PHYSIOLOGICAL AND ANATOMICAL REACTION OF GRAPE (*Vitis vinifera* L.) GENOTYPES TO ANTHRACNOSE

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
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MASTER OF SCIENCE
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BOTANY
(Minor Subject: Biochemistry)

By

Sarah Murria
(L-2015-BS-235-M)

Department of Botany
College of Basic Sciences and Humanities

LUDHIANA – 141004

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CERTIFICATE I

This is to certify that the thesis entitled **"Physiological and anatomical reaction of grape (*Vitis vinifera* L.) genotypes to anthracnose"** submitted for the degree of **M.Sc.** in the subject of **Botany** (Minor subject: **Biochemistry**) of the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Sarah Murria (L-2015-BS-235-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

(Dr. Nirmaljit Kaur)
Major Advisor
Senior Botanist
Department of Botany
Punjab Agricultural University
Ludhiana- 141004.

CERTIFICATE – II

This is to certify that the thesis entitled, "**Physiological and anatomical reaction of grape (*Vitis vinifera* L.) genotypes to anthracnose**" submitted by **Sarah Murria** (Admn. No. **L-2015-BS-235-M**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **M.Sc.** in the subject of **Botany** (Minor subject: **Biochemistry**) has been approved by the Student's Advisory Committee after an oral examination on the same.

(Dr. Nirmaljit Kaur)
Major Advisor

(Dr. Adarsh Pal Vig)
External Examiner
Associate Professor
Department of Botanical and Environmental Sciences
GNDU, Amritsar

(Dr. Seema Bedi)
Head of the Department

(Dr. Neelam Grewal)
Dean Postgraduate Studies

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ABSTRACT

The present investigation was undertaken with the objective to screen five grapevine varieties against anthracnose caused by *Elsinoe ampelina* (de Bary) Shear under field conditions; and to evaluate the anatomical, physiological and biochemical characteristics in relation to reaction of the grapevine varieties to the disease. On the basis of Percent disease incidence (PDI) worked out in the leaves, the variety Punjab MACS Purple was characterized as resistant, Pusa Navrang and Flame Seedless as moderately resistant and Beauty Seedless and Perlette as susceptible varieties. The stomatal characteristics viz., stomatal size and stomatal index were significantly high in most susceptible variety Perlette. The variety Punjab MACS Purple which is resistant to the pathogen had compact spongy mesophyll and visibly high trichome density. Significant Pearson correlation coefficient has been found between PDI and stomatal size and stomatal index. The physiological and biochemical constituents viz., chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, total soluble sugars, total soluble proteins, free amino acids, total phenols, ascorbic acid, proline, α-tocopherol and MDA content was quantified from the healthy and infected leaves of the five grapevines varieties. The chlorophyll a, chlorophyll b and total chlorophyll were higher in the healthy leaves as compared to the infected ones. These pigments were high in the resistant variety and low in the susceptible variety. The carotenoids were higher in the healthy leaves as compared to the infected ones. This was high in the susceptible variety and low in the resistant variety. The total soluble sugars, total soluble proteins, free amino acids, total phenols, ascorbic acid, proline and α-tocopherol were significantly higher in the resistant variety Punjab MACS Purple and minimum in the susceptible variety Perlette whereas the MDA content was low in the Punjab MACS Purple and maximum in the Perlette. The activity of the peroxidase and polyphenol oxidase was significantly higher in the infected leaves as compared to their healthy counterparts in all the grape varieties. Among different varieties, peroxidase and polyphenol oxidase was maximum in Punjab MACS Purple and minimum in Perlette. Highest Pearson correlation coefficient of PDI has been observed and chlorophyll b followed by total phenols. The characteristics viz., stomatal size, stomatal index, pigments and total phenols can therefore be used as markers for evaluating grapevine genotypes against *Elsinoe ampelina*.

