conditions and free moisture in the form of dew or rain are necessary for infection and rainy conditions lead to their epidemic build up (Thind and Arora, 2004). Pampanagouda and Benagi (2005) observed that anthracnose fungus grows well at a maximum temperature of 30-34^{0}C, when the minimum temperature was almost constant, maximum and minimum relative humidity were in increasing trend and rains were received throughout the period.

2.4 Background of Research Area-2

2.4.1 Biochemical basis of tolerance to anthracnose

Sugar content in the leaves is one of the most important parameter to categorize the variety to a particular reaction, *viz*., resistant or susceptible. Mohanraj *et al*. (1972) studied possible role of sugars in the anthracnose disease resistance mechanism in grapevine and found that young leaves of Anab-e-Shahi, which were susceptible to *Gloeosporium ampelophagum*, contained a higher percentage of sugars than resistant old leaves of Anab-E-Shahi or resistant young or old leaves of anthracnose. The anthracnose of grapevine has been reported to be high sugar disease (Mohanraj *et al*., 1972). Bindra and Kapoor (1979) found higher sugar content in diseased than healthy leaves. Shankar and Jindal (2001) reported that total sugars were higher in diseased leaves (7.10 mg/g) than in healthy leaves. The susceptible genotype contain highest high amount of total sugars and reducing sugars than resistant varieties. Beauty Seedless, which was the most susceptible genotype, had the highest sugar content. Prakash and Khirbhat (2011) reported lower content of sugar in fruit rot (*Colletotrichum capsici*) resistant chilli varieties compared to susceptible varieties.

Saharan *et al*. (2000) studied changes in phenolics compounds and oxidative enzymes in healthy and alternaria blight infected leaves of cluster bean. Total phenols increased in all the varieties with the advancement of crop age, while ortho-dihydro phenols decreased. Shankar and Jindal (2001) recorded the highest total phenol contents in resistant genotype (H-144) and least in the susceptible genotype

74-10. Ruelas *et al*. (2006) reported that phenolic acids, chlorogenic, vanillic and caffeic acid in tomato fruit are phytoanticipins and during a pathogenic attack, tomato fruit respond by increasing the concentration of vanillic acid in the epicarp. Prasath and Ponnuswami (2008) carried out biochemical analysis of chilli genotypes against *Colletotrichum annum* and found highest phenol contents in resistant genotypes (Acc. 16 (PCB 81) followed by moderately resistant and the least in the susceptible genotypes (Hybrid 6). Kulkarni (2009) reported that healthy leaves of resistant and moderately resistant genotype contained higher amount of total phenols than susceptible one. Kaur *et al.* (2011) analyzed physiological and biochemical traits of *Capsicum annum* L. germplasm for resistance to *Colletotrichum annum*. High content of total phenols was observed in resistant genotypes such as Jaun, Breek-1 and Breek-2. Increase in total phenols upon infection in resistant genotypes than that in moderately resistant and susceptible genotypes indicates the influence of total phenols in disease resistance.

Enzymes are known to play decisive role in host pathogen interaction. Certain oxidative enzymes (polyphenol oxidase, peroxidase and phenylalanine lyase) of host pathogen interaction defend the host from being diseased (Agrios, 1978). In the presence of oxygen, polyphenol oxidase oxidizes the phenolic compounds that are in the form of O-diphenol to O-quinone. Marutyan *et al*. (1979) studied metabolic changes in grapevine leaves during mildew infection and reported that the activity of phenol oxidase enzyme is generally higher in infected tissues of resistant varieties than in the infected tissue of susceptible genotype. Shankar and Jindal (2001) suggested highest peroxidase activity in grape genotype MA x RR 76-3, whereas polyphenol oxidase activity was highest in H-144 both of which fall under resistant category. The lowest enzyme activity was observed in the most susceptible genotype. Furthermore, the enzyme activity in diseased leaves was higher than that in corresponding healthy leaves. Campos *et al.* (2004) observed positive correlation among peroxidase and polyphenol oxidase activities and anthracnose resistance in bean. Higher impulses in enzymatic activity were observed in cultivars with higher disease resistance. According to Kavitha and Umesha (2008) in bacterial wilt resistant tomato cultivars, level of polyphenol oxidase and peroxidase enzymes were increased in comparison with highly susceptible tomato cultivars. Singh *et al.* (2011) investigated defensive enzyme activity of *Brassica juncea* genotypes during

pathogenesis of *Alternaria*. They observed higher PPO activity in infected leaves as comparison to healthy one and the resistant genotypes expressed more PPO activity than the susceptible one. Zhou *et al.* (2012) studied correlation between resistance of eggplant and defence related enzyme, and detected that the activities of PPO and PAL were significantly positively correlated with resistance. Resistance was significantly positively correlated with activity of POD but significantly negatively correlated with the content of malondialdehyde (MDA). Thus, it was concluded that the activity of the enzymes is directly related to resistance in the host.

Leaf pigment composition is sensitive to plant stress, with a range of abiotic and biotic factors responsible for either loss of photosynthetic pigments (e.g. chlorophylls) or the production of photoprotective pigments such as zeaxanthin or ß-carotene (Demmig-Adams and Adams, 1992). Changes in leaf pigment content of grapevines has previously been examined in response to growth light, water stress, cold-temperature stress and virus infection (Bertamini *et al.*, 2004; Bertamini *et al*., 2005; Bertamini and Nedunchezhian, 2004; Chaumont *et al.*, 1997; Flexas *et al.*, 2000; Hendrickson *et al*., 2004; Maroco *et al.*, 2002; Medrano *et al*., 2002; Sampol *et al.*, 2003). Gupta *et al.* (1987) studied biochemical changes in sesame in relation to *Alternaria* leaf spot disease. They observed higher phenol and chlorophyll content and lower reducing sugars in resistant than in susceptible plants. Blanchfield *et al.* (2006) investigated response of leaf pigments to phylloxera infestation in Pinot Noir and Cabernet Sauvignon grapevines grown under field conditions. A reduction in the leaf chlorophyll content and an increase in photoprotective pigment concentrations were observed in leaves of phylloxera infested grapevines compared to uninfested vines. Kulkarni *et al*. (2009) recorded higher amount of chlorophyll ‘a’ and ‘b’ in resistant and moderately resistant genotypes than susceptible genotypes in both healthy and diseased leaves. In all the varieties, there was decrease in chlorophyll ‘a’ and chlorophyll ‘b’ in diseased leaves. Total chlorophyll also had the same trend. Lobato and Goncalves (2009) reported 15.2% decrease in chlorophyll and 30.5% decrease in total carotenoids content in susceptible cultivars of bean infected by *Colletotrichum lindemuthianum* causing anthracnose.

Accumulation of proline occurs in response to many abiotic stresses including drought, salinity and frost as well as biotic stresses such as pathogen

infection (Hare *et al.*, 1997). Goicoechea *et al.* (2000) observed modifications in the concentration of proline in foliar tissues of pepper under *Verticillium dahliae* infection. There was an increase in proline concentration in foliar tissues of infected plants while it did not change in the leaves of control plants. Sivritepe *et al.* (2009) studied level of proline accumulation in response to two spotted spider mite. Mite infestation caused an increase in proline contents by 6.7 and 4.2-fold in the leaves of Muscule and Sultana grapevines respectively. Naglaa *et al.* (2011) reported significant increase in proline content in powdery mildew infected leaves of flax lines as compared with either resistant or susceptible parents.

The studies have shown that some of the quality and biochemical factors like chlorophyll, reducing sugars, total sugars, total phenols and enzymes like polyphenol oxidase and peroxidase and malondialdehyde content, play a vital role in causing resistance and delayed susceptibility in grape germplasm against anthracnose infection. Higher phenolics appear to be responsible for rendering resistance and sugars are playing important role for rendering susceptibility as indicated by positive correlation with sugar and negative correlation with phenols (Abusaleha *et al.*, 1989).

CHAPTER 3 Materials and Methods

The present study entitled "Biochemical basis of anthracnose [Elsinoe ampelina de Bary) Shear] tolerance in grape" was carried out at laboratory and farm of Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi along with facilities available at the Divisions of Plant Pathology and Plant Physiology during 2012 and 2013.

3.1 Plant Materials

3.1.1 Grape genotypes

The experimental materials for the present study consisted of 45 grape genotypes, including table purpose, wine making varieties and rootstocks. The details of these genotypes are given below:

a. Table and juice purpose: Pusa Urvashi, Tas, Centennial Seedless, Fakri, Hybrid 70-56 (Hur x Beauty Seedless), Alumwick, Cardinal, Pearl of Csaba, Tas-E-Ganesh, Black Muscat, Julesky Muscat, Black Prince, Anab-E-Shahi, Perlette, Bharat Early, Kishmish Beli, Banqui-Abyad, Hur, Pusa Seedless, Pusa Navrang, Beauty Seedless, Hybrid (BA x BS) x BE, Hybrid 75-32 (Banqui-Abyad x Perlette), Victory, Hybrid 72-51 (Angoor Kalan x Pusa Seedless) and Hybrid 71-50 (Banqui-Abyad x Beauty Seedless).

b. Wine making: Syrah, Tempranillo, Cabernet Sauvignon, Merlot, Sauvignon Blanc, Pinot Noir, Ugni Blanc, Chenin Blanc and Chardonnay.

c. Rootstock: Ten rootstock genotypes used in the study were;

Table 3.1. Rootstock and their parentage selected for the study.

S. No.	Rootstock	Parentage/ Species
1	Dog Ridge	*V. champini*
2	H-144	Cheema Sahebi x Catawba
3	Male hybrid (74-9)	Banqui-Abyad x Victory
4	Salt Creek	*V. champini*
5	SO4	*V. berlandieri* x *V. Riparia*
6	110-R	*V. berlandieri x V. Rupestris*
7	St. George	*V. rupestris*
8	1613	(*V. riparia* x *V. vinifera*) x (*V. candicans* x *V. labrusca*)
9	3309-C	*V. riparia* x *V. Rupestris*
10	Degrasett	*V. champini*

All these 45 genotypes are maintained at the Grape Germplasm Block of the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi

3.1.2 Climate and Soil

Climate of Delhi is categorized as semi-arid, sub-tropical with hot dry summer and cold winter and it falls in the Agro-Eco-region-IV. The mean annual temperature is 25^0C. May and June are the hottest months with maximum temperature of 40 to 45^0C. December and January are the coldest months with a temperature of 7^0C however, the minimum temperature dips to as low as 1^0C. The mean annual rainfall is 710 mm of which as much as 75 per cent is received during monsoon season (June to September). Sometime occasional winter showers are also received during December-March. Furthermore, frost occurs occasionally during the period December-January. The average relative humidity varies from 34.1 to 97.9 per cent and average wind speed is 0.45 to 3.96 m per sec. The meteorological data was collected from Agromet Observatory, Division of Agricultural Physics, IARI, New Delhi and are given as Annexure I. Soils of IARI represent a typical alluvium profile of Yamuna origin. The entire farm is covered under several soil series. The soil type ranges from sandy loam to clay loam. The texture up to depth of about 150 cm appears almost uniform. As per USDA textural classification, major portion of the area belongs to sandy loam class. Porosity in general is about 40% and soil belongs to good class as far as its permeability is concerned.

3.2 Morphological observations

Following parameters were recorded for observing the morphological traits so as to segregate them in to different groups based on the occurrence of disease intensity. The different characters for which the data were recorded include:

Leaf characters: (i) Leaf length, (ii) Leaf width, (iii) Leaf area, and (iv) Leaf texture.

Vine characters: (i) Surface texture, (ii) Inter nodal length, and (iii) Vine thickness at 7^{th}-8^{th} nodes.

3.3 Disease screening and calculation of DSI

From each genotype, about 120 leaves were sampled during second fort night of September when anthracnose symptoms were fully developed. Each leaf then graded as: 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 based on the estimated percentage of lesions over the whole leaf area: 0, 0.1-5.0, 5.1-10.0, 10.1-25.0, 25.1-40.0, 40.1-55.0, 70.1-85.0 and > 85.0, respectively and evaluation of natural infection and disease severity index (DSI) was calculated as suggested by Wang *et al.* (1998).

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

Table 3.2. Resistance levels of grape genotypes based on Disease Severity Index suggested by Wang *et al.* (1998).

Disease Severity Index	Disease reaction
0.0 - 0.9	Immune
1.0 - 5.0	Extremely resistant
5.1 - 10.0	Highly resistant
10.1 - 25.0	Resistant
25.1 - 40.0	Moderately resistant
40.1 - 55.0	Moderately susceptible
55.1 - 70.0	Susceptible
70.1 - 85.0	Highly susceptible
85.1 - 100.0	Extremely susceptible

3.4 Biochemical studies

Out of 45 grape genotypes screened, 20 genotypes comprising genotypes from each sub-group, *i.e.* rootstock, wine making and table purpose categories were randomly selected for biochemical analyses (Table 3.3). The leaf tissues from the different genotypes were sampled during April-May (Disease-free conditions) and August-September (during peak occurrence of disease). The leaf sample were collected in three replications, in ice box and stored at -20^{o}C until processing for biochemical estimation.

Table 3.3. Genotypes selected for biochemical estimation.

Category	Genotype(s)
Rootstock	Male Hybrid, Hybrid-144, 3309-C, Dog Ridge, 110-R.
Wine purpose	Chardonnay, Merlot, Syrah, Tempranillo, Ugni Blanc.
Table purpose	Anab-E-Shahi, Alumwick, Cardinal, Centennial Seedless, Fakri, Perlette, Hur, Pusa Seedless, Pusa Urvashi, Hybrid 70-56.

Different biochemical parameters estimated in healthy leaves and after occurrence of disease under field conditions are given in detail hereunder;

3.4.1 Chlorophylls and carotenoids

Chlorophyll content in the leaves was measured by DMSO method (Hiscox and Israelstam, 1979). In this method, 50 mg of finely chopped leaf was taken and filled in test tubes poured with 10 ml of AR grade dimethyl sulphoxide (DMSO). The filled tubes were covered with aluminium foil and kept in hot air oven at 65^{0}C for 4 h. Subsequently, the tubes were shaken to allow the pigment to distribute uniformly and the absorbance was read at 645, 663 and 470 nm wavelengths in a

spectrometer (UV-VIS double beam PC 8 scanning auto cell spectrophotometer, UVD 3200 (Labomed Inc, USA) using pure DMSO as a blank reading. The following formulae were used for estimation of chlorophyll 'a', chlorophyll 'b', total chlorophylls and total carotenoids contents.

Chlorophyll 'a' (mg/g FW) = $(12.7 \times A_{663}) - (2.69 \times A_{645}) \times V / (1000 \times W)$

Chlorophyll 'b' (mg/g FW) = $(22.9 \times A_{645}) - (4.68 \times A_{663}) \times V / (1000 \times W)$

Total Chlorophylls (mg/g FW) = $(20.2 \times A_{645}) - (8.02 \times A_{663}) \times V / (1000 \times W)$

Total Carotenoids (mg/g FW) = $(1000 \times A_{470}) - (3.27 \times$ Chl.-a + $104 \times$ Chl.b/ 299

Where, A = Absorbance at given wave length; V = Final volume of solvent in ml, W = Weight of plant sample in grams.

3.4.2 Total phenols

The total phenols content of leaves was determined according to the method described by Malick and Singh (1980). Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml Folin-Ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. A blue colour was developed in each tube because the phenols undergo a complex redox reaction with phosphors-molibdic acid in Folin-Ciocalteau reagent in alkaline medium resulting in a blue coloured complex, molybdenum blue. The test solutions were warmed for 1 min., cooled and absorbance was measured in a spectrophotometer (UV-VIS double beam PC 8 scanning auto cell spectrophotometer, UVD 3200 (Labomed Inc, USA) at 650 nm against the reagent used as a blank. A standard calibration plot was generated at 650 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catechol equivalent of phenol per gram of sample.

Preparation of standard curve for estimation of total phenols

Twenty mg of analytical grade catechol (SRL Chem Co., Mumbai) was dissolved in small volume of double-distilled water and volume was made up to 100 ml. In five stoppered test tubes (10 ml), 0.2, 0.4, 0.6, 0.8 and 1.0 ml aliquot of catechol was taken in each and volume was made up to 3 ml with double-distilled water followed by addition 0.5 ml Folin-Ciocalteau reagent. After 3 minutes, 2 ml of 20% sodium carbonate were added and mixed vigorously. A blank was run simultaneously taking 3 ml of distilled water. All the tubes were placed in water bath (58^oC) for one minute, cooled down to room temperature and absorbance was measured at 750 nm using UV-

VIS double beam PC 8 scanning auto cell spectrophotometer, (UVD 3200, Labomed Inc, USA).

3.4.3 Total reducing sugars

Reducing sugars were estimated following the method suggested by Somogyi (1952). Hundred milligrams composite leaf sample was weighed and extracted with hot 80 per cent ethanol twice (5 ml each time). Supernatant was collected and evaporated to dryness by keeping in water both at 80^{o}C. Then, 10 ml distilled water was added to dissolve the sugars. From this, 0.2 ml aliquot was pipetted out to separate test tubes and volume was made to 2 ml with distilled water, 1 ml of alkaline copper tartarate was added followed by keeping in water both at 100^{0}C for 10 minutes. Tubes were cooled and 1 ml of arseno-molybolic acid reagent was added to all the tubes. Then, volume was made to 10 ml with distilled water and absorbance was read at 620 nm after 10 minutes. From the standard curve, amount of reducing sugars present in the sample was calculated.

Preparation of standard curve

For the preparation of standard curve, stock solution was prepared by dissolving 100 mg glucose in 100 ml distilled water. Working solution was prepared from the stock solution by diluting 10 ml of stock to 100 ml with distilled water (100 µg/ ml). For plotting a standard curve, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of the working standard solution was pipetted out into a series of test tubes. Rest of the procedure was similar to steps followed for estimation of reducing sugars in leaf samples as mentioned previously.

Calculation

$$\text{Reducing sugars (\%)} = \frac{X}{10} \times 10 \text{ mg of glucose}$$

Absorbance corresponds to 0.1 ml of test = X mg of glucose

3.4.4 Total soluble sugars

The sucrose content was measured by the anthrone reagent method (Hedge and Hofreiter, 1962). Hundred milligram leaf sample was hydrolyzed by keeping it in boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cooled to room temperature. It was neutralized with sodium carbonate; volume was made up to 100 ml and centrifuged. 0.5 and 1 ml aliquots were taken for analysis. Then 4 ml of Anthrone reagent was added and test tubes were placed in a boiling water bath for 8 minutes.