Keywords: Anthracnose, *Elsinoe ampelina*, enzymes, phenols, pigments, stomatal characteristics

Signature of Major Advisor **Signature of the student**

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ਮੌਜੂਦਾ ਜਾਂਚ ਅੰਗੂਰਾਂ ਦੇ ਪੰਜ ਜੀਨੋਟਾਇਪ ਨੂੰ (*ਇਲਸੀਨੋ ਐਂਪੀਲੀਨਾ* ਡੀ ਬੈਰੀ) ਸ਼ੀਅਰ ਨਾਲ ਹੋਣ ਵਾਲੀ ਐਂਥਰਾਕਨੋਜ ਦੇ ਵਿਰੁੱਧ ਸਕਰੀਨ ਕਰਨ ਦੇ ਉਦੇਸ਼ ਅਤੇ ਰੋਗ ਪ੍ਰਤੀ ਅੰਗੂਰ ਜੀਨੋਟਾਇਪ ਦੀ ਐਨੋਟੀਮੀਕਲ ਫਿਜਾਲਜੀਕਲ ਅਤੇ ਬਾਇਓਕੈਮੀਕਲ ਦੀ ਪ੍ਰਤੀਕਿਰਿਆ ਦਾ ਆਂਕਲਤ ਕਰਨ ਲਈ ਕੀਤੀ ਗਈ। ਪੱਤਿਆ ਉੱਪਰ ਰੋਗ ਪ੍ਰਤੀਸ਼ਤਤਾ (PDI) ਨੂੰ ਦੇਕਿਆ ਗਿਆ ਅਤੇ ਇਸਦੇ ਅਧਾਰ ਤੇ ਪੰਜਾਬ (MACS) ਪਰਪਲ ਕਿਸਮ ਪ੍ਰਤੀਰੋਧੀ, ਪੂਸਾ ਨਰੰਗ ਅਤੇ ਫਲੇਮ ਸੀਡਲੈਸ ਦਰਮਿਆਨੀ ਪ੍ਰਤੀਰੋਧੀ ਅਤੇ ਬਿਊਟੀ ਸੀਡਲੈਸ ਅਤੇ ਪਰਲੈਟ ਸੰਵੇਦਨਸ਼ੀਲ ਪਾਈਆਂ ਗਈਆਂ। ਸਟੋਮੈਟਾ ਚਿਤਰਾਂਕਨ ਜਿਵੇਂ ਸਟੋਮੈਟਾ ਅਕਾਰ, ਸਟੋਮੈਟਾ ਸੂਚਕਾਂਕ ਆਦਿ ਸਭ ਤੋ ਸੰਵੇਦਨਸ਼ੀਲ ਕਿਸਮ ਪਰਲੈਟ ਵਿਚ ਸਭ ਤੋਂ ਜ਼ਿਆਦਾ ਸਨ। ਪੰਜਾਬ (MACS) ਪਰਪਲ ਜੋ ਪ੍ਰਤੀਰੋਧੀ ਕਿਸਮ ਸੀ ਵਿਚ ਗੁੰਦਵੇ ਸਧੋਜੀ ਮੀਸੋਫਿਲ ਅਤੇ ਉੱਚ ਟਰਾਈਕੋਸ ਘਣਤਾ ਦੇਖਣ ਨੂੰ ਮਿਲੀ।(PDI) ਅਤੇ ਸਟੋਮੈਟਲ ਅਕਾਰ ਅਤੇ ਸਟੋਮੈਟਾ ਸੂਚਕਾਂਕ ਵਿਚ ਅਰਥਪੂਰਨ ਸਹਿਸਬੰਧ ਦੇਖਣ ਨੂੰ ਮਿਲਿਆ।