After rapid cooling, green colour intensity was measured at 630 nm. A graph was prepared by plotting concentrations of standards on X axis versus absorbance on the Y axis. From graph the amount of carbohydrate present in samples was calculated and expressed as mg/g sample.

$$\text{Amount of carbohydrate present in 100 mg of sample} = \frac{\text{mg of glucose}}{\text{Vol. of test sample (100 ml)}}$$

3.4.5 Proline

The proline content in matured leaves of each treatment was estimated by rapid colorimetric method as suggested by Bates *et al.* (1973). Leaf sample (0.5 g) was homogenized in 5 ml of 3 per cent sulpho-salicylic acid in pre-chilled mortar and pestle. Then, it was centrifuged at 10,000 rpm for 10 minutes at 4^{o}C. The supernatant was diluted to 10 ml with double-distilled water and from it 0.1 ml was taken in test tube and volume was made to 1 ml with double-distilled water. Thereafter, it was reacted with 5 ml of acid ninhydrin reagent and 5 ml of glacial acetic acid for one hour at 100^{o}C in hot water bath. Thereafter, the reaction was terminated by keeping the solution on an ice bath. Then, 4 ml toluene was added and mixed vigorously with the help of a vortex stirrer for 20-30 seconds. The chromophore containing toluene layer (light pink) was aspirated from the aqueous phase, warmed to room temperature and then absorbance was read at 520 nm on UV-VIS spectrophotometer (UV-VIS double beam PC 8 scanning auto cell spectrophotometer, UVD 3200 (Labomed Inc, USA) using pure toluene as a blank. The proline concentration in the samples was determined from a standard curve prepared by using analytical grade proline (SRL Chem. Co. Mumbai) and calculated on fresh weight basis according to the following formula.

$$\text{Proline (}\mu\text{g/ g FW)} = \frac{\text{Concentration (}\mu\text{g) x 10 x 20 x dilution factor}}{\text{1000 x weight of sample}}$$

Preparation of standard curve for proline estimation

Stock solution of proline was prepared by dissolving 25 mg of analytical grade proline in small amount of double-distilled water and making the volume to 100 ml for preparation of standard curve. Working solutions were prepared by serially diluting the standard proline solution with water to get 0-25 µg of proline per ml. Simultaneously, a tube containing 1 ml distilled water was prepared to serve as blank. Five ml of acid ninhydrin reagent and 5 ml of glacial acetic acid was added and mixed well. The tubes were then kept at 100^{o}C in hot water bath for one hour. Thereafter, the

reaction was terminated by keeping the solution on ice bath. Then, 4 ml toluene was added and mixed vigorously with the help of a vortex for 20-30 seconds. The chromophore containing toluene layer was aspirated from the aqueous phase, warmed to room temperature and then absorbance was read at 520 nm on UV-VIS spectrophotometer (UVD 3200, Labomed Inc, USA) using pure toluene as a blank. The readings were so obtained.

3.4.6 Extraction of enzyme

One gram of leaf sample was taken in a pre-chilled mortar and homogenized thoroughly with 10 ml of ice-cold extraction medium which consisted of phosphate buffer (pH 6.8; 0.2 M), PVP (15 mg/g) and Triton X-100 (5 µg/g). Extracted homogenate was transferred to ice-cold centrifuge tubes. The homogenate was centrifuged at 11,000 rpm for 20 min. at 2^{o}C. Floating particles present in the supernatant were removed by filtering through cotton wool. The pellet was discarded. The supernatant was dialyzed against 0.2 M phosphate buffer for two days with two changes of buffer. The dialysate was used for assay.

3.4.7 Peroxidase activity (EC 1.11.1)

The activity of peroxidase in leaf sample was determined by the method proposed by Thomas *et al.* (1981). The assay utilized guiacol as the enzyme substrate. The enzyme extract was prepared by homogenizing 1 g of clean leaf sample. The reaction mixture was prepared in tubes by adding 3 ml of phosphate buffer (0.1 M; pH 7.0), 30 µl of H_2O_2 (20 mM), 50 µl of guiacol (20 mM) as enzyme substrate and 50 µl of enzyme extract. The reaction mixture was incubated in cuvette for exactly 10 minutes at room temperature. The absorbance was read at 436 nm wavelength on UV-VIS spectrophotometer (UVD 3200, Labomed Inc, USA). A decrease in absorbance was recorded at 30 second intervals till the constant reading was obtained. The peroxidase activity was expressed as number of absorbance units per gram fresh weight of leaf.

3.4.8 Polyphenol oxidase activity (EC 1.14.18.1)

Poly phenol activity was determined as per the method suggested by Matto and Diamond (1963). The reaction mixture for the assay of polyphenol oxidase (PPO) contained 1 ml of 0.05 M catechol, varying amounts of enzyme extracts and 0.2 M phosphate buffer (pH 6.8) in a final volume of 5 ml. The reaction was initiated by adding enzyme extract as the last component. The rate of increase in absorbance at 410 nm was measured against the blank every 30 seconds up to 30 minutes. The changes in

absorbance were plotted and enzyme activity was calculated from the linear part of the curve. The specific activity of the enzyme was expressed as the units per mg protein.

3.8.9 Phenylalanine ammonia lyase (EC 4.3.1.24)

Phenylalanine ammonia lyase activity was measured as described by Lisker *et al.* (1983). Leaf material was homogenized in 25 mM Tris-HCl buffer, pH 8.8 (w/v, 1:1) centrifuged at 8,000 r.p.m. for 30 min. at 4^0C. The reaction mixture contains 1 ml of enzyme extract, 0.5 ml substrate L-phenylalanine and 0.4 ml of 25 mM Tris-HCl buffer. After incubation of 2 h at 4^0C, activity was stopped by the addition of 0.06 ml 5N HCl and the absorption was read at 290 nm. The enzyme activity was expressed as μmol of trans-cinnamic acid/ mg protein/ h.

3.4.10 Malondialdehyde (MDA)

Malondialdehyde (MDA) level was assayed by thiobarbituric acid (TBA) method (Hagege *et al*., 1990). About 1.5 ml of enzyme fluid was mixed with 2.5 ml of 0.5% thiobarbituric acid (prepared with 5% trichloroacetic acid), boiled for 15 min., then icebathed to room temperature. The mixture was centrifuged for 10 min. (4000 rpm), and then the optical density of supernatant was measured at 450, 532 and 600 nm. Samples with buffer instead of crude enzyme fluid were used as blank control.

$$\text{MDA } (\mu \text{ mol/ g}) = 6.45\ (A_{532}-A_{600}) - 0.56\ A_{450}$$

Where, A is the difference in optical density between treatment and control.

3.5 Statistical analysis

The experiment was conducted in completely randomised block design with three replications. Data for all the parameters were subjected to analysis of variance (ANOVA). Correlations (r) among DSI and biochemical traits was computed by using statistical analysis system software (SAS version 2.).

CHAPTER 4 **Research Paper-I**

Comparative field reaction of grape (*Vitis* sp.) genotypes to anthracnose [*Elsinoe ampelina* (de Bary) Shear] under sub-tropical conditions

4.1 Abstract

Anthracnose is one of the detrimental diseases of grapevine in humid regions. To lessen the risk of crop damage and to reduce fungicide application, resistant cultivars are needed. In, this study, 45 grape genotypes including rootstocks, wine making and table purpose varieties were screened under field conditions against anthracnose. Disease severity index was calculated during the peak disease incidence, *i.e.* second fort night of September. Results showed that Dog Ridge, Male Hybrid and H-144 were extremely resistant (DSI = <5.0); St. George, SO4, and 110-R were highly resistant; Salt Creek and Pusa Navrang were resistant; Cabernet Sauvignon, Ugni Blanc and Centennial Seedless were susceptible; Anab-E-Shahi, Fakri, Kishmish Beli and Cardinal were highly susceptible, while Hybrid 70-56, Perlette and Alumwick were extremely susceptible (DSI = >85.0) to the disease. In general, genotypes belonging to rootstock group showed higher resistance (DSI = 5.88) followed by wine making genotypes (DSI = 42.1), whereas table purpose genotypes were least resistant (DSI = 57.11). Correlation studies between DSI and weather parameters indicated that relative humidity (r = 0.912) and minimum temperature (r = 0.779) were positively correlated with occurrence of anthracnose disease under sub-tropical condition, whereas maximum temperature (r = -0.560) and sunshine hours (r = -0.706) were negatively correlated.

Key words: *Elsinoe ampelina* [*Elsinoe ampelina* (de Bary) Shear], anthracnose, field reaction, *Vitis* species, resistance.

4.2 Introduction

Grape (*Vitis* sp.) is among the oldest plants (90-95 million years) on the earth, existing almost at the time when dinosaurs flourished as evidenced by the recent discoveries from western Kazakhstan (Shanmugavelu, 2003). In India, grapes were introduced into the northern parts from Iran and Afghanistan by Muslim invaders in 12^{th} century. Nearly 94% of the cultivated area falls in the tropical region (Maharashtra, Karnataka, Tamilnadu and Andhra Pradesh). At present, the total area under grapes in India is estimated to be 111 thousand hectare with an annual production of 2.22 million tones, which is 1.6% of total fruit production. Among the

different states Maharashtra leads in grape production. Over the time, different pests and diseases spread over grape vineyards and have became one of the important yield reducing factors in the Indian vineyards.

Anthracnose caused by *Elsinoe ampelina* is one of the most important and destructive disease of grape in many countries including India. It can cause serious damage to grape production in conducive environmental conditions, affect the yield quantitatively and qualitatively and increase the production cost significantly. The disease was first reported from Indian grape vineyards in 1903 near Pune (Butlar, 1905) and since then it has been reported from Maharashtra, Karnataka, Punjab, Haryana, Andhra Pradesh, Uttar Pradesh and Tamil Nadu. In general, the disease is controlled by fungicides but in fruit crop it is somewhat difficult and hazardous for human health, if residues are beyond permitted levels, makes it difficult to export to European Union countries. Moreover, their application needs special attention. Hence, development and use of resistant and tolerant varieties are one of the most important and environmentally sound strategies for management of grapevine anthracnose. Thus, the available germplasm must be thoroughly studied, screened and compared as sources of resistance.

Shelke *et al*. (1997) screened five varieties, *viz*., Kishmish Charni, Thompson Seedless, Tas-E-Ganesh, Sonaka and Manik Chaman against anthracnose and found all the genotypes to be moderately susceptible. Thind *et al.* (1997) evaluated six grape cultivars. Of which Perlette and Beauty Seedless were found to be most susceptible. Earlier, Jindal *et al*. (2002) screened 48 grape genotypes for their field reaction to anthracnose and found that *V. vinifera* (7.49) and its hybrids were highly resistant to anthracnose, whereas BA x BS 74-10 (75.81) was the most susceptible to the disease. Yuan *et al.* (2006) evaluated grape germplasm for resistance to anthracnose disease and found that ‘Black Eye’, ‘Mario’, 'Niunai', 'Rizamat', and ‘Rosario Bianco’ were sensitive, while ‘Campbell Early’, ‘Niagara’ and ‘Honey Red’ were tolerant to anthracnose. Poolsawat *et al*. (2012) carried out field evaluation of grape genotypes for resistance against anthracnose. Resistance evaluations classified ‘Wilcox 321’, ‘NY88.0507.01’, ‘NY65.0550.04’ and ‘Illinois 547-1’ as resistant lines useful as parents for future breeding programmes.

Keeping above information in view the present study was, therefore, undertaken to screen the grape germplasm against anthracnose incidence and identify

anthracnose resistant grape genotypes among germplasm available at IARI, New Delhi.

4.3 Materials and 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.

4.3.1 Plant material

Forty five grape genotypes including rootstocks, wine making and table purpose varieties were selected for this study (Table 4.1). For screening of germplasm, evaluation of disease incidence was carried out on leaves and canes.

Table 4.1 Grape genotypes taken for the study.

Category	Genotype(s)
Rootstock	Dog Ridge, Degrasett, Male Hybrid, Salt Creek, St. George, H-144, SO4, 3309, 110-R, 1613.
Wine purpose	Chardonnay, Cabernet Sauvignon, Chenin Blanc, Merlot, Pinot Noir, Syrah, Sauvignon Blanc, Tempranillo, Ugni Blanc.
Table purpose	Anab-E-Shahi, Alumwick, Bharat Early, Beauty Seedless, Banqui-Abyad, Black Muscat, Black Prince, Cardinal, Centennial Seedless, Fakri, Hur, Hybrid (Hur x BE) x BS, Julesky Muscat, Kishmish Beli, Pusa Urvashi, Pearl of Csaba, Perlette, Pusa Seedless, Pusa Navrang, Tas, Tas-E-Ganesh, Victory, Hybrid 70-56, Hybrid 75-32, Hybrid 72-151, and Hybrid 71-50.

4.3.2 Methodology for screening of grape germplasm against anthracnose under field conditions

Screening of grape germplasm against anthracnose was carried out during August - September when anthracnose symptoms widely appeared on the leaves and canes. For germplasm screening, three vines of each genotype were randomly selected and around 120 leaves were evaluated during August-September. Evaluation of natural infection and disease severity index was calculated as suggested by Wang *et al.* (1995). 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).

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

Resistance level of each genotype was arbitrarily rated in nine categories based on mean DSI of its cane and leaves (Table 4.2).

Table. 4.2. Disease rating for anthracnose infection. (Wang *et al*., 1998)

Disease Severity Index	Disease reaction
0.0 - 0.9	Immune
1.0 - 5.0	Extremely resistant
5.1 - 10.0	Highly resistant
10.1 - 25.0	Resistant
25.1 - 40.0	Moderately resistant
40.1 - 55.0	Moderately susceptible
55.1 - 70.0	Susceptible
70.1 - 85.0	Highly susceptible
85.1 - 100.0	Extremely susceptible

4.4 Results and Discussion

Forty five genotypes, *i.e.* rootstocks, wine purpose and table purpose genotypes were grouped based on the disease severity under field conditions.

4.4.1 Morphological characterization of grape Genotypes

The data pertaining to different morphological parameters, namely, leaf length, leaf width, leaf area, leaf texture, inter nodal length; vine thickness and vine surface were recorded for 45 genotypes and has been presented in Table 4.3. Leaf length data showed that majority of the genotypes had medium leaf lenght (6.6-8.5 cm) comprising of 22 genotypes followed by long (>8.5 cm) including 14 genotypes, whereas nine genotypes had short leaf length. As evident most of the rootstocks had small leaves, while wine purpose genotypes had medium sized leaves, while table purpose had either medium or long leaf length. The leaf width in rootstocks ranged from 7.67 cm in 3309-C to 13.1 cm in 1613. In wine grapes it ranged from 7.87 cm in Pinot Noir to 14.0 cm in Syrah. In table purpose genotypes, the minimum (8.05 cm) width was noted in Pearl of Csaba with maximum in Pusa Urvashi (12.9 cm). Leaf area showed highest variability and ranged from 15.98 cm^2 in Dog Ridge to 142 cm^2 in Tash-E-Ganesh. Leaf area was not correlated with disease severity index. Inter nodal lenght at 7-8th nodes were shortest in Dog Ridge (3 cm), while it was longest in Julesky Muscat (8.6 cm). Mean vine thickness ranged from 0.35 cm in 110-R to 0.97 cm in Alumwick. Both leaf and vine surface were observed and it was found that trichomes (hairs) were present in eight out of forty five genotypes, i.e., Black Prince, H-144, 110-R, Chenin Blanc, 1613, Black Muscat, Fakri and

Tempranillo. Trichomes on vine were noted only in two genotypes, *i.e.*, 110-R and Black Prince, while others were found to have smooth or uneven surface. There was no direct correlation noted between vegetative parameters and DSI. This suggested that these variables are due to genetic make-up of individual genotype. Presence of wax (glabrous) or trichomes on leaf/vine of a genotype could not be directly related with anthracnose resistance in grape but disease incidence was lower in the genotypes, where trichomes were present. This aspect need to be illustrated further and studied in detail to make a clear cut conclusion on such a interrelationship. Interestingly, two genotypes, *i.e.*, Black Prince and 110-R where trichomes were present on leaves and vines were ranked as highly resistant and extremely resistant respectively, along with H-144, Male Hybrid and 3309-C which suggest that unlike mildew, tolerance or resistance of the genotypes against anthracnose is not solely based on mechanical barrier like glabrous surface or presence of trichomes but also on different biochemical constituents in foliar tissues which may play significant role in disease severity or resistance.

4.4.2 Field screening of grape genotypes

As evident from Table 4.4a, out of the ten rootstocks, four were extremely resistant (Dog Ridge, H-144, Male Hybrid and 3309-C), five were highly resistant (Degrasett, St. George, 110-R, 1613 and SO4) and one was resistant (Salt Creek) to the anthracnose disease. Among rootstocks, H-144 showed the lowest DSI (1.64 = extremely resistant), whereas highest DSI (17.98) was recorded in Salt Creek among all rootstocks.

Of the nine wine making genotypes four genotypes, namely, Chardonnay (28.5), Chenin Blanc (36.8), Merlot (31.6) and Tempranillo (32.0) showed moderate resistance against disease. Genotypes Cabernet Sauvignon (48.7), Pinot Noir (41.30) and Syrah (44.0) were grouped as moderately susceptible, whereas Sauvignon Blanc (56.0) and Ugni Blanc (60.3) were found to be susceptible (Table 4.4b).

As evident from the Table 4.4c, the table purpose varieties showed least resistance against the disease as compared to rootstocks and wine making genotypes. Among table purpose genotypes, eight genotypes were found to be moderately susceptible (Bharat Early, Beauty Seedless, Black Muscat, Pusa Urvashi, Hur, Tas, Hybrid 71-50 and Pearl of Csaba), three were susceptible (Hybrid (Hur x BE) x BS, Centennial Seedless and Tas-E-Ganesh), nine were highly susceptible (Anab-E-Shahi, Banqui-Abyad, Cardinal, Fakri, Kishmish Beli, Pusa Seedless, Victory, Hybrid 75-32 and Hybrid 72-151), whereas, three genotypes were found to be extremely susceptible (Alumwick, Perlette and Hybrid 70-56). Among table purpose genotypes, Black Prince (5.57) followed by Pusa Navrang showed the lowest (18.8) disease severity index.

In present study field screening of some grape genotypes was conducted to know the disease reaction of different genotypes against anthracnose and to identify sources of resistance which can be involved in breeding programmes for developing resistant hybrids. Field screening of grape genotypes against anthracnose indicated that among 45 genotypes tested four were extremely resistant, six were highly resistant, two were resistant, five were moderately resistant, 11 were moderately susceptible, five showed susceptibility, nine were highly susceptible and three varieties/ hybrids were extremely susceptible. None of the genotypes studied were immune to the disease (DSI = 0.0). Resistance was in general highest in rootstocks (DSI = 5.88) followed by wine making varieties (DSI =42.1), while table purpose varieties / hybrids showed the lowest resistance among all genotypes (DSI = 57.1).

Among hybrids, Hybris-144 and Male Hybrid showed extreme resistance against the disease, Hybrid 71-50 was found as moderately susceptible, whereas Hybrid 75-32 and Hybrid 72-151 were categorized as highly susceptible to the disease. Disease reaction of different grape genotypes against anthracnose has previously been examined by various workers worldwide under different growing conditions (Singh and Bakshi, 1971; Goyal *et al*., 1971; Bedi *et al*., 1986; Shelke *et al*., 1990; Jindal and Shankar, 2002; Yuan *et al*., 2006; Li and Wang, 2008; Yanminal *et al*., 2010; Poolsawat *et al*., 2012).

Table 4.4a. Disease severity index and disease reaction of some grape rootstocks to anthracnose under sub-tropical conditions.

Genotype	Leaf DSI	Cane DSI	Mean	Disease reaction
Dog Ridge	4.28	1.96	3.12	ER
Degrasett	8.23	2.98	5.60	HR
H-144	2.14	1.17	1.64	ER
Male Hybrid	3.85	1.07	2.47	ER
St. George	7.50	4.12	5.81	HR
Salt Creek	22.1	13.83	17.98	R
SO4	9.28	3.13	6.20	HR
110-R	7.58	4.85	6.20	HR
3309-C	4.29	2.43	3.36	ER
1613	9.71	3.17	6.44	HR
Mean	7.89	3.87	5.88	

Table 4.4b Disease severity index and disease reaction of wine making genotypes to anthracnose under sub-tropical conditions.

Genotype	Leaf DSI	Cane DSI	Mean	Rating
Chardonnay	34.2	22.8	28.5	MR
Cabernet Sauvignon	54.2	43.1	48.7	MS
Chenin Blanc	40.0	33.6	36.8	MR
Merlot	37.8	25.5	31.6	MR
Pinot Noir	49.7	33.0	41.3	MS
Syrah	45.7	34.3	44.0	MS
Sauvignon Blanc	62.7	49.3	56.0	S
Tempranillo	38.5	25.4	32.0	MR
Ugni Blanc	68.5	52.2	60.3	S
Mean	47.9	35.4	42.1	

4.4.3 Role of Weather Factors on Disease Development

Among the epidemiological factors, minimum and maximum temperatures, relative humidity and sunshine hours were recorded. Weather data of two growth periods, *viz*., April-May (No disease incidence) and August-September (High disease incidence) revealed that, the disease development was observed from 2nd week of August to 2nd week of September, *i.e.*, autumn. During May-June, the maximum and minimum temperatures ranged from 35^0-40^{0}C and 19^0 - 25^{0}C, respectively with corresponding relative humidity ranging from 30-54% and sunshine hours from 6-8 h (avg.) during each fortnight. During May-June period there was no disease incidence. During August-September, occurrence of anthracnose was noted. During this period there was a distinct decrease in maximum temperature, which marked the increase in atmospheric relative humidity. During this period, maximum temperature and relative humidity ranged between 31^0-34^{0}C and 75-82%, respectively. However, minimum temperature was almost constant. The sunshine hours was also reduced by two hours during the disease appearance period. Findings of this study indicate that high relative humidity coupled with mean maximum temperature falling between 31-34^{0}C and drop in sunshine hours favoured weather conditions for high incidence of anthracnose. It can be inferred that during the summer months when the vine was in active growth stage, there was large variation in maximum and minimum temperature with concurred decrease in relative humidity and slight increase in sunshine hours. In contrast, during the severe disease occurrence, the difference in maximum and minimum temperature was minimum with gradual increase in relative humidity and sunshine period by two hours, *i.e.*, post rainy season creating ideal weather conditions for maximum disease intensity. Hence, it can be concluded that drop in maximum temperature up to 35^{0}C with rise in minimum temperature up to 26^{0}C and maintenance of relative humidity up to 80% and gradual rise in sunshine hours from 4 to 6 was the ideal condition for appearance of maximum disease intensity. Correlation studies revealed that the disease severity index (DSI) was positively correlated with relative humidity (r = 0.912) and minimum temperature (r = 0.779), whereas DSI was negatively correlated with maximum temperature (r = -0.560) and sunshine hours (r = -0.706).

Environmental factors play an important role in development of disease epidemics. Anthracnose of grape also has been reported to be greatly influenced by epidemiological factors, minimum and maximum temperatures, relative humidity and sunshine hours (Rao and Satyanarayan, 1991). Suhag (1971) related the disease to pre-monsoon showers, which not only increased the humidity but also decrease the maximum temperature. Kumar *et al*. (1999) investigated development of anthracnose disease of kidney bean in relation to weather variables and revealed that heavy ad frequent rainfall with moderate temperature (19^0C -25^0C) and high relative humidity (70%) favored the spread of disease. Robert *et al*., 2001 reported that warm and wet weather with temperature around 27^0C and high humidity (a mean of 80%) are optimum for anthracnose disease development in capsicum. In present investigation also, it was found that incidence of disease increased due to decrease in maximum temperature up to 31^0-33^0C and increase in atmospheric relative humidity by up to 82%. Results also confirmed with Garg *et al*. (2009). They observed that diurnal temperature ranged from 26.0^0C -32.0^0C with a relative humidity of 81-88% coupled with rainfall (13mm) are congenial conditions for anthracnose incidence in *Capsicum annum*.

Table 4.3. Morphological characterization of some grape genotypes.

Genotype(s)	Leaf length (cm)			Leaf width (cm)	Leaf area (cm^2)	Leaf texture	Inter-nodal length (cm)	Vine thickness at 7-8th node (cm)	Vine surface texture
	Short 4.5-6.5	Medium 6.6-8.5	Long > 8.6						
Rootstock									
Dog Ridge	6.07	-	-	9.55	15.98	Smooth	3.00	0.49	Smooth
Degrasett	6.49	-	-	10.1	17.09	Smooth	4.36	0.46	Smooth
H-144	-	-	13.2	12.8	120.1	Hairs present on abaxial surface	7.57	0.45	Smooth
Male Hybrid	-	7.10	-	7.82	22.55	Smooth	5.40	0.47	Smooth
St. George	5.43	-	-	8.42	35.12	Smooth	4.67	0.44	Smooth
Salt Creek	-	6.65	-	11.7	24.63	Smooth	5.35	0.78	Smooth
SO4	6.21	-	-	9.8	29.97	Smooth	4.87	0.44	Smooth
110-R	5.16	-	-	11.3	17.99	Smooth, Hair on petiole	3.85	0.35	Hairs present
3309-C	5.20	-	-	7.67	31.76	Glabrous	4.32	0.56	Smooth
1613	-	-	9.32	13.1	41.11	Hairs on abaxial surface	4.57	0.51	Rough
Wine purpose									
Chardonnay	-	6.87	-	8.35	75.62	Hairs absent	3.87	0.48	Smooth
Cabernet Sauvignon	-	7.81	-	9.50	69.41	Hairs present on abaxial surface	3.77	0.76	Rough
Chenin Blanc	5.90	-	-	8.57	65.19	Rough, undulating with tufted hairs on abaxial surface	3.75	0.63	Smooth
Merlot	-	7.72	-	9.87	73.98	Smooth	4.62	0.59	Rough
Pinot Noir	6.45	-	-	7.87	67.27	Smooth	5.87	0.75	Smooth
Syrah	-	-	10.50	14.0	85.31	Rough on both surfaces	4.0	0.64	Smooth
Sauvignon Blanc	-	7.93	-	9.80	78.04	Smooth	3.92	0.74	Smooth
Tempranillo	-	-	8.85	10.7	80.93	Very low density of hairs are present on abaxial surface	5.85	0.55	Rough
Ugni Blanc	-	7.92	-	10.8	71.65	Smooth	4.75	0.86	Smooth

Table purpose									
Anab-E-Shahi	-	7.90	-	9.82	121.31	Smooth	6.3	0.69	Rough
Alumwick	-	8.40	-	10.7	74.98	Rough	5.85	0.97	Smooth
Bharat Early	-	-	9.47	12.8	38.89	Rough	6.0	0.51	Smooth
Beauty Seedless	-	-	9.57	11.7	41.22	Smooth	3.62	0.65	Smooth
Banqui-Abyad	-	7.92	-	10.7	38.44	Smooth	7.85	0.60	Smooth
Black Muscat	-	8.30	-	9.77	39.00	Hairs present on abaxial surface	5.42	0.59	Smooth
Black Prince	-	-	9.58	12.6	29.14	Smooth on adaxial surface, hairs are present on abaxial surface.	6.82	0.63	Smooth
Cardinal	-	7.00	-	9.87	39.98	Smooth	4.97	0.58	Rough
Centennial Seedless	-	7.71	-	11.2	32.49	Rough	5.5	0.64	Smooth
Fakri	-	8.20	-	11.5	57.87	Very small hairs on abaxial surface	5.47	0.62	Smooth
Hur	-	-	10.12	12.8	45.58	Smooth	5.75	0.69	Smooth
Hybrid (Hur x BE) x BS	-	6.70	-	8.17		Smooth	5.27	0.82	Rough
Julesky Muscat	-	7.50	-	10.3	41.21	Smooth	8.6	0.92	Smooth
Kishmish Beli	-	8.40	-	11.2	34.43	Smooth	4.8	0.69	Smooth
Pusa Urvashi	-	-	9.0	12.9	56.89	Smooth	4.47	0.72	Smooth
Pearl of Csaba	5.50	-	-	8.05	29.32	Adaxial surface smooth, abaxial surface rough	5.62	0.79	Smooth
Perlette	-	-	9.25	11.4	53.23	Smooth	6.6	0.71	Smooth
Pusa Seedless	-	8.32	-	10.9	42.98	Smooth	5.45	0.76	Rough
Pusa Navrang	-	-	9.7	11.7	106.3	Smooth	4.22	0.65	Smooth
Tas	-	-	9.31	12.3	50.01	Smooth	5.85	0.75	Smooth
Tas-E-Ganesh	-	7.50	-	9.75	142.1	Smooth	4.92	0.92	Rough
Victory	-	-	8.69	11.5	38.28	Smooth	5.77	0.68	Smooth
Hybrid 70-56	-	7.57	-	9.70	53.23	Smooth	4.97	0.51	Smooth
Hybrid 75-32	-	8.12	-	9.90	43.59	Smooth	5.47	0.72	Smooth
Hybrid 72-151	-	-	9.22	11.27	59.18	Smooth	4.90	0.51	Smooth
Hybrid 71-50	-	8.43	-	10.14	42.35	Smooth	4.99	0.70	Smooth

Table 4.4c Disease severity index and disease reaction of table purpose varieties to anthracnose under sub-tropical conditions.

Genotype	Leaf DSI	Cane DSI	Mean	Rating
Anab-E-Shahi	84.1	59.6	71.8	HS
Alumwick	89.2	78.3	83.8	ES
Bharat Early	54.9	29.1	42.0	MS
Beauty Seedless	40.7	33.8	37.2	MS
Banqui-Abyad	75.0	67.9	71.4	HS
Black Muscat	43.5	37.6	40.6	MS
Black Prince	8.70	2.45	5.57	HR
Cardinal	77.0	67.0	72.0	HS
Centennial Seedless	62.1	49.3	55.7	S
Fakri	82.1	71.2	77.6	HS
Hur	59.8	48.6	54.2	MS
Hybrid (Hur x BE) x BS	69.5	53.8	61.6	S
Julesky Muscat	39.2	28.1	33.7	MR
Kishmish Beli	80.3	62.0	71.1	HS
Pusa Urvashi	48.1	32.4	40.7	MS
Pearl of Csaba	51.1	31.2	41.6	MS
Perlette	91.9	79.4	85.7	ES
Pusa Seedless	79.9	63.6	71.7	HS
Pusa Navrang	23.5	14.2	18.8	R
Tas	51.4	37.0	44.2	MS
Tas-E-Ganesh	69.2	44.7	56.9	S
Victory	84.4	62.7	73.4	HS
Hybrid 70-56	91.2	62.4	85.3	ES
Hybrid 75-32	84.2	71.0	74.5	HS
Hybrid 72-151	79.8	64.8	72.9	HS
Hybrid 71-50	53.2	28.9	41.1	MS
Mean	64.38	49.27	57.11	

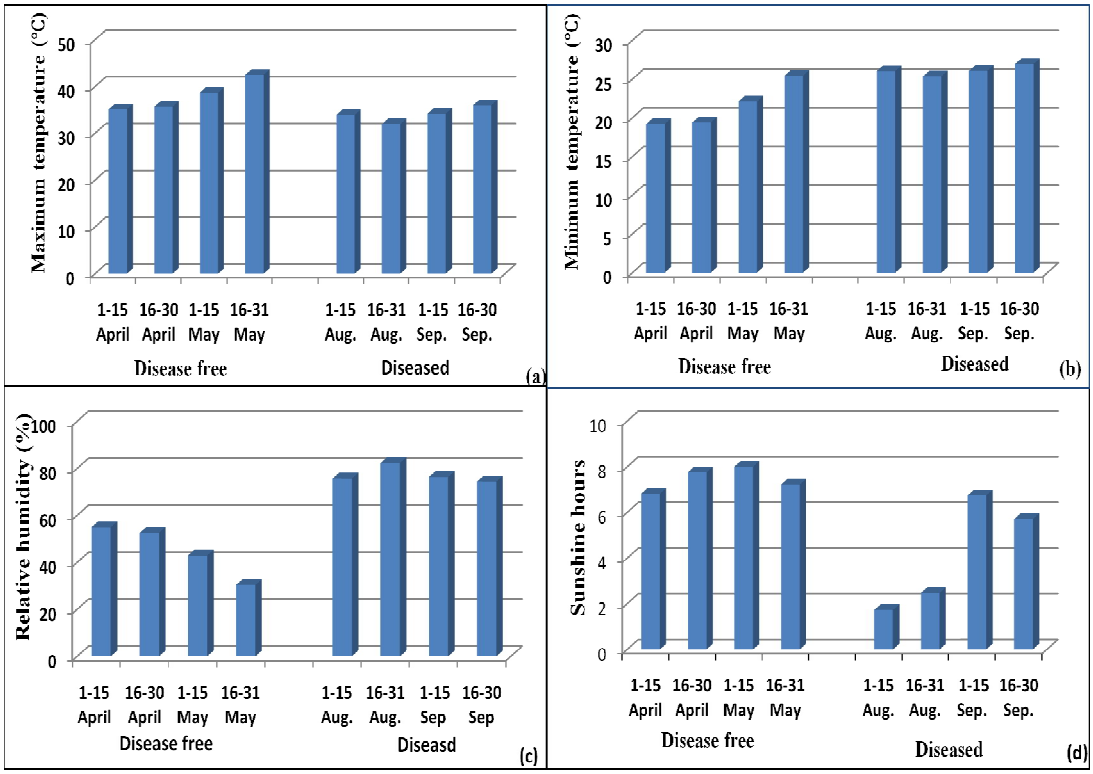

Fig. 4.1. Mean maximum & minimum temperature, relative humidity and sunshine hours during May-June and August-September (2012).

Table 4.5 Correlation (r) between weather parametars and Diseease Severity Index

	Sunshine	**Tmin**	**Tmax**	**Relative humidity**	**DSI**
Sunshine	1	-0.488	-0.491	-0.690	-0.706
T_{max}		1	-0.542	-0.448	-0.560
T_{min}			1	0.457	0.779
Relative humidity				1	0.912
DSI					1

CHAPTER 5 **Research Paper -II**

Biochemical analysis of *Vitis* sp. germplasm for resistance to anthracnose [*Elsinoe ampelina* (de Bary) Shear]

5.1 Abstract

To find out the biochemical basis of anthracnose resistance, the activities of defence related enzymes and the contents of some biochemical substances in healthy and anthracnose infected leaves were investigated in twenty diverse grape genotypes. The results revealed that upon disease infection, chlorophyll 'a', 'b', total chlorophylls and carotenoids contents declined irrespective of genotypes. It was observed that the concentration of total phenols and proline increased in resistant as well as susceptible genotypes as a part of defence system, whereas this increase was more pronounced in resistant genotypes. High contents of total and reducing sugars were observed in infected leaves of susceptible genotypes (7.91 mg/g FW; 3.53 mg/g FW) as compared to resistant genotypes (5.97 mg/g FW; 2.64 mg/g FW). Biochemical analysis revealed an increase in activities of defence related enzymes (polyphenol oxidase, peroxidase and phenylalanine ammonia lyase) and malondialdehyde content upon disease infection. Correlation studies between DSI and biochemical parameters showed that activities of PPO (r = -0.811), POD (r = -0 840) and PAL (r = -0.908) and MDA content (r = -0.758) were significantely negatively correlated with DSI, whereas, phenols (r = -8.37) and proline (r = -0.351) contents were found to be negatively correlated. These traits can be used as biochemical markers to identify anthracnose resistant genotypes, which can be included in breeding programmes. Carotenoids (r = 0.619), total sugars (r = 0.744) and reducing sugars (r = 0.785) showed positive correlations, wheares total chlorophyll was found to be negatively correlated (r = -0.435) with disease severity. The genotypes Black Prince (DSI = 5.57), Pusa Navrang (DSI = 18.8), Chardonnay (DSI = 28.5), Merlot (DSI = 31.6), Tempranillo (DSI = 32.0) and Chenin Blanc (DSI = 36.8) were found to have good resistance to anthracnose and could be used as donor parents in breeding programmes.

Keywords Anthracnose, biochemicals, Enzyme activity, Grape, Genotypes, Variability.

5.2 Introduction

Grape is one of the most widely cultivated fruit crop in the world and in India it is cultivated on an area of 1, 11,000 thousand hectares (NHB database, 2011). Most of the commercial grape varieties of India are highly susceptible to major fungal diseases, such powdery mildew, downy mildew and anthracnose under different agro-climatic conditions. Anthracnose is one of the most damaging diseases of grape and it is caused by fungus, *Elsinoe ampelina* (de Bary) Shear. Grape anthracnose can be identified by the "bird's eye" lesions on the berries and sunken black or grayish lesions on leaves and shoots. From these lesions conidia are developed. Chemical control of anthracnose is hazardous and uneconomical, hence development and use of resistant varieties is the most pragmatic way to keep the disease under check. Before initiating any resistance breeding programme, one must have thorough knowledge on the nature and basis of resistance to anthracnose as they help in formulating effective breeding strategies.