ਪੰਜ ਅੰਗੂਰ ਕਿਸਮਾਂ ਵਿਚੋਂ ਸਿਹਤਮੰਦ ਅਤੇ ਰੋਗੀ ਪੱਤਿਆਂ ਵਿੱਚ ਕਲੋਰੋਫਿਲ b, ਕੁਲ ਕਲੋਰੋਫਿਲ, ਕੈਰੋਟੀਨਾਇਡ ਕੁਲ ਘੁਲਣਸ਼ੀਲ ਸ਼ੂਗਰ, ਕੁਲ ਘੁਲਣਸ਼ੀਲ ਪ੍ਰੋਟੀਨ, ਮੁਕਤ ਅਮੀਨੋ ਏਸਿਡ, ਕੁਲ ਫਿਨੋਲ, ਐਸਕਰੋਬਿਕ ਏਸਿਡ ਪ੍ਰੋਲੀਇਨ, ਟੋਕੋਫਿਨੋਲ ਅਤੇ MDA ਮਾਤਰਾ ਵਰਗੇ ਬਾਇਓਕੈਮੀਕਲ ਪਟਕਾਂ ਪਰਿਮਾਣ ਕੀਤਾ ਗਿਆ ਇਹ ਪਿੰਗਮੈਂਟ ਪ੍ਰਤੀਰੋਧੀ ਕਿਸਮ ਵਿੱਚ ਜਿਆਦਾ ਜਦਕਿ ਸੰਵੇਦਸ਼ੀਲ ਕਿਸਮਾਂ ਵਿੱਚ ਘੱਟ ਪਾਏ ਗਏ। ਸਿਹਤਮੰਦ ਪੱਤਿਆਂ ਵਿਚ ਕੈਰੋਟੀਨ ਮਾਤਰਾ ਰੋਗੀ ਪੱਤਿਆਂ ਨਾਲੇ ਜਿਆਦਾ ਸੀ। ਇਹ ਸੰਵੇਦਸ਼ੀਲ ਕਿਸਮ ਵਿੱਚ ਪ੍ਰਤੀਰੋਧੀ ਕਿਸਮ ਨਾਲੋ ਵੱਧ ਸੀ। ਪ੍ਰਤੀਰੋਧੀ ਕਿਸਮ ਪੰਜਾਬ (MACS) ਪਰਪਲ ਵਿਚ ਕੁਝ ਘੁਲਣਸ਼ੀਲ ਸ਼ੂਗਰ, ਕੁਝ ਘੁਲਣਸ਼ੀਲ ਪ੍ਰੋਟੀਨ, ਮੁਕਤ ਅਮੀਨੋ ਏਸਿਡ ਕੁਝ ਫਿਤੋਲ, ਐਸਕੋਰਬਿਕ ਏਸਿਡ, ਪ੍ਰੋਨਾਇਨ ਅਤੇ ਏਕੋਟਿਰੋਲ ਵੱਧ ਸਨ ਜਦਕਿ ਪਰਲੈਟ ਸੰਵੇਦਨਸ਼ੀਲ ਕਿਸਮ ਵਿੱਚ ਸਭ ਤੋਂ ਘੱਟ ਸਨ ਜਦਕਿ (MDA) ਮਾਤਰਾ ਪੰਜਾਬ (MACS) ਪਰਪਲ ਵਿਚ ਘੱਟ ਅਤੇ ਸਭ ਤੋਂ ਜਿਆਦਾ ਪਰਲੈਟ ਵਿਚ ਸੀ। ਰੋਗੀ ਪੱਤਿਆ ਵਿਚ ਪਰਆਕਸੀਡੇਜ ਅਤੇ ਪੋਲੀਫਿਨੋਲ ਆਕਸੀਡੇਜ ਦੀ ਕਿਰਿਆ ਸਿਹਤਮੰਦ ਪੱਤਿਆ ਦੇ ਮੁਕਾਬਲੇ ਵੱਧ ਸੀ। ਪਰਆਕਸੀਡੇਜ ਅਤੇ ਪੋਲੀਫਿਨੋਲ ਆਕਸੀਡੇਜ ਇੰਜਾਇਮ ਸਭ ਤੋਂ ਜਿਆਦਾ ਪੰਜਾਬ (MACS) ਪਰਪਲ ਵਿਚ ਅਤੇ ਸਭ ਤੋਂ ਘੱਟ ਪਰਲੈਟ ਵਿਚ ਸੀ।(PDI) ਅਤੇ ਕਲੋਰੋਫਿਲ b ਅਤੇ ਕੁਝ ਫਿਨੋਲ ਵਿਚ ਪੀਅਰਸਨ ਸਹਿਸਬੰਧ ਕਾਫੀਸੈਂਟ ਸਭ ਤੋਂ ਜਿਆਦਾ ਸੀ। ਸਟੋਮੈਟਾ ਅਕਾਰ, ਸਟੋਮੈਠਲ ਸੂਚਕਾਂਕ, ਪਿਗਮੈਂਟ ਅਤੇ ਕੁਝ ਫਿਨੋਲ ਵਰਗੇ ਗੁਣਾ ਨੂੰ *Elsione ampelina* ਦੇ ਵਿਰੁੱਧ ਅੰਗੂਰ ਜੀਨੋਟਾਇਪ ਦੇ ਮੁਲਾਂਕਨ ਲਈ ਮਾਰਕਰ ਦੇ ਤੋਰ ਤੇ ਵਰਤਿਆ ਜਾ ਸਕਦਾ ਹੈ।

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ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਦਸਤਖ਼ਤ **ਵਿਦਿਆਰਥੀ ਦੇ ਦਸਤਖ਼ਤ**

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CHAPTER-I

INTRODUCTION

Grape (*Vitis vinifera* L.) is an important fruit crop cultivated all over the world. It is one of the oldest flora existing on the earth (approx. 90 - 95 million years ago) from the time when dinosaurs flourished as revealed by a discovery made in Western Kazakhstan (Shanmugavelu 2003). In India, grapes were introduced into the Northern parts from Iran and Afghanistan in the 12th century. Later it spread to southern parts of India. Grapevine is globally one of the most important fruit species due to the numerous uses of its fruits in the production of wine, grape juice and other foods. The grape is a fruiting berry of the deciduous woody vines of the family *Vitaceae* (Watson and Dallwitz 1992) and is commercially important crop of India. The grape is becoming popular for its high nutritive value, good taste, multipurpose use and better returns (Ghosh *et al* 2008). Grapes have high nutrient content that plays an important role in ensuring a healthy and active life. The fruits are a rich source of vitamin A, C, B_6 and folate. In addition to this, essential minerals like potassium, calcium, iron, phosphorus, magnesium and selenium are also present. Grapes contain flavonoids and resveratrol (Frankel and Meyer 1998) that are very powerful antioxidants which can reduce the damage caused by free radicals and can help in slowing down the process of aging. The fruit contains about 20 percent sugar in an easily digestible form besides being rich in calcium and phosphorus. It is grown mainly for wine making (82% production), raisin making (10% production) and for table purpose (8%) throughout the world. The production of wine grape varieties is increasing day by day as the wine industry is moving at a faster pace which is adding a potential value to the grape production (Karibasappa *et al* 2006). However, in India grapes are mostly consumed more as a fresh fruit and a limited quantity is used in the production of liquor, dry fruits like raisins, etc. Many studies have shown that increasing consumption of plant foods like grapes can decrease the risk of obesity and overall mortality.

The major producers of grape are Italy, France, Spain, USA, Turkey, China and Argentina. India stands amongst the top ten grape producing countries of the world. The area under grape is 1.2 Percent of the total area of fruit production crops in the country. Grapes contribute about 2.8 Percent production of total fruits in the country. In India, land occupied under grape cultivation is 136000 hectares and production is 2683000 tonne (Anonymous 2016). Among the different states, Maharashtra stands first giving about 80% production of the crop followed by Karnataka and Tamil Nadu. In Northern India, grape cultivation is practiced mainly in Punjab, Haryana, Uttar Pradesh, and Himachal Pradesh. In Punjab, the area under cultivation is 297 hectares and production is 8493 metric tons, with a productivity of 28.6 metric tons per hectare (Anonymous 2016).