Plants have to defend themselves against a variety of pathogens to ensure growth and development. Because of this, plants evolved defence system against pathogens such as physical and chemical barriers and activation of biochemical pathways for synthesis of antimicrobial compounds. Plants respond to pathogenic attack by synthesizing compounds, which activate the defence system in plants. Blanchfield *et al.* (2006) investigated response of leaf pigments to phylloxera infestation in Pinot Noir and Cabernet Sauvignon grapevines under field conditions. A reduction in the leaf chlorophyll content and an increase in photoprotective pigment concentrations were observed in leaves of phylloxera infested grapevines compared to uninfested vines. Ruelas *et al*. (2006) reported enhanced level of phenolic acids namely, chlorogenic, vanillic and caffeic acid in tomato fruits (phytoanticipins) during a pathogenic attack. Defensive enzymes are among the most and widely distributed proteins in the plants. Phenylalanine lyase, polyphenol oxidase and peroxidase were reported in plants treated with various biotic and abiotic stress inducers (Raghvendra *et al.*, 2007). In the presence of oxygen, PPO oxidizes to phenolics compounds that are in the form of diphenol to o-quinone (Raj *et al*., 2006). Campos *et al.* (2004) observed positive correlation among

peroxidase and polyphenol oxidase activities and anthracnose resistance in bean. Higher impulses in enzymatic activity were observed in cultivars with higher disease resistance. Zhou *et al.* (2012) studied correlation between resistance of eggplant and defence related enzymes, and detected that inherent resistance was significantly and positively correlated with activity of POD but significantly negatively correlated with the content of malondialdehyde (MDA). It was concluded that the activity of the enzymes is directly related to resistance in the host. Hence, synthesis of different biochemical help plants to defend disease and understanding of these defence mechanisms would certainly help in screening large germplasm for identifying sources of resistance.

5.3 Materials and Methods

5.3.1 Plant material

Out of forty 45 grape genotypes screened, 20 genotypes comprising genotypes from each sub-groups, *i.e*. rootstock, wine making and table purpose categories were taken for biochemical analyses. The leaf tissues from the different genotypes were sampled during April-May (Disease free conditions) and August-September (during peak occurrence of disease) and collected in ice box (-4^0C) and stored in deep freeze (-20^0C) before estimation.

Grape genotypes selected for biochemical estimation.

Category	Genotype(s)
Rootstock	Male Hybrid, Hybrid-144, 3309-C, Dog Ridge, 110-R.
Wine purpose	Chardonnay, Merlot, Syrah, Tempranillo, Ugni Blanc.
Table purpose	Anab-E-Shahi, Alumwick, Cardinal, Centennial Seedless, Fakri, Perlette, Hur, Pusa Seedless, Pusa Urvashi, Hybrid 70-56.

5.3.2 Biochemical estimation

Different biochemical parameters such as total phenols, total sugars, reducing sugars, proline, polyphenol oxidase, peroxidase, phenylalanine lyase, and malondialdehyde contents were estimated. Changes in leaf pigments (chlorophyll and carotenoids) were also estimated in healthy leaves and after occurrence of disease under natural field conditions. Chlorophyll was estimated as per the method given by Hiscox

and Israelstam (1979). The total phenols were estimated by the method of Malik and Singh (1980). Quantity of total sugars and reducing sugars were estimated by anthrone reagent methods of Hedge and Hofreiter (1962) and Somogyi (1952), respectively. Proline estimation was carried out as per the method given by Bates *et al*. (1973). Peroxidase and polyphenol activities were assayed according to the method of Thomas *et al*. (1980) and Matto and Diamond (1963), respectively. Activity of phenylalanine ammonia lyase was assayed as per the method given by Lisker *et al*. (1983). Malondialdehyde level was assayed by thiobarbituric acid (TBA) method (Hagege *et al*., 1990).

5.3.3 Statistical analysis

Data for all the parameters were recorded three times and analysis of variance (ANOVA) was carried out in Completely Randomized Design. Correlation (r) and coefficient of determination (R^2) among DSI and biochemical traits were computed by using statistical analysis system software (SAS version 2.).

5.4 Results and discussion

The 20 genotypes varied significantly for different biochemical and enzymatic activities. There was wide variation between healthy and diseased conditions.

5.4.1 Chlorophyll

Chlorophyll content could be used in initial screening of progenies in a breeding programme for high photosynthetic rates. Analysis of chlorophyll contents revealed that Chlorophyll ‘a’, chlorophyll ‘b’ and total chlorophyll decreased upon fungal infection compared to healthy leaves (Table 5.1). Similar results were also reported by Mesta *et al.* (2006), while working on alternaria blight of sunflower. In general, amount of chlorophyll ‘a’, ‘b’ and total chlorophylls were higher in table purpose genotypes followed by wine purpose and rootstock genotypes (Table 5.1). Resistant genotypes showed higher mean chlorophyll ‘a’ (1.07 mg/g FW), chlorophyll ‘b’ (0.29 mg/g FW) and total chlorophyll (1.76 mg/g FW) contents compared to susceptible genotypes (Chl ‘a’- 0.83 mg/g FW; Chl ‘b’- 0.2 mg/g FW and Total chl- 1.23 mg/ g FW) under diseased

conditions. Similar results were also reported by Gupta *et al.* (1987), while working on *Alternaria* blight of sesame. Highest content of total chlorophyll was found in Male Hybrid (2.2 mg/g FW) which showed extreme degree of disease resistance, whereas minimum total chlorophyll content was estimated in Centennial Seedless (0.75 mg/g FW), which was most susceptible genotype. Chlorophyll 'b' was found to be more sensitive, which was also reduced at higher rates in susceptible genotypes. Chlorophyll 'b' was estimated to be lowest in 70-56 (0.12 mg/g FW) followed by Alumwick (0.13 mg/g FW) during the disease occurrence stage.

5.4.2 Carotenoids

The observations (Table 5.1) revealed that total carotenoids content was higher in healthy leaves of susceptible genotypes compared to resistant genotypes. Significantly highest carotenoids content was observed in Anab-E-Shahi (2.34 mg/ g FW), whereas the minimum content was found in 110-R (1.12 mg/ g FW). This observation clearly indicates that resistant genotype (110-R) had lower content of total carotenoids compared to susceptible (Anab-E-Shahi) genotype. Among different categories the highest carotenoids content was observed in table purpose (1.85 mg/g FW) followed by wine making (1.40 mg/g FW) genotypes, whereas lowest carotenoids content was found in rootstocks (1.36 mg/g FW) upon disease infection (Table 5.1)

Abnormalities in the form and destruction of chloroplasts are common features of diseased tissue turning necrotic. A reduction in the leaf chlorophyll and carotenoid concentrations were observed in leaves of phylloxera infested grapevines compared to uninfested vines (Blanchfied *et al*., 2006). In present study, both chlorophyll and carotenoid contents were highest in table purpose genotypes followed by rootstocks and wine making genotypes, which decreased post fungal attack. Upon fungal infection, rootstocks showed the highest content of chlorophyll, whereas carotenoid content was highest in table purpose genotypes (Table 5.1).

5.4.3 Total phenols

The data presented in the Table 5.2 reveal that the diseased leaves had higher total phenol contents than healthy leaves in all the genotypes. This indicates that phenol biosynthesis was enhanced due to pathogen infection. Total phenols content was highest in rootstocks followed by wine making and the least in table purpose genotypes under both healthy as well as diseased conditions. Highly resistant genotype, Hybrid-144 showed the maximum amount of total phenols (5.71 mg/g FW) followed by Dog Ridge (4.95 mg/g FW), which was significantely different. Lowest total phenols content was estimated in Perlette (2.66 mg/g FW), which was classified as extremely susceptible genotype.

Phenolic compounds are part of plant defence system which can be acquired in the tissue under stress. They are known known to possesses insecticidal and antimicrobial activities (Barberan *et al*., 1988). This study indicated that resistant genotype (Dog Ridge) had higher content of phenols compared to susceptible genotypes (Perlette), which is also supported by findings of Bhawani Shankar *et al*. (2000). Prasath and Ponnuswami (2008) also found highest phenol contents in *Colletotrichum annum* resistant genotypes (Acc. 16 (PCB 81) of chilli followed by moderately resistant and the least in the susceptible genotypes (Hybrid 6). High phenolic contents in *Colletotrichum* resistant genotypes of *Capsicum annum* L. was also reported by Kaur *et al*. (2011).

5.4.4 Proline

The present investigation revealed that the mean proline content was higher in diseased (82.88 µg/g FW) leaves than healthy (42.63 µg/g FW) ones. The highest proline content was observed in Dog Ridge (98.68 µg/g FW) followed by 110-R (98.35 µg/g FW), which were categorized as extremely resistant (ER) and highly resistant (HR) respectively. In contrast, disease susceptible genotypes, namely, Cardinal (57.09 µg/g FW) and Syrah (62.80 µg/g FW) showed lower proline contents among the genotypes studied (Table 5.2).

Proline accumulation in higher plant in response to various environmental stresses is a widespread phenomenon, and is believed to play adaptive roles in stress

tolerance (Mattioli *et al*., 2009; Trovato *et al*., 2008; Verbruggen and Hermans, 2008; Szabados and Savour, 2010). Results of this study indicated that, diseased leaves of resistant genotypes had higher proline levels compared to of susceptible genotypes. This finding is supported by the earlier reports of Goicoechea *et al.* (2000) who found increase in proline level in foliar tissues of *Verticillium dahliae* infected plants, while its level did not change in the leaves of control plants. Sivritepe *et al*. (2009) also reported 6.7- and 4.2-fold increase in the leaves of Muscule and Sultana grapevines respectively due to mite infectation. Present investigation also revealed that susceptible genotypes had lower increase in proline content upon infection compared to resistant genotypes. Nagla *et al*. (2000) earlier obtained similar results in flax infected with powdery mildew. In general, table purpose genotypes showed lowest mean proline levels (77.86 µg/g FW) as compared to wine making genptypes and rootsocks.

5.4.5 Total sugars

The data in Table 5.3 indicates that total sugars in the leaf tissue during healthy stage varied with the genotypes. There was a general decline in total sugar levels during diseased conditions. Upon disease incidence, the highest total sugars were found in Hybrid 70-56 (8.89 mg/g FW) followed by Alumwick (8.83 mg/g FW), both of which grouped in extremely susceptible category. The lowest amount of total sugars in the leaf tissues were found in extremely resistant genotype 3309-C (5.68 mg/g FW). Among the three groups, mean total sugar contents were found to be highest in table purpose genotypes (7.9 mg/g FW), which in general showed higher susceptibility to disease (DSI = 57.11), whereas rootstocks recorded lower total sugars (5.97 mg/g FW), which had higher resistance to the disease (DSI = 5.88).

5.4.6 Reducing sugars

Higher levels of reducing sugars were estimated in infected leaves over healthy leaves of a genotype (Table 5.3). It also shows that table purpose varieties had high mean (3.53 mg/g FW) reducing sugars over wine making (3.09 mg/g FW) or rootstocks (2.64 mg/ g FW). Out of 20 genotypes, Hybrid 70-56 (3.99 mg/g FW) followed by Fakri

(3.97 mg/g FW) had higher reducing sugars contents, and accordingly both showed high degree of susceptibility to disease. Lowest reducing sugars content was found in Male Hybrid (2.46 mg/g FW), which was extremely resistant to anthracnose. Total sugars and reducing sugars were lowest in resistant genotypes and highest in highly susceptible genotypes.

It shows that higher total and reducing sugars may be associated with the susceptibility to anthracnose. Pathogen releases enzyme amylase, which converts carbohydrates into sugars. Leaf total and reducing sugars contents is one of the most important parameter, to categorize the genotype to a particular reaction, *viz*., resistant or susceptible. Results of this study indicated that total as well as reducing sugars content increased upon disease infection. This finding is in accordance with the earlier reports of Bhavani Shankar *et al.* (2001). They observed that anthracnose infected leaves of susceptible genotypes (Pusa Seedless, Cardinal, AK x PS. 72-52 and BA x BS. 74-10 contained higher total and reducing sugars. Similar results were also shown by Dhanumjayarao *et al.* (2006) while working on powdery mildew of grape. They reported that highly susceptible variety, Gold had more total sugars (5.75 mg/g FW) whereas, resistant variety Victory had lowest (3.30 mg/g FW) among all varieties studied. It was also reported that, Hur contained more reducing sugars (3.82 mg/g FW) over other varieties. Rubired, which is resistant against powdery mildew of grape, had the lowest (2.11 mg/g FW) reducing sugars over other varieties. Recently, Prakash *et al.* (2011) also reported lower content of sugar in fruit rot (*Colletotrichum capsici*) resistant varieties compared to susceptible varieties of chilli.

5.4.7 Polyphenol oxidase

Activity of polyphenol oxidase in leaves from different grape genotypes significantly varied with their degree of resistance to anthracnose. Its activity was higher in diseased leaves (66.1 g/min.) as compared to corresponding healthy leaves (55.77 g/min.) in all the genotypes. This could be due to the stress created due to pathogen infection and post progression establishment. As observed from Table 5.4, there was a

wide variation in PPO activity among rootstocks (91.26 g/min.), wine making type (84.8 g/min) and table purpose genotypes (42.2 g/min.) Among different genotypes, PPO activity was highest in Dog Ridge (118.13 g/min.) followed by 110-R (90.70 g/min), both of which grouped in extremely resistant category. The lowest PPO activity was found in highly susceptible genotype Anab-E-Shahi (34.5 g/min.).

5.4.8 Peroxidase

Results of peroxidase activity showed that, it was higher in diseased leaves (2.06 g/min.) compared to the healthy leaves (1.56 g/min.). The, POD activity in leaves of resistant genotypes was found significantly higher (2.86 g/min.) than those in the leaves of susceptible genotypes (1.48 g/min). The highest activity of POD was found in Dog Ridge (3.6 g/min.) followed by 110-R (3.4 g/min.), which were categorized as extremely resistant and highly resistant respectively, whereas Fakri (Highly susceptible) showed the lowest activity of POD (1.2 g/min.). As evident from the Table 4, rootstocks in general showed the highest activity of POD (2.86 g/min.) followed by wine making type (2.31 g/min). The lowest POD activity was found in table purpose genotypes (1.48 g/min).

Defensive enzymes are among the most influential and ubiquotes protein in the plants. Enhanced PPO and peroxidase activities were reported in plants exposed to various biotic and abiotic inducer activities (Raghvendra *et al*., 2007). Present study revealed that in disease resistant genotypes, the activities of PPO and POD were alleviated in comparison with highly susceptible genotypes. Bhavani Shankar *et al.* (2001) reported that anthracnose resistant grape genotypes possessed higher peroxidase (0.084 OD/min./g) and polyphenol oxidase activities. Similarly, Kavitha and Umesha (2008) reported that in bacterial wilt resistant tomato cultivars, the activities of PPO and POD were enhanced in comparison with highly susceptible cultivars. Singh *et al.* (2011) also reported increased in enzymatic activity upon disease infection in alternaria leaf spot in resistant *Brassica* genotypes.

5.4.9 Phenylalanine ammonia lyase

In the present investigation, it was observed that PAL activity was increased 2- to 3-fold upon disease infection (7.40 µmol of trans-cinnamic acid/ mg protein/ h) in all the genotypes compared to healthy leaves (5.90 µmol of trans-cinnamic acid/ mg protein/ h) but this increase was more significant in resistant genotypes than susceptible genotypes (Table 5.5). The highest PAL activity was observed in Dog Ridge (13.56 µmol of trans-cinnamic acid/mg protein/ h) followed by 3309-C (12.06 µmol of trans-cinnamic acid/ mg protein/ h), both of which were placed in extremely resistant category (ER), whereas lowest PAL was observed in Pusa Urvashi (3.50 µmol of trans-cinnamic acid/ mg protein/ h), which shows moderate susceptibility to the disease. This indicates that resistant genotypes had higher PAL activity than susceptible genotypes. Among rootstock, wine making type and table purpose genotypes, highest activity of PAL was observed in rootstock (11.76 µmol of trans-cinnamic acid/ mg protein/ h) followed by wine making types (7.86 µmol of trans-cinnamic acid/ mg protein/h), whereas table purpose genotypes showed the lowest PAL activity (4.77 µmol of trans-cinnamic acid/ mg protein/ h).

5.4.10 Malondialdehyde

Results revealed that compared to healthy leaves (2.18 nmol /g), infected leaves showed increase in the levels of malondialdehyde (2.89 nmol /g). Among all the genotypes the highest level of MDA was observed in Dog Ridge (5.33 nmol /g), whereas Centennial Seedless showed the lowest (1.80 nmol /g) content, which was significantly different (Table 5.5). This indicates that MDA levels were higher in resistant compared to susceptible genotypes. In general, table purpose genotypes showed lower levels of MDA (2.44 nmol/g) amongst all three groups, *i.e.* rootstock, wine making type and table purpose genotypes, whereas it was highest in rootstocks (4.32 nmol/g).

Phenylalanine ammonia lyase is the key enzyme catalyzing the biosynthesis of phenolics and lignin from the aromatic amino acid phenylalanine (Cartea *et al.*, 2010). A higher increase in the PAL activity and MDA content were observed in leaves of resistant genotypes upon infection as compared to susceptible ones. Earlier, Prasatha and

Ponnuswami (2008) also reported increased activity of phenylalanine ammonia lyase in resistant genotypes followed by moderate resistant hybrids. An increase in PAL activity results in increase in concentration of phenolic compounds, which are substrates for oxidative enzymes such as PPO and POD and better defence against the disease progression.