Grapes have special components that make them even more essential to our health, giving them "super food" status and reducing the risk of the diseases like cancer, heart diseases, high B.P., constipation, allergies, etc. The chemical composition of *Vitis vinifera* fruits because of the presence of resveratrol is one of the strongest antioxidants known as reported by Caimi *et al* (2003). Research shows that antioxidant status and administration in red wine has been mainly concerned with vitamins C and E and coronary artery disease. Fruits of *Vitis vinifera* have been used for thousands of years because of their nutritional and medicinal benefits. They are rich in sugars, flavonoids, anthocyanins and proanthocyanins (Rapport and Jockwood 2001) organic acids, tannin, mineral salts and vitamins. Grape skin, especially from the red and black species is rich in resveratrol which is a derivative of stilbene. Resveratrol is one of the strongest known natural antioxidants. It is found in a large quantity in black grape juice, skin and seed. Resveratrol has an antioxidant potential 50 times higher than vitamin C and E together. This substance prevents the oxidation of low-density lipoproteins (LDL) cholesterol, lowers total cholesterol levels (Whitehead *et al* 1995) also demonstrates an antibacterial action, reduces the risk of cardiovascular diseases and can prevent the cancer development (Chen *et al* 1998). It has been reported that the compounds present in grape juice can prevent platelet aggregation, oxidative damage to DNA and coronary diseases and atherosclerosis (Frankel and Mayer 1998). Grapes are multiplied exclusively by rooting of hardwood cuttings. In its natural habitat, the crop bears fruit during the hot and dry period and it becomes dormant during the period of severe cold. It tolerates frost during the resting stage but is very susceptible during growing period. Temperature ranging from $15^0 – 35^0$ C is ideal for shoot growth and normal physiological processes of the grapevine. Vines do not grow and fruit well when the temperature falls below 10^0 C.

The productivity of grapes is adversely affected by the attack of insects, fungal, bacterial and viral diseases. Anthracnose or "Bird's eye spot" of grapes (*Vitis vinifera* L.) caused by a fungus *Elsinoe ampelina* (de Bary) Shear is one of the major diseases under tropical and sub-tropical regions. In India, this disease is posing a threatening potential towards grape cultivation. It is one of the most prevalent and destructive disease of the vineyards. According to a report, there is about 10-46.5% yield loss in different genotypes due to this malady (Suhag and Grower 1972). The disease becomes very much destructive if it gets established in a vineyard. In India, the disease is widespread in Maharashtra, Karnataka, Haryana, Punjab, Uttar Pradesh, Tamil Nadu and Andhra Pradesh. The disease appears every year in North India from the month of July to September. The disease causes peak damage in the month of August resulting in a great loss in the product. However, the disease pervades from March to October in the parts of South India causing peak damage from the months of March to July. This malady causes 15-30% losses annually in Maharashtra state as reported by Deshmukh (2006). Anthracnose of grapes is causing a very

serious damage to the viticulture industry in Punjab (Thind *et al* 1992; Chandermohan *et al* 2002).

The disease attacks all the aerial parts of the vines, such as fruits, leaves, tendrils and petioles. Symptoms appear on leaves as numerous circular spots which later on become enlarged with lesions around the edges. They become sunken and give reddish black appearance. The necrotic centres of these lesions drop out producing a shot-hole appearance (Michael and Erincik 2008). On fruits, the lesions are sunken and they appear more reddish black in colour. These spots can enlarge upto a diameter of ¼ inches. These centres turn whitish grey and are surrounded by reddish-brown margins giving a resemblance of bird's eye and so the common name of the disease (Hartman and Kaiser 2008).

Grape anthracnose is important to the wine industry, as it decreases quality and quantity of the berries produced as well as kills the vines, leading to large economic losses, particularly in the middle summer months (Singhrot *et al* 1982). The disease can be checked by the application of fungicides like Topsin-M, Bavistin, Aureofungin etc. (Thind *et al* 1997). Frequent sprays of these fungicides are usually required to protect the crop, but they are costly and can be hazardous for the human health as well as the environment (Poolsawat *et al* 2012). Due to these problems, the attention towards the use of resistant cultivars is pre-requisite. So, to reduce the losses caused by diseases selection of such genetic resources should be done that show tolerance towards them (Shobharani and Ravikumar 2003; Clifford *et al* 2011). So the idea is to discover the inbuilt plant defense mechanism against different pathogens. When fungal pathogens attack plants, their chemical stressors like suppressors, toxins, elicitors and cells of the host plant respond in various methods (Yamada *et al* 1997). Some plants get infected as they are not able to create any defense mechanism against the pathogen, while the plants which overcome the stress is due to series of resistant reactions which cause the survival of the host (Park and Ikeda 2008). Plants possess defense mechanisms for preventing unfavorable interactions with other living organisms. There is production and accumulation of secondary metabolites especially phenols and synthesis of defense peptides and proteins. These biochemicals are synthesized during the normal development. Defense mechanisms in a susceptible plant are induced more slowly than those of a resistant plant. Thus, the time required to induce an array of defense responses appears to be a key factor ensuing in a resistant phenotype. Therefore, it can be an alternative management strategy for selecting a less-susceptible variety. Biochemical parameters viz., polyphenol oxidase, peroxidase activities, phenols and sugars were reported in plants treated with various biotic and abiotic inducers (Gurjar *et al* 2015). So, it is of interest to know which biochemical constituent is associated with anthracnose resistance. The current study was thus taken up on the basis of percent disease index to characterize biochemical parameters to identify grape varieties tolerant to anthracnose. The use of resistant cultivars should be