5.5 Correlation Studies

The level of resistance or susceptibility of different grape genotypes was correlated with different biochemical parameters, *i.e*., total phenols, total sugars, reducing sugars, proline, changes in the activities of leaf pigments (chlorophyll 'a', 'b', total cholorophylls and carotenoids) and activity of defence related enzymes (PPO, POD, PAL and MDA). Correlation studies indicated that the activities of PPO and POD were significantaly negatively correlated with disease severity index of -0.811 and -0.840, respectively. In similar way, the sharp increase in PPO and POD activities were observed due to infestation by anthracnose pathogen in chilli plants (Bharath *et al*., 2004; Borua, 2000). Similar results were earlier reported by Jindal and Rao (2000) and Shankar and Jindal (2001). The disease severity index showed negative correlation with the activities of PAL (r = -0.908) and MDA (r = -0.758). 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 disease severity index (r = -0.908), which suggests that this trait would be useful in breeding for anthracnose resistant hybrids. The correlation studies also revealed that total and reducing sugars were positively correlated with disease severity index, having correlation coeffecient of 0.744 and 0.785, respectively. Dhanumjayarao *et al*. (2006) also reported that total sugars had positive correlation with disease severity (r = 0.743). Similarily, reducing sugars were also significantely positively correlated with disease index. Pigments namely, chlorophyll 'a' (r = -0.378), 'b' (r = -0.462), total chlorophylls (r = -0.435) and total carotenoids (r = 0.619) showed non significant correlation with disease severity index. Earlier, Shen and

Huo (2009) also reported that cholophyll content of different non-heading chinese cabbage was positively correlated with resistance to the downy mildew. Recently, Zhou *et al*. (2012) also reported negative correlation of total chlorophyll content with disease incidence (r = -0.732) and disease index (r = 0.657) in *Verticillium* wilt infected eggplant genotypes. Total phenols showed higher negative correlation (r = -0.837) with DSI, whereas proline was found to be moderately negatively correlated (r = -0.351) with disease severity index. The study revealed that there was distinct variations in different biochemicals and in healthy and diseased conditions. The parameters like PAL (r= -0.908), POD (r = -0.840) , PPO (r = -0.811), MDA (r = -0.783), and total phenols (r = -0. 837), were found to be significantly correlated with disease severity index, hence these parameters could be taken for screening progenies/ germplasm for identifying genotypes with higher anthracnose tolerance in grape. It is well established that the species of the genus *Vitis* (*V. rotundifolia*) that have a higher content of polyphenols are more resistant to disease infection caused by powdery mildew and, when infected, produce larger amounts of polyphenol than genotypes which are more susceptible (Dai *et al*., 1994). MDA content increases due to disease infection, which causes lipid peroxidation and some level of stress mitigation, membrane damage and photo-oxidation of chlorophyll. In this study an attempt was made to correlate different biochemical parameters with disease severity index and some parameters were found to be significantly correlated with disease severity. However, further studies are needed on relevant biochemical processes, to reveal the mechanism of resistance to anthracnose of grapevine.

Table 5.1. Chlorophyll 'a', 'b', total chlorophylls and carotenoids contents in healthy and diseased leaves of some grape genotypes as affected by anthracnose incidence.

Genotype	Chlorophyll 'a' (mg/g FW)		Chlorophyll 'b' (mg/g FW)		Total chlorophyll (mg/g FW)		Carotenoids (mg/g FW)	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
Rootstock								
Male Hybrid	1.08^{j}	1.11^{ab}	0.18^{jk}	0.34^{a}	2.06^{f}	2.20^{a}	1.28^{gh}	1.13^{f}
3309-C	1.18^{ji}	1.06^{abcd}	0.12^{k}	0.22^{cd}	2.48^{c}	1.61^{f}	1.71^{de}	1.48^{e}
110-R	1.38^{bcde}	1.07^{abc}	0.46^{bcd}	0.31^{ab}	1.91^{g}	1.96^{c}	1.44^{g}	1.12^{f}
Dog Ridge	1.10^{i}	0.95^{cdef}	0.39^{fg}	0.33^{ab}	2.21^{f}	1.15^{h}	1.63^{fg}	1.42^{e}
H-144	1.25^{efgh}	1.19^{a}	0.40^{efg}	0.25^{cd}	2.22^{f}	1.88^{cd}	1.71^{de}	1.65^{cd}
Mean	**1.20**	**1.07**	**0.31**	**0.29**	**2.17**	**1.76**	**1.55**	**1.36**
Wine purpose								
Chardonnay	1.35^{cdef}	1.00^{bcd}	0.47^{bcde}	0.28^{cd}	2.94^{ab}	1.48g	1.64^{fg}	1.45^{e}
Chenin Blanc	1.26^{ef}	1.13^{ab}	0.39^{i}	0.30^{bc}	2.00^{g}	0.91^{jk}	1.55^{g}	1.38^{e}
Syrah	1.22^{fghi}	0.89^{fgh}	0.23^{j}	0.31^{abc}	2.02^{g}	1.83^{de}	1.83^{cd}	1.62^{cd}
Tempranillo	1.35^{cdef}	1.05^{bcde}	0.42^{efg}	0.31^{abc}	2.64^{de}	2.13^{ab}	1.22^{h}	1.17^{f}
Merlot	1.17^{ij}	1.01^{bcdef}	0.25^{ij}	0.23^{e}	1.97^{g}	2.09^{b}	1.64^{fg}	1.40^{e}
Mean	**1.27**	**1.01**	**0.35**	**0.28**	**2.31**	**1.68**	**1.57**	**1.40**
Table purpose								
Pusa Urvashi	1.41^{bc}	0.72^{kl}	0.56^{a}	0.32^{ab}	2.64^{de}	1.83^{cd}	1.64^{fg}	1.59^{d}
Centennial Seedless	1.47^{bc}	0.76^{jkl}	0.50^{abc}	0.20^{de}	2.96^{ab}	0.75^{l}	2.08^{b}	1.98^{ab}
Pusa Seedless	1.42^{abcd}	0.85^{hij}	0.52^{abc}	0.32^{ab}	2.84^{ab}	1.01^{i}	2.19^{ab}	2.03_{a}
Hybrid 70-56	1.54^{ab}	0.81^{ijk}	0.53_{ab}	0.12^{g}	3.04^{a}	0.86^{k}	1.98^{bc}	1.86^{b}
Alumwick	1.52^{ab}	0.96^{cdef}	0.40^{efg}	0.13^{fg}	3.09^{a}	1.19^{h}	1.87^{cd}	1.81^{b}
Cardinal	1.43^{abcd}	0.85^{hij}	0.46^{bcd}	0.19^{ef}	2.85^{bc}	1.78^{e}	1.76^{d}	1.69^{c}
Anab-E Shahi	1.29^{def}	0.93^{efgh}	0.45^{cdef}	0.16^{fg}	2.60^{de}	1.14^{h}	2.55^{a}	2.34^{a}
Perlette	1.68^{a}	0.88^{ghi}	0.36^{gh}	0.17^{ef}	2.20^{f}	1.16^{h}	2.15^{ab}	2.12^{ab}
Hur	1.43^{abcd}	0.94^{defg}	0.52^{abc}	0.21^{ef}	2.70^{cd}	1.64^{f}	1.69^{ef}	1.57^{cde}
Fakri	1.37^{cde}	0.64l	0.36^{gh}	0.18^{efg}	2.72^{cd}	0.96^{ij}	1.63^{fg}	1.53^{de}
Mean	**1.46**	**0.83**	**0.46**	**0.20**	**2.76**	**1.23**	**1.95**	**1.85**
Grand mean	**1.34**	**0.94**	**0.39**	**0.24**	**2.49**	**1.48**	**1.75**	**1.60**
CV (%)	**5.27**	**6.98**	**9.45**	**10.95**	**3.95**	**3.64**	**5.00**	**2.91**

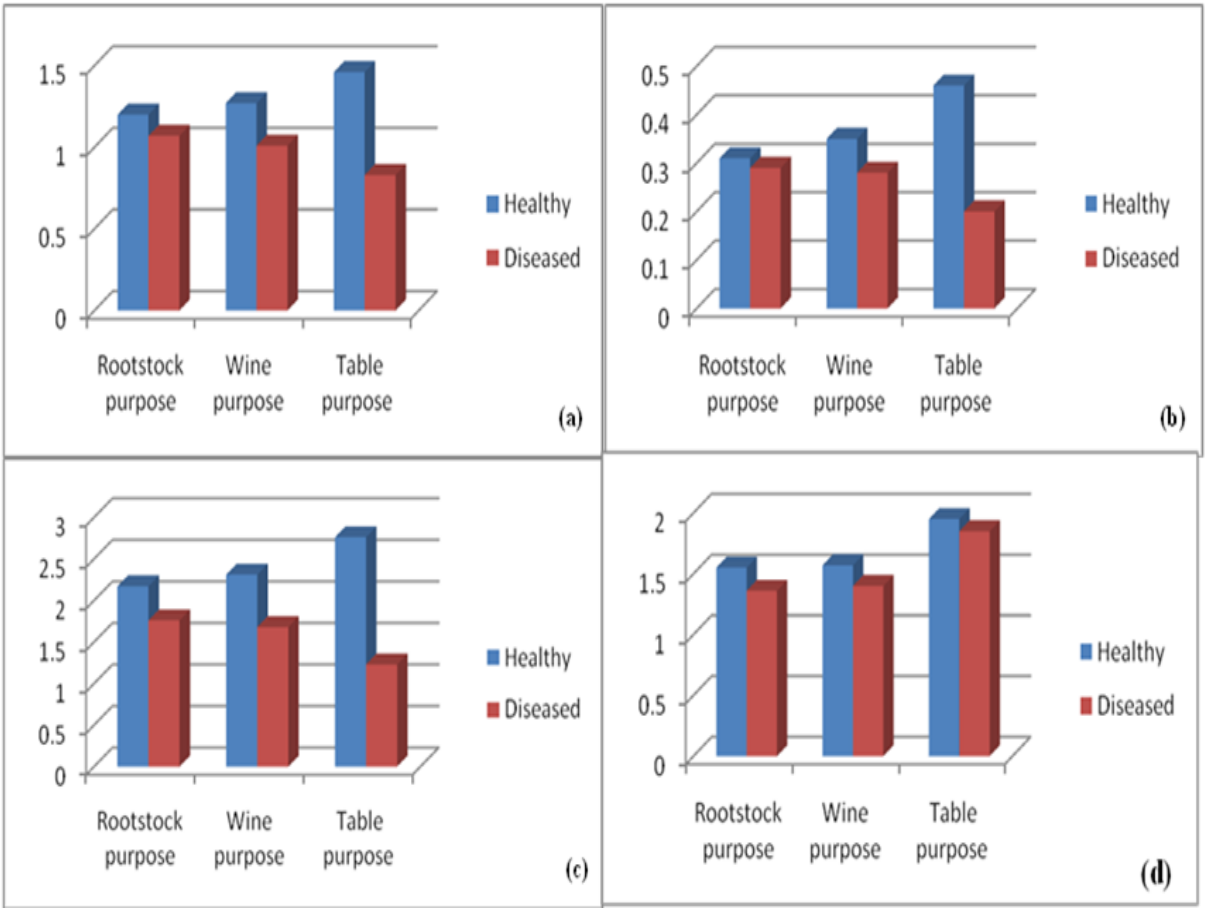

Fig. 5.1. Comparative chlorophyll ‘a’, ‘b’ , total chlorophylls and carotenoids contents (mg/g FW) in healthy and anthracnose infected leaves of some rootstock, wine and table purpose grape genotypes.

Table 5.2. Total phenols and proline contents in healthy and diseased leaves of some grape genotypes as affected by anthracnose incidence.

Genotype	Total phenol (mg/g FW)		Proline (μg/g FW)	
	Healthy	Diseased	Healthy	Diseased
Rootstock				
Male Hybrid	4.18^{a}	4.93^{bc}	35.27^{hi}	96.16^{ab}
3309-C	3.87^{cd}	4.86^{c}	40.29^{fg}	95.56^{ab}
110-R	3.41^{f}	4.05^{g}	51.79^{b}	98.35^{a}
Dog Ridge	405^{ab}	4.95^{b}	57.13^{a}	98.60^{a}
H-144	4.19^{a}	5.71^{a}	51.92^{b}	82.07^{ef}
Mean	**3.94**	**4.90**	**47.28**	**94.15**
Wine purpose				
Chardonnay	3.77^{d}	4.68^{d}	52.62^{b}	81.48^{efg}
Chenin Blanc	3.93^{bcd}	4.42^{e}	49.59^{c}	73.23^{gh}
Syrah	3.81^{cd}	4.32^{f}	39.85^{g}	62.80^{i}
Tempranillo	3.61^{e}	4.27^{f}	51.76^{b}	92.31^{abcd}
Merlot	4.10^{a}	4.72^{d}	50.16^{c}	94.24^{abc}
Mean	**3.84**	**4.48**	**48.80**	**80.81**
Table purpose				
Pusa Urvashi	3.22^{g}	3.92^{h}	36.03^{h}	85.91^{cde}
Centennial Seedless	2.97^{h}	3.46^{j}	40.68^{fg}	88.07^{bcde}
Pusa Seedless	2.87^{h}	3.55^{i}	34.99^{i}	74.10^{fgh}
Hybrid 70-56	2.52^{i}	2.93^{m}	34.64^{i}	66.40^{hi}
Alumwick	2.12^{j}	3.53^{ij}	47.78^{d}	89.00^{bc}
Cardinal	2.43^{i}	3.08^{l}	18.63^{k}	57.09^{j}
Anab-E-Shahi	3.13^{g}	3.26^{k}	47.96^{d}	91.47^{ab}
Perlette	2.46^{i}	2.66^{h}	23.02^{j}	69.52^{hi}
Hur	2.96^{h}	3.29^{k}	40.82^{f}	73.30^{gh}
Fakri	2.57^{i}	3.15^{l}	42.73^{e}	83.70^{de}
Mean	**2.73**	**3.28**	**36.73**	**77.86**
Grand mean	**3.38**	**4.02**	**42.63**	**82.88**
CV (%)	**2.67**	**1.23**	**1.24**	**5.70**

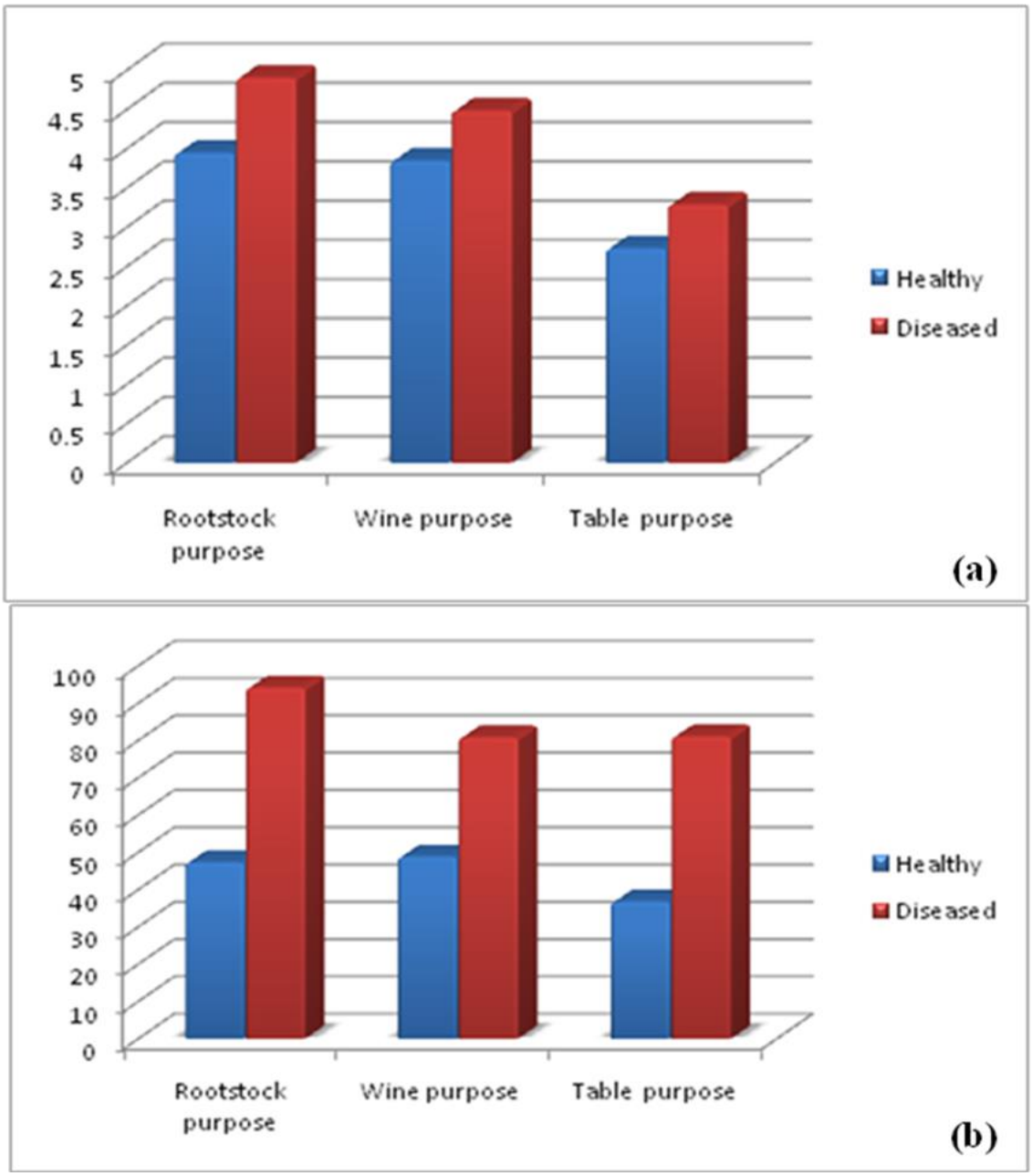

Fig. 5.2. Comparative (a) total phenols (mg/g FW) and (b) proline (µg/g FW) contents in healthy and anthracnose infected leaves of rootstock, wine and table purpose grape genotypes.

Table 5.3. Total sugars and reducing sugar contents in healthy and diseased leaves of some grape genotypes as affected by anthracnose.