exploited to control grape disease in an effective and economical way (Lu 1997). With the intent to have grape production free from residues an attempt has to be made to find resistant cultivars on the basis of physiological and anatomical studies. Hence, the characterization of biochemical and anatomical parameters that render tolerance to grape anthracnose needs to be worked out. The current study was taken up with the following objectives:

i. Evaluation of grape genotypes for susceptibility to grape anthracnose on the basis of disease severity and anatomical studies

ii. Characterization of physiological and biochemical parameters as indicator for identifying grape genotypes tolerant to anthracnose

CHAPTER-II

REVIEW OF LITERATURE

Grapevine is cultivated worldwide and is one of the important horticultural crops. The grape itself is used for a myriad of products. Grapes have been used for treating metabolism disorders, liver, kidney and lung disorders and cardiovascular problems. Grapes help in improving metabolism and have diuretic, anti-inflammation, light laxative effects. They manoeuvre nervous exhaustion, high blood pressure, hypertension, bronchitis and gout. Eating grapes strengthens the body to recover from anaemia, gastritis with high acidity, metabolism disorder, chronic insomnia and constipation. A recent study says that purple coloured Concord grape juice helps in preventing cancer (American Institute for Cancer Research). Grapes exhibit antimutagenic and antioxidant properties that are very effective in combating all kinds of cancer.

The primary component of mature grapes is water making upto 75-85 percent of their weight. Approximately 15-25 percent is in the form of sugars, a higher percentage than in many other fresh fruits. The organic acids tartaric, malic and citric make up 0.5-1.0 % of the fruit, pectin about 0.25% and there are other nutritional components. If all these are summed up, they amount to over 99% of the weight of grapes.

Grapes are high in carbohydrates and not a particularly good source of dietary fibre. However, these are a useful source of many minerals and vitamins B6, C, E and K. They are also a source of antioxidant compounds through the phenolics in their skins and possibly seeds (Yilmaz and Toledo 2004).

Though grape is a temperate crop but in India, it is mainly cultivated in the peninsular parts, especially Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh. The major producers of grape are Italy, France, Spain, USA, Turkey, China and Argentina. India stands amongst the top ten grape producing countries of the world. The area under grape is 1.2% of the total area of fruit production crops in the country. Grapes contribute about 2.8% production of total fruits in the country. In India, land occupied under grape cultivation is 136000 hectares and production is 2683000 tonne (Anonymous 2016). Among the different states, Maharashtra stands first giving about 80% production of the crop followed by Karnataka and Tamil Nadu. In Northern India, grape cultivation is practiced mainly in Punjab, Haryana, Uttar Pradesh, and Himachal Pradesh. In Punjab, the area under cultivation is 297 hectares and production is 8493 metric tons, with a productivity of 28.6 metric tons per hectare (Anonymous 2016).

Worldwide, grapes are mainly utilised for wine making, however in India, exclusively table grape varieties were cultivated so far. It's only in the last decade that several growers have ventured into wine making and a number of small and medium

wineries are established making about one thousand MT of red and white wines. At present, there are 46 wineries in India, out of which 43 are in Maharashtra and 22 in Nasik, eight each in Sangli and Pune, three in Solarpur and one each in Usmanabad and Buldhana districts. Currently, total grape wine production in India is 1.04 crore litres, of which 94.79 lakh litres is produced in Maharashtra. The country also dries grapes to prepare over 7000 MT of raisins, which are ample to meet the local demands.

Raisin export is about 355 MT per year. Grape by-product like grape seed oil is commercially used for cooking and by the cosmetic industry. The winery waste i.e. grape Marc, consisting of skin and seeds, is composted and used as manure. Grape has assumed importance owing to its export value and also the very cohesive association of the grape growers into associations and societies in building up the grape industry of India. However, due to the wet and warm climate in the areas of viticulture, many pathogens and insect pests attack the vines thus reducing the quantity and quality of the yield. Among the diseases, powdery mildew, downy mildew and anthracnose are damaging diseases which can cause upto 100% crop loss.

Anthracnose or 'bird's eye spot' of grapes caused by *Elsinoe ampelina* (de Bary) Shear, is an economically important disease of grapes in the warm and wet tropical and sub-tropical regions of India. Worldwide anthracnose is reported to be caused by *Elsinoe ampelina* (de Bary) Shear (Mirica 1998) (syn. *E. viticola* Raciborski), anamorph *Sphaceloma ampelinum* (de Bary) (syn. *Gloeosporium ampelophagum* (Pass.) Sacc, *Ramularia ampelophaga* (Pass.) (Pearson and Goheen 1988). In India, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* have also been reported as causal pathogens (Kumar *et al* 1994, Chowdappa *et al* 2009). The fungus can infect the grapevine at any stage of its life cycle, from seedling to fruit development and can reduce the quantity and quality of the fruit and weakens the vine. To prevent such loss, various approaches can be followed but the most economic and environment friendly way is to exploit the in-built capacity of the host plant to fight against the pathogen.

Occurrence and Distribution

Anthracnose is one of the chronic problems which pose a serious threat to the successful future of viticulture industry. It is a serious disease of the commercial grape varieties in all the grape growing regions of the country during humid and rainy weather. It is prevalent in Karnataka, Andhra Pradesh and Maharashtra (Gandhi 1956), Haryana and Punjab (Chohan 1965), Uttar Pradesh (Prasad and Nirvan 1965) and Tamil Nadu (Ramakrishnan and Sundaram 1955). Singhrot *et al* (1982) compared the shift in productivity between infected and healthy vines and recorded extreme reduction in number of leaves, leaf area & percent bud sprouting in the anthracnose affected vines. In Northern India, the disease appears almost every year and is most damaging and widely prevalent and cause reduction in the quality and

quantity after pre-monsoon showers. The disease was widespread and appeared in severe form during monsoons in almost all the districts of Punjab (Thind *et al* 1998). A survey by Kumar and Thind (1992) in different vineyards of Punjab and Haryana during March to September in 1990 revealed that the disease was less severe during early growing season but increased with onset of rainy season and was at peak from July to September. In Southern India, the disease prevails from March to October with peak damage during May to July. In Karnataka, Jamadar and Lingaraju (2011) observed the drastic reduction in productivity of grape cultivars due to this malady.

The disease attacks all the aerial parts of the vines, such as fruits, leaves, tendrils and petioles. Symptoms appear on leaves as numerous circular spots which later become enlarged with lesions around the edges. They become sunken and give reddish black appearance. The necrotic centres of these lesions drop out producing a shot-hole appearance (Michael and Erincik 2008). On fruits, the lesions are sunken and they appear more reddish black in colour. These spots can enlarge upto a diameter of ¼ inches. These centres turn whitish grey and are surrounded by reddish-brown margins giving a resemblance of bird's eye and so the common name of the disease (Hartman and Kaiser 2008).

While these lesions may be very apparent and easy to identify, they can sometimes be confused for hail damage. Hail damage typically appears on only one side of the plant. Also, anthracnose lesions will have darker and more raised edge. Anthracnose lesions on leaves and petioles look very similar to those on shoots. However, on leaves, the lesions have dry grey or white centers that eventually fall off, leaving a hole. This response by the plant is called a shot-hole. If the lesions spread and the infection makes it into the vascular system of the leaf, the anthracnose prevents the proper development of the leaf and leads to malformation or to the drying of the leaf.