Genotype	Total sugars (mg/g FW)		Reducing sugars (mg/g FW)	
	Healthy	Diseased	Healthy	Diseased
Rootstock				
Male Hybrid	5.16^{l}	6.22^{i}	2.22^{fgh}	2.46^{m}
3309-C	4.78^{m}	5.68^{l}	2.08^{h}	2.67^{kl}
110-R	5.73^{i}	6.16^{i}	2.31^{fg}	2.54^{lm}
Dog Ridge	5.55^{j}	5.82^{k}	2.22^{fgh}	2.76^{jk}
H-144	4.03^{o}	5.97^{j}	2.36^{fg}	2.78^{jk}
Mean	**5.05**	**5.97**	**2.24**	**2.64**
Wine purpose				
Chardonnay	5.47^{k}	6.14^{i}	2.44^{ef}	2.95^{hi}
Chenin Blanc	5.62^{j}	6.48^{h}	2.03^{h}	3.08^{gh}
Syrah	4.99^{m}	6.48^{h}	2.21^{fgh}	3.31^{ef}
Tempranillo	4.49^{n}	6.20^{i}	3.01^{bc}	3.19^{fg}
Merlot	5.12^{l}	6.75^{g}	2.83^{cd}	2.91^{ij}
Mean	**5.13**	**6.41**	**2.50**	**3.09**
Table purpose				
Pusa Urvashi	5.11^{l}	6.86^{g}	2.64^{de}	3.11^{g}
Centennial Seedless	5.95^{h}	7.09^{f}	2.67^{d}	3.40^{de}
Pusa Seedless	6.60^{e}	7.15^{f}	2.38^{fg}	3.28^{ef}
Hybrid 70-56	7.41^{c}	8.89^{a}	2.72^{d}	3.99^{a}
Alumwick	7.76^{a}	8.83^{a}	3.22^{ab}	3.56^{c}
Cardinal	6.22^{g}	7.69^{e}	3.02^{bc}	3.52^{cd}
Anab-E-Shahi	7.63^{b}	8.07^{d}	2.16^{gh}	3.93^{a}
Perlette	6.32^{f}	8.57^{b}	3.25^{a}	3.74^{b}
Hur	6.87^{d}	7.79^{e}	3.18^{ab}	2.87^{j}
Fakri	6.16^{g}	8.20^{c}	3.13^{ab}	3.97^{a}
Mean	**6.60**	**7.91**	**2.84**	**3.53**
Grand mean	**5.81**	**7.01**	**2.59**	**3.19**
CV (%)	**0.70**	**1.00**	**4.75**	**2.64**

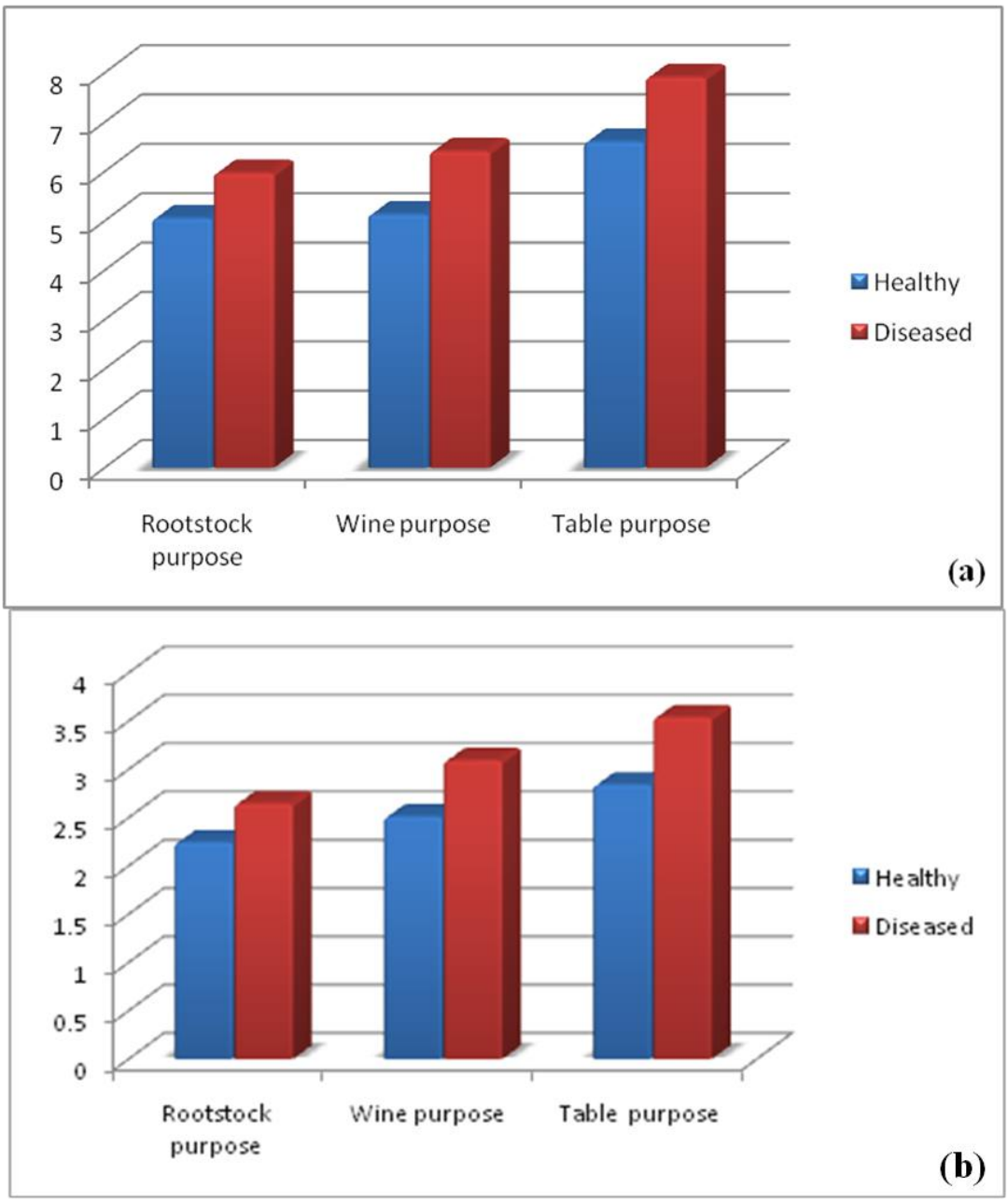

Fig. 5.3 Comparative (a) total sugars and (b) reducing sugar contents (mg/g FW) in healthy and diseased leaves of rootstock and table purpose grape genotypes.

Table 5.4. Peroxidase and polyphenol oxidase activities in healthy and diseased leaves of some grape genotypes as affected by anthracnose incidence.

Genotype	Peroxidase activity (g/min.)		PPO activity (g/min.)	
	Healthy	Diseased	Healthy	Diseased
Rootstock				
Male Hybrid	2.06^{d}	2.40^{d}	50.00^{g}	74.60^{g}
3309-C	2.40^{c}	2.71^{c}	78.16^{c}	89.46^{b}
110-R	2.90^{b}	3.40^{b}	81.06^{b}	90.70^{a}
Dog Ridge	3.20^{a}	3.60^{a}	94.80^{a}	118.13^{a}
H-144	1.80^{e}	2.20^{fg}	61.46^{g}	83.40^{d}
Mean	**2.47**	**2.86**	**73.10**	**91.26**
Wine purpose				
Chardonnay	1.81^{ef}	2.15^{g}	78.03^{c}	86.70^{c}
Chenin Blanc	1.80^{ef}	2.40^{de}	82.33^{b}	89.03^{b}
Syrah	1.93^{de}	2.29^{ef}	69.23^{e}	79.13^{f}
Tempranillo	1.21^{gh}	2.42^{d}	81.00^{b}	88.43^{b}
Merlot	1.62^{f}	2.29^{e}	72.20^{d}	80.73^{e}
Mean	**1.67**	**2.31**	**76.56**	**84.80**
Table purpose				
Pusa Urvashi	1.00^{hij}	1.40^{jk}	38.60^{i}	46.56^{i}
Centennial Seedless	0.80^{jk}	1.50^{j}	32.00^{j}	39.30^{l}
Pusa Seedless	1.20^{gh}	1.80^{h}	36.63^{i}	44.53^{j}
Hybrid 70-56	1.10^{ghi}	1.30^{kl}	31.46^{j}	39.40^{l}
Alumwick	1.30^{g}	1.70^{hi}	39.16^{i}	43.23^{k}
Cardinal	1.19^{gh}	1.67^{i}	42.10^{h}	50.60^{h}
Anab-E-Shahi	1.11^{ghi}	1.79^{h}	28.20^{k}	34.53^{m}
Perlette	0.70^{k}	1.20^{l}	25.33^{l}	39.40^{l}
Hur	0.80^{jk}	1.30^{kl}	32.06^{j}	35.12^{m}
Fakri	0.90^{ijk}	1.20^{l}	44.37^{h}	49.70^{h}
Mean	**1.01**	**1.48**	**34.99**	**42.23**
Grand mean	**1.56**	**2.06**	**55.77**	**66.13**
CV(%)	**8.25**	**2.97**	**2.82**	**0.99**

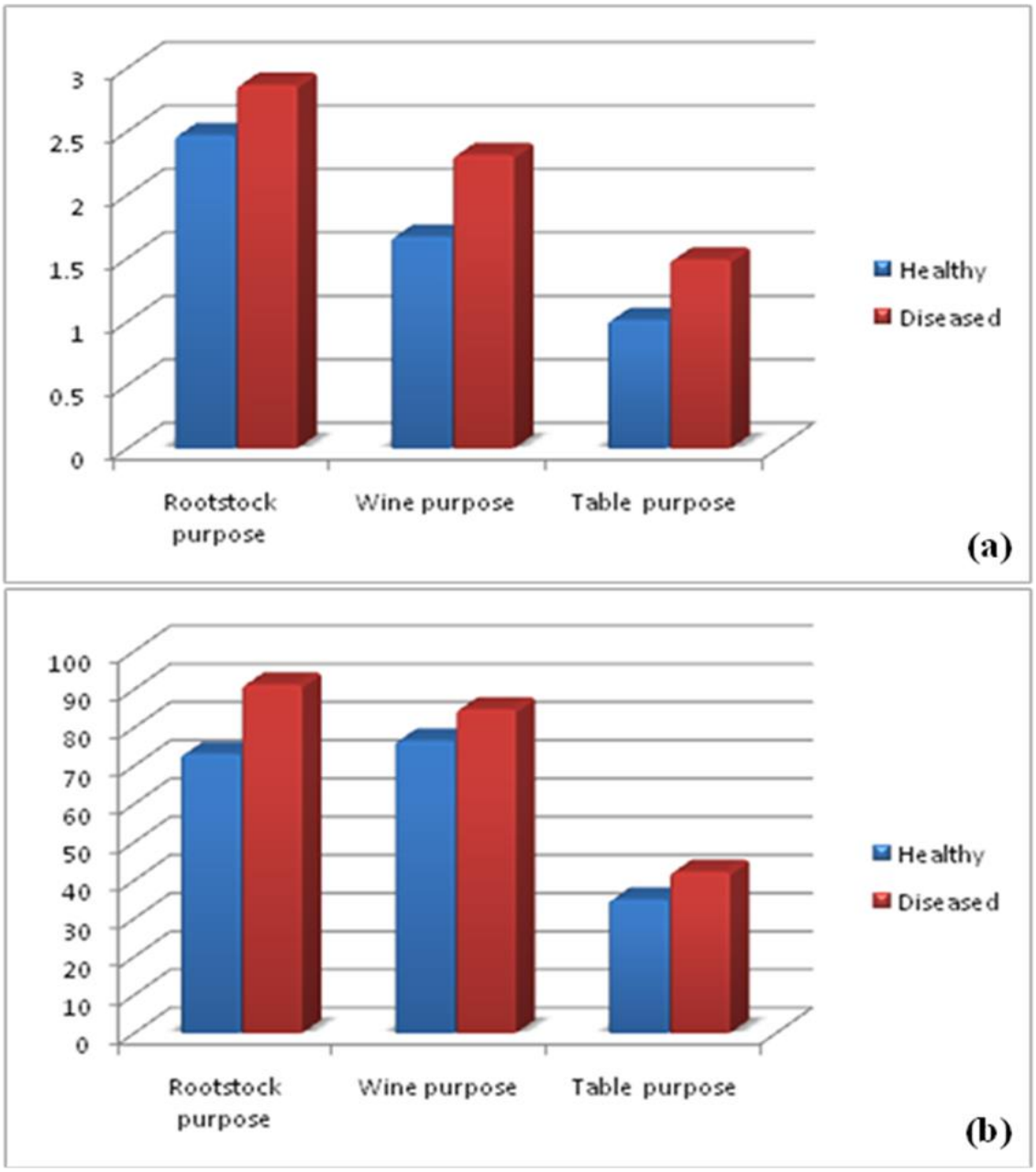

Fig. 5.4. Comparative polyphenol oxidase and peroxidase activities (g /min.) in healthy and anthracnose infected leaves of some rootstock, wine purpose and table purpose grape genotypes.

Table 5.5. Phenylalanine ammonia lyase activity and malondialdehyde content in healthy and diseased leaves of some grape genotypes as affected by anthracnose incidence.

Genotype	PAL activity in (µmol of trans cinnamic acid/ mg protein/ h)		MDA (nmol/ g)	
	Healthy	Diseased	Healthy	Diseased
Rootstock				
Male Hybrid	4.23^{ef}	11.40^{c}	2.40^{c}	3.80^{bc}
3309-C	8.40^{c}	12.06^{b}	3.60^{b}	4.13^{b}
110-R	9.13^{b}	11.36^{c}	3.76^{ab}	4.30^{b}
Dog Ridge	10.16^{a}	13.56^{a}	4.20^{a}	5.33^{a}
H-144	8.26^{c}	10.40^{d}	3.86^{ab}	4.03^{bc}
Mean	**8.04**	**11.76**	**3.56**	**4.32**
Wine purpose				
Chardonnay	6.86^{d}	7.20^{g}	1.80^{def}	2.36^{de}
Chenin Blanc	7.16^{d}	7.86^{f}	2.20^{cd}	2.60^{d}
Syrah	8.33^{c}	8.43^{e}	1.80^{def}	2.10^{ef}
Tempranillo	7.90^{c}	8.46^{e}	1.63^{ef}	2.26^{def}
Merlot	6.80^{d}	7.36^{fg}	1.46^{f}	2.20^{def}
Mean	**7.41**	**7.86**	**1.78**	**2.30**
Table purpose				
Pusa Urvashi	3.40^{g}	3.50^{h}	1.40^{f}	2.13^{ef}
Centennial Seedless	3.20^{g}	4.76^{jkl}	1.53^{f}	1.80^{g}
Pusa Seedless	4.20^{ef}	4.90^{ij}	2.06^{cde}	2.43^{de}
Hybrid 70-56	3.73^{fg}	4.40^{klm}	1.86^{def}	2.56^{d}
Alumwick	4.46^{e}	5.40^{hi}	1.43^{f}	3.53^{c}
Cardinal	3.60^{fg}	4.10^{m}	1.80^{def}	2.43^{de}
Anab-E-Shahi	4.50^{e}	5.30^{ij}	1.60^{ef}	2.50^{d}
Perlette	3.13^{g}	4.23^{lm}	1.40^{f}	1.90^{g}
Hur	4.26^{ef}	5.90^{h}	1.76^{def}	2.60^{d}
Fakri	4.60^{e}	5.20_{ij}	1.73^{def}	2.50^{d}
Mean	**3.91**	**4.77**	**1.66**	**2.44**
Grand mean	**5.90**	**7.40**	**2.18**	**2.89**
CV (%)	**6.76**	**4.24**	**11.87**	**10.43**

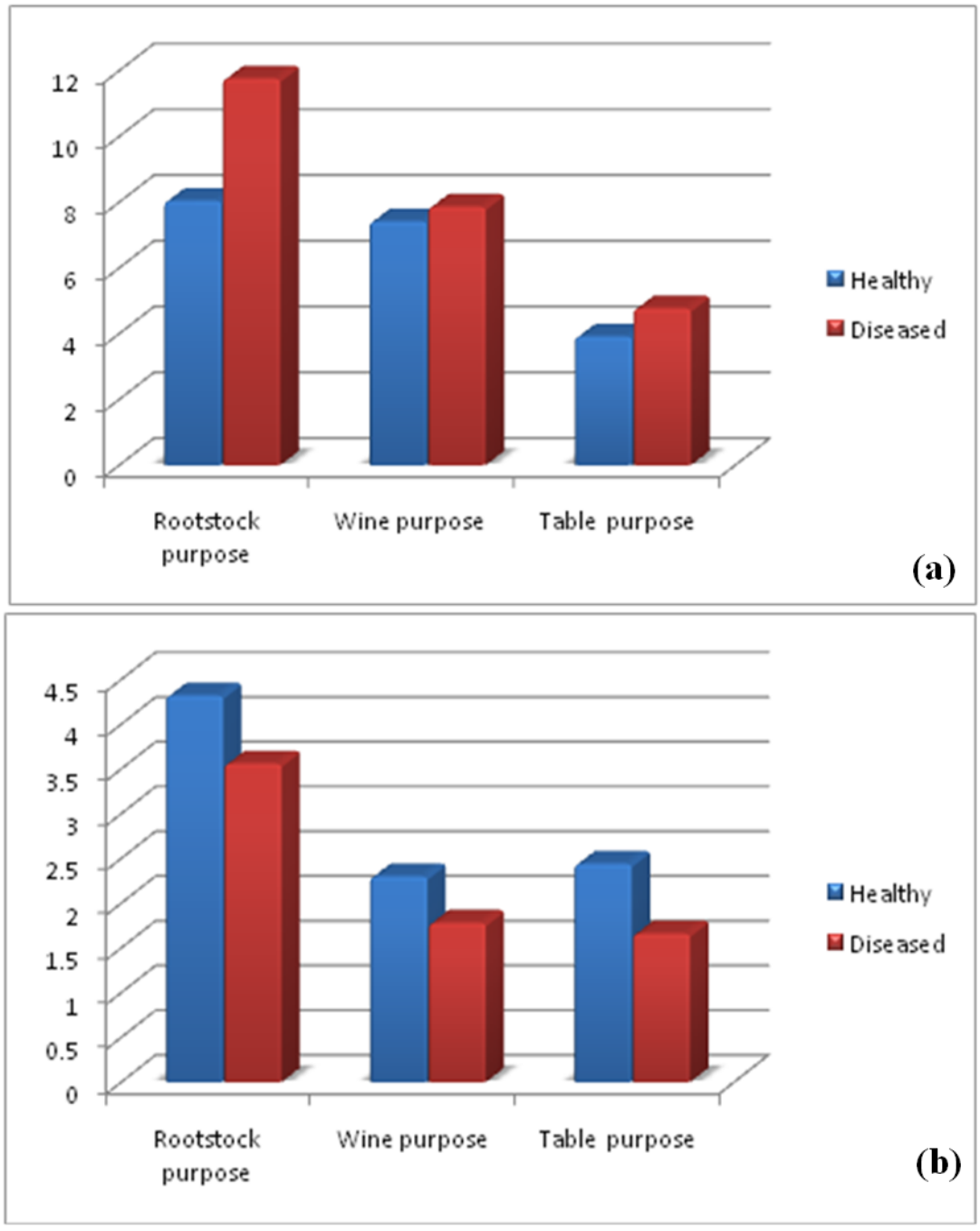

Fig. 5.5. Comparative (a) phenylalanine ammonia lyase activity (µmol of trans-cinnamic acid/ mg protein/ h) and (b) malondialdehyde content in healthy and anthracnose infected leaves of rootstock, wine purpose and table purpose grape genotypes.

Table 5.6. Correlation of different biochemical parameters with Disease Severity Index.

	Total phenols	Proline	Total sugars	Reducing sugars	PPO	POD	MDA	PAL	Chl a	Chl b	Total chl	Carotenoids	DSI
Total phenols	**1.000**	**0.327**	**-0.844**	**-0.729**	**0.824**	**0.705**	**0.621**	**0.797**	**-0.271**	**-0.627**	**-0.591**	**-0.366**	**-0.837**
Proline		**1.000**	**-0.177**	**-0.248**	**0.254**	**0.414**	**0.244**	**0.403**	**-0.435**	**-0.201**	**-0.281**	**0.012**	**-0.351**
Total sugars			**1.000**	**0.684**	**-0.827**	**-0.748**	**-0.554**	**-0.739**	**0.136**	**0.582**	**0.666**	**0.413**	**0.744**
Reducing sugars				**1.000**	**-0.708**	**-0.703**	**-0.632**	**-0.700**	**0.288**	**0.412**	**0.348**	**0.511**	**0.785**
PPO					**1.000**	**0.901**	**0.686**	**0.869**	**-0.275**	**-0.775**	**-0.616**	**-0.533**	**-0.811**
POD						**1.000**	**0.767**	**0.899**	**-0.414**	**-0.736**	**-0.670**	**-0.520**	**-0.840**
MDA							**1.000**	**0.842**	**-0.353**	**-0.524**	**-0.524**	**-0.397**	**-0.758**
PAL								**1.000**	**-0.489**	**-0.766**	**-0.727**	**-0.475**	**-0.908**
Chlorophyll a									**1.000**	**0.415**	**0.508**	**0.506**	**-0.378**
Chlorophyll b										**1.000**	**0.701**	**0.537**	**-0.462**
Total chl											**1.000**	**0.520**	**-0.435**
Carotenoids												**1.000**	**0.619**
DSI													**1.000**

CHAPTER 6 Discussion

Anthracnose of grapes is an economically devastating disease caused by the fungus, *Elsinoe ampelina* (de Bary) Shear. Symptoms usually appear as numerous circular spots, which enlarge then become sunken and produce lesions with round edges. Once established in a vineyard, the disease can be very destructive. The pathogenic fungus, which attacks all aerial parts of the plants, such as fruits, leaves, tendrils and petioles, is of considerable economic importance. The fungus over-winters in dormant and dead canes, making it very difficult to control. Strategies for the control of anthracnose in grapevines, such as developing resistant cultivars are necessary in order to reduce the production cost and environmental impacts of fungicide applications in areas of high disease pressure. For this purpose, the selection of genetic resources showing tolerance to anthracnose is a prerequisite for any breeding programme. Variations in resistance or tolerance to anthracnose among grape species have been reported. For instance, *Vitis vinifera* was highly susceptible, while *V. labrusca* and their hybrid were resistant, or moderately resistant, and *V. rotundifolia* was immune to *E. ampelina* (Kitajima, 1989; Mortensen, 1971; Olmo, 1971; Vidhyasekaran and Charan, 1972; Wang *et al.,* 1998). Within a given species, grape genotypes behave differently in their response to the intensity of disease occurrence. Besides, inherent characteristic of the genotype the tolerance/ resistance level is also dependent on host-plant relationship and prevailing environmental conditions.

Keeping in view all these aspects, the present study was conducted to screen the grape genotypes under field conditions belonging to three different categories, *i.e.*, rootstocks, wine and table purpose, against anthracnose and to know the relationship between biochemical parameters and disease resistance. Severity of disease was also correlated with weather parameters, *viz*. maximum and minimum temperatures, atmospheric relative humidity and duration of sunshine hours to know the ideal conditions for development of the disease.

Besides this, some morphological traits, *i.e*., leaf length, width, leaf area, leaf texture, vine thickness, internodal length and vine and leaf surface texture were also recorded.

The presence of trichome on leaf and vine did not prove restriction in infection unlike mildews of grape where tolerance is correlated with presence of hairiness and glabrous leaves or vine surface. It was observed that most of the rootstocks had small leaves; wine purpose genotypes had the medium sized leaf, while table purpose had either medium or long leaves length. It was found that there was no direct relationship between vegetative parameters and disease severity index. No clear relation between ampelo-graphic characteristics and susceptibility or resistance to anthracnose could be determined.

The cuticle, stomata and cell wall are the first barriers to infection for an invading microorganism. In order to penetrate the cuticle, pathogens must either use physical force and/ or produce cutin-degrading enzymes (Kolattukudy, 1985). The cell wall is a highly organised barrier around the cell and prevents invasion by all microorganisms except those that can overcome this chemical and physical barrier. For pathogens that infect through direct penetration, such as E. necator, the thickness of the cuticle can affect the success with which a pathogen invades a host (Martin, 1964), though Ficke *et al*. (2004) found cuticle thickness not to be a factor in ontogenic resistance in grape. More studies are needed to conclude if morphological traits play any role or not in anthracnose resistance.

The grape genotypes including rootstock, wine and table purpose genotypes were screened under field conditions against anthracnose during August-September when anthracnose symptoms were fully developed. Results revealed that table purpose genotypes in general were susceptible to the disease as most of them belonged to *V. vinifera* except Black Prince, which showed the highest degree of resistance among table purpose genotypes (DSI = 5.75) and categorized as highly resistant (HR) type. Among all the genotypes screened the lowest DSI was noticed in Hybrid-144 (DSI = 1.64), whereas Perlette showed

highest disease severity index (DSI = 85.70). Among rootstocks, Salt Creek showed highest disease severity index (DSI = 17.98) and was least resistant to the disease among all the rootstocks. Of the nine wine making genotypes four genotypes, namely, Chardonnay (28.5), Chenin Blanc (36.8), Merlot (31.6) and Tempranillo (32.0) showed moderate resistance against disease. No genotype was found to be immune against disease. Inherent resistance in grape genotypes is believed to be due to unique genetic make up and also the parents involved in their pedigree. Besides, differential inherent bio-synthesis of different biochemicals as a result of host-pathogen relationship could be the key in selecting genotypes for use as parents in breeding. Disease reaction of different grape genotypes against anthracnose has previously been examined by various workers worldwide (Singh and Bakshi, 1971; Goyal *et al*., 1971; Bedi *et al*., 1986; Patil and Rao, 1990; Shelke *et al*., 1990; Jindal *et al*., 2000; Yuan and Keun, 2006; Li and Wang, 2008; Yanminal *et al*., 2010; Poolsawat *et al*., 2012). Jindal and Shankar (2001) screened 48 genotypes against anthracnose and reported that out of these genotypes H-144 (Cheema Sahebi x Catwaba), *Vitis parviflora* (7.49) and its hybrids were resistant, while Banqui-Abyad x Beauty Seed1ess 74-10 (75.81) was the most susceptible. Yuan and Keun (2006) found that ‘Black Eye’, ‘Mario’, ‘Niunai’, ‘Rizamat’, and “Rosario Bianco' were sensitive, while 'Campbell Early', 'Niagara', and 'Honey Red' were tolerant to anthracnose. Very recently, Poolsawat *et al*. (2012) carried out field evaluation of grape genotypes for resistance against anthracnose and classified ‘Wilcox 321’, ‘NY88.0507.01’, ‘NY65.0550.04’ and ‘Illinois 547-1’ as resistant lines useful as parents for future breeding programmes.

Anthracnose incidence has been reported to be greatly influenced by the epidemiological factors such as rainfall, relative humidity and temperature. Correlation of weather parameters and disease severity index showed that relative humidity (r = 0.912) and minimum temperatures (r = 0.779) were positively correlated with disease severity whereas maximum temperature (r = -

0.560) and sunshine hours (r = -0.706) were found to be negatively correlated. During high anthracnose incidence period (August-September), the maximum and minimum temperature ranged between 31^0-34^0C and 25^0- 26^0C respectively with corresponding high relative humidity (76-80%) and lowering sunshine hours (4-6 h.). Hence, it can be concluded that drop in maximum temperature up to 35^0C with rise in minimum temperature up to 26^0C and rise of relative humidity up to 80% and gradual decline in sunshine hours to 4 to 6 hours may conditioning for appearance of maximum disease intensity. Earlier, Pampanagouda and Benagi (2005) also observed that maximum temperature of 30-34^0C with constant minimum temperature and increased minimum and maximum relative humidity due to rains favoured conditions for rapid development or spread of anthracnose fungus on plants.

Some of the biochemical substances are energy materials of self defence reactions, or products of injury, which have some correlation with plant resistance (Deng, 2006). In present investigation, the relationship between the resistance of different grape genotypes to anthracnose and biochemical characters were systematically analyzed and identified indicators for resistance were defined. Several biochemicals including enzymes are enhanced as a result of hyper-sensitive response caused due to fungal infection. The cellular damage cause rise in phenolics leading to necrosis and enhanced antioxidant enzyme activities. It is possible to use these indicators for the screening of anthracnose resistance levels of different grape genotypes. Among biochemical parameters; proline, total phenols, reducing and total sugars, malondialdehyde and activities of certain enzymes like polyphenol oxidase, peroxidase and phenylalanine ammonia lyase were found to be enhanced. In present study, reduction in the contents of chlorophylls and carotenoids in disease affected leaves in comparison to healthy leaves 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.

Results of present investigation revealed highest content of total chlorophylls in susceptible genotypes. Zhou *et al*. (2012) also reported that total chlorophylls were negatively correlated with disease incidence (r = -0.732) and disease index (r = 0.657) in *Verticillium* wilt infected eggplant genotypes. Total sugars and reducing sugar content in the leaves is one of the most important parameters to categorize genotypes to particular disease reaction, *viz*., Resistant or susceptible. Present investigation revealed that, disease affected leaves of susceptible genotypes had higher content of total sugars (7.91 mg/g FW) compared to resistant genotypes (5.77 mg/g FW). Hybrid 70-56 (3.99 mg/g FW) followed by Fakri (3.97 mg/g FW) had highest contents of reducing sugars, and accordingly both of which showed high degree of susceptibility to disease. Lowest content of reducing sugars was found in Male Hybrid (2.46 mg/g FW), which was extremely resistant to the disease. Total sugars (r = 0.744) and reducing sugars (r = 0.785) showed significant positive correlation with disease severity index. Findings of this study are in the support of the results of Mohan Raj *et al*. (1972), Bindra and Kapoor (1979) and Prakash *et al.* (2011).

During abiotic stress, proline was proposed to act as a compatible osmolyte, carbon and nitrogen storage and pH stabilizer (Hare and Cress, 1997). Proline accumulation increased during stress; therefore, level of proline may surpass that of other amino acids to become the dominant amino acid, suggesting that proline is a major player in signalling. Results of present experiment also indicated increase in proline accumulation due to the stress caused by the pathogen *Elsinoe ampelina* (de Bary) Shear. In general, rootstocks had higher proline content followed by wine purpose and table purpose genotypes. These results get support from earlier findings of Goicoechea *et al.* (2000), Sivritepe *et al.* (2009) and Naglaa *et al.* (2011). 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 first stage of the defence mechanism of the plants involves rapid accumulation of phenols at infection site, which function to slow down the growth of pathogens. Results of this study showed increase in phenol content in diseased leaves (4.04 mg/g FW) as compared to healthy leaves (3.38 mg/g FW). Similar results were earlier reported by Ruelas *et al.* (2006) who observed that during pathogenic attack, tomato fruit respond by increasing the concentration of vanillic acid in the epicarp. Among all genotypes, Hybrid-144 showed the highest amount of total phenols (5.71 mg/g FW), whereas lowest phenol content was observed in Perlette (2.66 mg/g FW). Total phenols were found to be significant negative correlated with disease severity index (r = -0.837). The results of present investigation get the support from earlier findings of Jindal (2001), Prasath and Ponnuswami (2008) and Kaur *et al.* (2011). Raj *et al.* (2004) opined that proline -a stress induced amino acid was prominent in negating infection of pearl millet downy mildew disease.

Enzymes are known to play decisive role in host pathogen interaction. They are involved in biosynthesis of different compounds with biocidal activities. Activities of defensive enzymes (PPO, POD and PAL) and MDA contents were investigated in present investigation. Higher enzymatic activities of PPO (66.13 g/min.), POD (1.56 g/min.) and PAL (7.40 µmol of trans-cinnamic acid/mg protein/h) were recorded in infected leaves as comparison to healthy one (55.77 g/min.; 1.56 g/min.; 5.90 µmol of trans-cinnamic acid/mg protein/h, respectively) and the resistant genotypes expressed more enzymatic activities than the susceptible ones. In similar way, the sharp increase in PPO and POD activities were observed due to infestation by anthracnose pathogen in chilli plants (Bharath *et al.*, 2004; Borua, 2000). Correlation studies indicated that the activities of PPO (r = -0.811), POD (r = -0.840) and PAL (r = -0.908) were significantely negatively correlated with disease severity index. Findings of this study get support from earlier reports of Campos *et al.* (2004) where they observed positive correlation among peroxidase and polyphenol oxidase activity

and anthracnose resistance in bean. Several workers believe that the characterization of germplasm based on biochemical composition is useful in identifying suitable sources and potential accessions for a given trait. The genotypes could be Rootstocks accordingly may be grouped into high phenol accumulators, high carbohydrate and high sugar accumulators. It is well established that the species of the genus *Vitis* (*V. rotundifolia*) that have a higher content of polyphenols are more resistant to disease infection caused by powdery mildew and, when infected, produce larger amounts of polyphenols than genotypes which are more susceptible (Dai *et al.*, 1994). Dai *et al.* (1994) classified the grape species *V vinifera* as susceptible, *V rupestris* as intermediate and *V rotundifolia* as resistant. The resistant variety showed an enriched content of gallic acid derivatives and catechin tannins compared to the sensitive varieties. Relationship between agronomical traits and biochemical variation has been demonstrated for comparison between tolerant and susceptible genotypes within bunch and/ or within muscadine genotypes that allows a better description of the biochemical and genetics of vascular tissues during disease occurrence in grapes (Jain and Basha, 2003).

In response to *Botrytis cinerea* infection, the leaves of *Vitis* spp. produce the stilbene, resveratrol, etc. Stilbene is converted into the antifungal trimer E-viniferin. In grapevine leaves, an efficient elicitation factor may alter the activity of the rate-limiting enzyme, leading to stilbene formation. Stilbene synthase activity is the rate-limiting step, and various biotic and UV-light treatments were able to activate the stilbene synthase gene to different extents (Schroder *et al*., 1988).

Our study was performed in a natural environment, and it was noted that there was an increase in MDA content due to disease infection, which reflects lipid peroxidation and therefore causing some level of stress mitigation, membrane damage and photo-oxidation of chlorophyll. Levels of carotenoids and activities of the antioxidant enzymes, catalase, ascorbate peroxidase and

peroxidase were elevated by during stress caused due to abiotic stress like high UV-B application as reported by Berli *et al.* (2009).

Grapevine species (*Vitis* sp.) are prone to several diseases, fungi being the major pathogens compromising its cultivation and economic profit around the world. Knowledge of the complexity of mechanisms responsible for resistance to fungus infection of cultivars, is necessary for strategies to be defined which will improve resistance in highly susceptible crop species. The differences in transcripts and metabolites detected in terms of the metabolic pathways and their possible role in plant defence against pathogen attack, as well as their potential interest to discriminate among resistant and susceptible grapevine cultivars has to be elucidated (Figueiredo *et al.*, 2008). Plant defence is attributed to both the constitutive mechanisms and active mechanisms induced by the invading pathogen. The success of active resistance depends on both the rapidity and the intensity of plant response to infection. The specific interaction between host and pathogen is crucial to the success of the resistance or the pathogen invasion, and is mediated by many pathways involved in producing or detecting elicitors, enhancers, suppressors and secondary signals.

Although there is extensive research going on disease control management and breeding programmes for resistant varieties but there is necessity to rich diverse genotypes to strengthen the breeding programmes. In this study an attempt was made to correlate different biochemical parameters with disease severity index. Among all the parameters activity of phenylalanine ammonia lyase ($r = -0.908$), peroxidase ($r = -0.840$) and polyphenol oxidase ($r = -0.811$) were significantly correlated with disease severity index, hence selection for these traits will be useful in screening of grape germplasm to find out the resistant genotypes for developing anthracnose resistant hybrids. Further studies are needed on relevant biochemical processes, to reveal the mechanism of resistance to anthracnose of grapevine. Similar results were earlier reported by Jindal and Rao (2000) and Shankar and Jindal (2001).

CHAPTER 7 Summary and Conclusion

Anthracnose disease of grape is an important yield reducing factor in different commercial viticulture areas. In sub-tropical regions the crops escape the damage as it occurs after harvesting of crop. However, in the last few years due to erratic weather pattern its occurrence during crop season could be increased which reduce the yield and quality. Hence, a study entitled "**Biochemical basis of anthracnose [*Elsinoe ampelina* de Bary) Shear] tolerance in grape (*Vitis* sp.)"** was conducted to screen 45 grape diverse genotypes based on the field reaction to anthracnose and also to know the biochemical basis of resistance towards disease incidence. The study was carried out during 2012-2013. The salient findings are summarized hereunder:

7.1 Field Screening and disease severity

- Eight leaf and vine parameters were examined and results indicated that there was considerable variation among the genotypes with regard to their unique morphology. None of the morphological parameter had much correlation with disease incidence. Though, the trait like occurrence of trichomes (hairs) on under surface of leaf and vine did not lead to low incidence of anthracnose.
- Disease severity index was calculated based on occurrence of symptoms during August-September and it was found to be highest in Perlette (85.7) followed by Hybrid 70-56 (85.3), whereas it was lowest in H-144 (1.64).
- Based on disease reaction, Dog Ridge (93.12), H-144 (1.64), Male hybrid (2.47) and 3309-C (3.36) rootstock were grouped as extremely resistant. Among wine purpose genotypes, Chardonnay (28.5), Chenin Blanc (36.8), Merlot (31.6) and Tempranillo (32.0) were found to be moderately resistant, whereas Sauvignon Blanc and Ugni Blanc were susceptible. Among table purpose genotypes, Black Prince followed by Pusa Navrang had good resistance against anthracnose, whereas Perlette and Hybrid 70-56 were found to be highly susceptible. No genotype was found to be immune.

b. Role of weather parameters in disease development

- Drop in maximum temperature up to 34^0C with rise in minimum temperature up to 26^0C and maintenance of relative humidity up to 80% and decrease in sunshine hours (4-5) created ideal conditions for appearance of maximum disease intensity.

c. Biochemical basis of resistance

- Leaf pigments, namely, chlorophyll and carotenoids decreased with disease progression and severity. The highest total chlorophyll content was estimated in H-144 (1.19 mg/ g FW); whereas, Pusa Urvashi had lowest (0.72 mg/ g FW) contents. Cultivar, Anab-E-Shahi showed the highest (2.34 mg/g FW) carotenoids among all genotypes studied.
- Total and reducing sugars were found to be increased due to disease appearance. The highest total sugars (8.89 mg/g FW) were found in Hybrid 70-56, whereas lowest (5.68 mg/g FW) were found in extremely resistant genotype 3309-C. The lowest content of reducing sugars (2.46 mg/g FW) was found in Male Hybrid, which was extremely resistant to the disease. Table purpose genotypes had the highest increment in total and reducing sugars amongst all three groups studied.
- Increase in proline accumulation upon pathogen infection was noticed in all the genotypes. The highest proline content (98.68 µg/ g FW) was observed in Dog Ridge, whereas Cardinal showed the lowest (57.09 µg/g FW) content among all the genotypes studied.
- Irrespective of genotypes, the total phenols content in disease affected leaves (4.04 mg/g FW) was higher than healthy leaves (3.38 mg/g FW). Hybrid-144 had the highest (5.71 mg/g FW) total phenols, whereas lowest (2.66 mg/ g FW) was estimated in Perlette.
- Infected leaves had enhanced activities of polyphenol oxidase (PPO) (66.13 g/min.), peroxidase (POD) (1.56 g/min.) and phenylalanine ammonia lyase (PAL) (7.40 µmol of trans-cinnamic acid/mg protein/h) and MDA content (2.18

nmol/ g) in comparison to healthy leaves (55.77 g/ min.; 1.56 g/min.; 5.90 µmol of trans-cinnamic acid/mg protein/ h and 2.44 n mol/g, respectively). The resistant genotypes expressed more enzymatic activities than the susceptible counterparts. Amongst the groups, table purpose genotypes showed lower MDA levels and increase in enzymatic activities, while it was highest in rootstocks.

d. Disease incidence and Correlation studies

- Correlation estimated between disease severity and biochemical parameters showed that total chlorophylls (r = -0.435), activities of PPO (r = -0.811), POD (r = -0.840), PAL (r = -0.908) and MDA (r = -0.758) contents were negativly correlated with disease severity whereas, total and reducing sugars had positive correlations (0.744 and 0.781). Total phenols showed higher negative correlation (r = -8.37) with DSI, whereas proline was found to be moderately negatively correlated (r = -0.351) with disease severity index.

Conclusion

From the present study it can be concluded that occurance of anthracnose incidence under field condition was due to the maximum (31^0-33^0C) and minimum (25^0-26^0C) temperatures, high relative humidity (75-82%) and decline in sunshine hours from normal of 6-8 to 4-5 hours. Out of the 45 genotypes screened, Black Prince, Pusa Navrang, Chardonnay, Chenin Blanc, Merlot and Chenin Blanc were identified as potential donor parents for developing anthracnose resistant hybrids. Total phenol content, and activities of PAL, PPO and POD were identified as biochemical parameters for screening genotyppes and progenies for anthracnose tolerance.

Grape in India, is greatly affected by occurrence of several diseases, *viz*. powdery mildew, anthracnose, downy mildew, *Cercospora* leaf spot etc. Of these diseases, anthracnose [*Elsinoe ampelina* de Bary) Shear] of grape commonly referred as 'bird's eye spot' is an important disease causing upto 15-30% yield reduction and loss in quality. The present investigation was undertaken to screen 45 diverse grape genotypes including rootstocks, wine making and table purpose varieties under sub-tropical conditions based of eight morphological parameters and the prevailing weather conditions. It was found out that there was no direct correlation of leaf and vine parameters on disease incidence. Disease severity index (DSI) calculated during the peak disease incidence, *i.e.* 1st September showed that Dog Ridge, Male Hybrid and H-144 to be extremely resistant (DSI = <5.0); St. George, SO4, and 110-R as highly resistant; Salt Creek and Pusa Navrang as resistant; Sauvignon, Ugni Blanc and Centennial Seedless as susceptible; Anab-E-Shahi, Fakri, Kishmish Beli and Cardinal as highly susceptible, while Hur, Hybrid 70-56, Perlette and Alumwick were extremely susceptible (DSI = >85.0) to anthracnose. In general, genotypes belonging to rootstock group showed higher resistance (DSI = 5.88) followed by wine making genotypes (DSI = 42.1), whereas table purpose genotypes were least resistant (DSI = 57.11). Correlation studies between DSI and weather parameters indicated that relative humidity (r = 0.912) and minimum temperature (r = 0.779) were positively correlated with occurrence of anthracnose disease under sub-tropical conditions, whereas maximum temperature (r = -0.560) and sunshine hours (r = -0.706) were negatively correlated. Twenty genotypes representing the three groups were analysed for different biochemical parameters, *i.e.* pigments, biochemical substances and activities of defence related enzymes in healthy and anthracnose infected leaves. The chlorophyll 'a', 'b', total chlorophylls and carotenoids contents declined upon disease infection. The concentrations of total phenols and proline increased in resistant as well as susceptible genotypes, but the increase was more pronounced in resistant genotypes. Higher contents of total and reducing sugars were observed in infected leaves of susceptible genotypes (7.91 mg/g FW; 3.53 mg/ g FW) as compared to resistant genotypes (5.97 mg/g FW; 2.64 mg/g FW). Biochemical analysis revealed an increase in activities of defence related enzymes peroxidase (PPO), polyphenol oxidase (POD), phenylalanine ammonia lyase (PAL) and

malondialdehyde (MDA) content upon disease infection. Correlation between DSI and biochemical parameters showed that activities of PPO (r = -0.811), POD (r = -0 840) and PAL (r = -0.908), MDA content (r = -0.758) were significantely negatively correlated with DSI, whereas total phenols (r = -8.37) and proline (r = -0.351) contents were found to be negatively correlated. These traits can be used as biochemical markers to identify anthracnose resistant genotypes, which can be included in breeding programmes. Carotenoids (r = 0.619), total and reducing sugars (r = 0.744; r = 0.785) showed positive correlations, wheares total chlorophyll was found to be negatively correlated (r = -0.435). The genotypes Black Prince (DSI = 5.57), Pusa Navrang (DSI = 18.8), Chardonnay (DSI = 28.5), Merlot (DSI = 31.6), Tempranillo (DSI = 32.0) and Chenin Blanc (DSI = 36.8) were found to have good resistance/tolerance to anthracnose and could be used as donor parents in breeding programmes. It was also revealed high relative humidity coupled with higher mean maximum temperature (31-34^{0}C) and drop in sunshine hours from 8-10 to 4-6 hours favoured weather conditions for high incidence of anthracnose.

अंगूर में ऐंथ्रेक्नोज़ [एल्सिनोय एम्पेलीना (डी बैरी) शीयर] का जैव–रासायनिक आधार

सार

भारत में अंगूर, कई रोगों यथा, चूर्णी फफूँद, ऐंथ्रेक्नोज, मृदुरोमिल फफूँद, *सर्कोस्पोरा* पर्ण धब्बा आदि के आ जाने से दुष्प्रभावित होता है। इन रोगों में, ऐंथ्रेक्नोज [एल्सिनोथ एम्पेलीना (डी बैरी) शीयर], जैसे सामान्यतया 'बर्ड्स आई स्पॉट' कहा जाता है, एक महत्वपूर्ण रोग है जिसके कारण उपज में 15–30% की क्षति एवं गुणवत्ता में कमी आती है। प्रस्तुत अध्ययन का उद्देश्य, आठ आकारिकीय प्राचलों एवं विद्यमान मौसम संबंधी परिस्थितियों के आधार पर उपोष्ण कटिबंधी परिस्थितियों क अन्तर्गत प्रकंदों, शराब के निर्माण एवं घरेलू उद्देश्य के लिए प्रयुक्त किस्मों के समावेश वाले, अंगूर के विविध 45 जीनप्ररूपों का विविक्तकर निरीक्षण करना था। यह पाया गया कि रोग आपतन हेतु पत्ती एवं लता के प्राचलों का कोई सीधा सहसंबंध नहीं था। सर्वाधिक रोग–आपतन वाले समय अर्थात 1 सितम्बर, पर रोग उग्रता घातांक (डी एस आई) की गणना ने दर्शाया कि ऐंथ्रेक्नोज़ के लिए डॉग रिज, मेल हायब्रिड एवं एच–144, अत्यंत रोगरोधी (डी एस आई = < 5.0); सेंट जॉर्ज, एस ओ 4 एवं 110–आर, उच्च स्तरीय रोगरोधी; साल्ट क्रीक एवं पूसा नवरंग, रोगरोधी; सौविगनॉन, अग्नि ब्लेंक एवं सेंटीनियल सीडलैस सुग्राही हैं; अनाब–ए–शाही, फाकरी, किशमिश बेली एवं कार्डिनल उच्च स्तरीय सुग्राही हैं जबकि हूर, हायब्रिड 70–56, पर्लेट एवं एलमविक अत्यधिक सुग्राही (डी एस आई = > 85.0) हैं। सामान्य रूप से, प्रकंद समूह के अन्तर्गत आने वाले जीनप्ररूपों ने उच्चतर प्रतिरोधिता (डी एस आई = 5.88) दर्शायी, तत्पश्चात शराब बनाने वाले जीनप्ररूपों (डी एस आई = 42.1) का स्थान रहा जबकि घरेलू आहार में काम आने वाले जीनप्ररूपों की रोगरोधिता सबसे कम (डी एस आई = 57.11) थी। डी एस आई एवं मौसम संबंधी प्राचलों के बीच सहसंबंध–अध्ययनों ने दर्शाया कि उपोष्ण कटिबंधी परिस्थितियों के अन्तर्गत आपेक्षित आर्द्रता (आर = 0.912) एवं न्यूनतम तापमान (आर = 0.912) का ऐंथ्रेक्नोज़ रोग आने के साथ घनात्मक सहसंबंध था जबकि अधिकतम तापमान (आर = –0.560) एवं धूप वाले घन्टों (आर = –0.706) का ऋणात्मक सहसंबंध था। तीन समूहों के बीस जीनप्ररूपों का विभिन्न जैवरासायनिक प्राचलों अर्थात स्वस्थ एवं ऐंथ्रेक्नोज़ से संक्रमित पत्तियों में वर्णकों, जैव रासायनिक पदार्थों एवं रक्षा–संबंधी एन्ज़ायमां की सक्रियताओं, हेतु विश्लेषण किया गया। रोग–संक्रमण होने पर हरितवर्ण 'ए', 'बी', कुल हरितवर्ण एवं कैरोटिनॉयड्स के अंश कम हो गए। कुल फीनोल्स एवं प्रोलीन की सान्द्रताएं रोगरोधी एवं सुग्राही दोनों में बढ़ गईं किन्तु रोगरोधी जीनप्ररूपों में यह बढ़ोतरी अधिक थी। रोगरोधी जीनप्ररूपों (5.97 मि ग्रा/ग्रा एफ डब्ल्यू; 2.64 मि ग्रा/ग्रा एफ डब्ल्यू) की तुलना में सुग्राही जीनप्ररूपों की सवंमित पत्तियों (7.91 मि ग्रा/ग्रा एफ डब्ल्यू; 3.53 मि ग्रा/ग्रा एफ डब्ल्यू) में कुल शर्करा एवं अपचायक शर्करा के अधिक अंश देखे गए। जैवरासायकि विश्लेषण ने दर्शाया कि रोग–संक्रमण होने पर

रक्षा–संबंधी एन्ज़ायमों परॉक्सीडेज़ (पी पी ओ), पॉलीफीनोल ऑक्सीडेज़ (पी ओ डी), फिनायलएलेनाइन आमोनिया लायेज़ (पी ए एल) की सक्रियताओं एवं मैलोंडीएल्डिहायड (एम डी ए) अंश में बढ़ोतरी होती है। डी एस आई एवं जैवरासायनिक प्राचनों के बीच सहसंबंधी दर्शाता है कि पी पी ओ (आर = –0.811), पी ओ डी (आर = –0.840) एवं पी ए एल (आर = –0.908) की सक्रियताएं तथा एम डी ए अंश (आर = –0.758) की डी एस आई के साथ महत्वपूर्ण रूप से ऋणात्मक सहसंबंध है जबकि कुल फीनोल्स (आर = –8.37) एव प्रोलीन (आर = –0.351) अंशो के साथ ऋणात्मक सहसंबंध पाया गया। ऐंथ्रेक्नोज़ रोगरोधी जीनप्ररूपों की पहचान करने के लिए इन गुणों का, जैव रासायनिक चिह्नकों के रूप में उपयोग किया जा सकता है। जिनका प्रजनन–कार्यक्रमों में समावेश हो सकता है। कैरोटिनॉयड्स (आर = 0.619), कुल एवं अपचायक शर्कराओं (आर = 0.785) ने धनात्मक सहसंबंध दर्शाए जबकि कुल हरितवर्ण का सहसंबंध ऋणात्मक (आर = 0.435) पाया गया। जीनप्ररूपों ब्लैक प्रिंस (डी एस आई = 5.57), पूसा नवरंग (डी एस आई = 18.8), कार्डोनाय (डी एस आई = 28.5), मर्लोट (डी एस आई = 31.6), टेम्प्रानिलो (डी एस आई = 32.0) एवं चेनिन ब्लैंक (डी एस आई = 36.6) में ऐंथ्रेक्नोज़ के लिए अच्छी प्रतिरोधिता सहनशीलता पायी गई और इनका प्रजनन–कार्यक्रमों में दाता जनाकों के रूप में उपयोग हो सकता है। यह भी देखा गया कि ऐंथ्रेक्नोज़ रोग के उच्च आपतन के लिए अधिकतम तापमान का उच्चतर औसत (31–34°सें) और 8–10 घन्टों के बजाय धूप के घन्टे 4–6 घन्टे हो जाने की मौसम संबंधी परिस्थितियाँ अनुकूल होती हैं।

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ANNEXURES

Annexure 1

Mean fortnightly weather data during April-May and August-September 2012.

Sl. No.	T_{max} (^{0}C)	T_{min} (^{0}C)	Relative humidity (%)	Sunshine hours
1st-15th April	35.14	19.20	54.96	6.82
16th-30th April	35.67	19.40	52.56	7.76
1st-15th May	38.70	22.13	43.00	8.01
16th-31st May	42.54	25.42	30.37	7.22
1st-15th August	33.94	26.05	75.63	1.72
16th-31st August	31.98	23.36	82.40	2.47
1st-15th September	34.20	26.08	76.31	6.74
16th-30th September	36.00	26.93	74.40	6.17

Annexure 2

2.1 ANOVA for total phenol content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	25.47	1.34	170.23
Error	40	0.31	0.01	
Total	59	25.78		

2.2 ANOVA for total phenol content in anthracnose infected leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	39.19	2.06	844.50
Error	40	0.10	0.00	
Total	59	39.29		

2.3 ANOVA for proline content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	5769.09	303.64	1096.28
Error	40	11.08	0.28	
Total	59	5780.16		

2.4 ANOVA for proline content in anthracnose infected leaves

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	12101.71	636.93	27.54
Error	40	925.09	23.13	
Total	59	13026.80		

2.5 ANOVA for total sugar content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	60.57	3.19	1858.64
Error	40	0.07	0.00	
Total	59	60.64		

2.6 ANOVA for total sugar content in anthracnose infected leaves

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	61.72	3.25	640.36
Error	40	0.20	0.01	
Total	59	61.92		

2.7 ANOVA for reducing sugar content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	9.84	0.52	33.71
Error	40	0.61	0.02	
Total	59	10.46		

2.8 ANOVA for reducing sugar content in anthracnose infected leaves

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	12.71	0.67	93.35
Error	40	0.29	0.01	
Total	59	12.99		

2.9 ANOVA for polyphenol oxidase activity in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	28987.79	1525.67	634.03
Error	40	96.25	2.41	
Total	59	29084.04		

2.10 ANOVA for polyphenol oxidase activity in anthracnose infected leaves

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	36069.79	1898.41	4524.62
Error	40	16.78	0.42	
Total	59	36086.57		

2.11 ANOVA for peroxidase activity in healthy leaves

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	27.75	1.46	89.98
Error	40	0.65	0.02	
Total	59	28.40		

2.12 ANOVA for peroxidase activity in anthracnose infected leaves

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	26.70	1.41	381
Error	40	0.15	0.00	
Total	59	26.85		

2.13 ANOVA for malondialdehyde content in healthy leaves

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	52.64	2.77	30.73
Error	40	3.61	0.09	
Total	59	56.25		

2.14 ANOVA for malondialdehyde content in anthracnose infected leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	47.27	2.49	37.60
Error	40	2.65	0.07	
Total	59	49.91		

2.15 ANOVA for phenylalanine ammonia lyase activity in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	296.60	15.61	100.82
Error	40	6.19	0.15	
Total	59	302.79		

2.16 ANOVA for phenylalanine ammonia lyase activity in anthracnose infected leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	529.75	27.88	290.43
Error	40	3.84	0.10	
Total	59	533.59		

2.17 ANOVA for chlorophyll ‘a’ content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	1.00	0.05	7.96
Error	40	0.26	0.01	
Total	59	1.27		

2.18 ANOVA for chlorophyll 'b' content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	0.60	0.03	21.13
Error	40	0.06	0.00	
Total	59	0.65		

2.19 ANOVA for total chlorophyll content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	9.04	0.48	49.05
Error	40	0.39	0.01	
Total	59	9.42		

2.20 ANOVA for carotenoid content in healthy leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	9.99	0.53	80.28
Error	40	0.26	0.01	
Total	59	10.25		

2.21 ANOVA for chlorophyll 'a' content in anthracnose infected leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	1.14	0.06	13.93
Error	40	0.17	0.00	
Total	59	1.32		

2.22 ANOVA for chlorophyll 'b' content in anthracnose infected leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	19	0.81	0.04	58.79
Error	40	0.03	0.00	
Total	59	0.84		

2.23 ANOVA for total chlorophyll content in anthracnose infected leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	**19**	**12.53**	**0.66**	**236.28**
Error	**40**	**0.11**	**0.00**	
Total	**59**	**12.64**		

2.24 ANOVA for carotenoid content in anthracnose infected leaves.

Source	D.F.	S.S	M.S.	F-Cal
Treatment	**19**	**10.08**	**0.53**	**219.99**
Error	**40**	**0.10**	**0.00**	
Total	**59**	**10.18**		