Grape vines are susceptible to anthracnose before flowering all the way through fruit softening and colouration. Essentially, the berries are susceptible to the pathogen throughout the growing season. Anthracnose presents itself on the berries as small reddish circles, around a quarter inch in diameter that will become sunken with a narrow dark brown to black border. Eventually, the center of the lesion changes colour from violet to white or grey and becomes velvety. These lesions often look like a shooting target or bulls'eye. If the disease spreads to and affects the pulp of the berry, it causes cracking, which opens the berry to secondary infections.

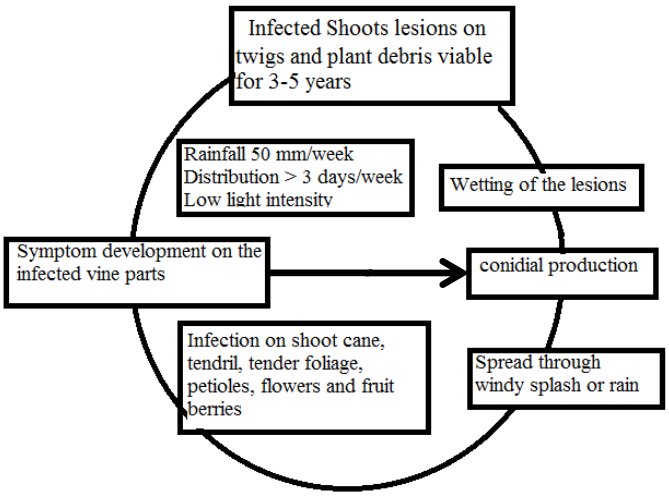
Disease Cycle

Late in the season, the grape anthracnose fungus produces sclerotia, which are located primarily at the edge of the infected lesions on shoots. Unlike acervuli, sclerotia serve as the overwintering structures. Because the fungus over-winters in dormant and dead canes—one-year-old wood that starts to become lignified-disease control becomes very difficult. Large

numbers of conidia are disseminated from sclerotia in the spring when there are wet periods of 24 hours and temperature is above 36°F (2°C). The infection rate escalates with increases in temperature. Development of disease symptom is also temperature-dependent: within 13 days at 36°F, or within 4 days at 90°F. Simultaneously, ascospores are produced on the lesions of infected canes or berries left on the trellis system or on the vineyard floor to carry out the infection. These ascospores are formed in asci, which are in cavities within a stroma - the dense structural tissue that produces fruiting bodies in fungi of pseudothecium. Pseudothecium of grape anthracnose, the sexual fruiting body of the fungus, has asci containing eight four-celled ascospores. The fungus also overwinters as pseudothecium, but the importance of ascospores in disease development is not clearly understood. The study done (Mirica 1998) validated that the ascospores do germinate and infect the tissue and produce the *Sphaceloma* phase which shows the existence of the perfect stage of *Elsinoe ampelina*. Spores germinate on the surface of the grapevine leaf after 2 hour incubation at room temperature (26°C) reaching a maximum after 24 hr. More spores (95%) germinate on the lower surface than on the stomata. Appisori are formed when the germ tube reaches stomata (Naik *et al* 1987).

Conidia and ascospores overwinter on the ground and on infected tissue and become the source of primary inoculum. Throughout spring and summer; the fungus produces acervuli on the exterior of the necrotic areas at their mature stage. Under wet conditions, acervuli form conidia. The conidia from acervuli become the secondary sources of infection for the remainder of the growing seasons. The disease cycle of *Elsinoe ampelina* is as follows:

1) The fungus overwinters by forming both pseudothecium and sclerotia
2) The spores from both structures cause primary inoculum and form mycelium on the infected lesions,
3) Acervuli disseminate conidia which becomes the source of secondary inoculum. As mentioned earlier, grape anthracnose is dependent upon moisture and temperature. It can be exacerbated during heavy rainfall and hail, or by overhead irrigation. Grape anthracnose can be found where ever grapes are grown, however it is more prevalent in certain areas with warm and wet conditions. Both primary and secondary inoculum is spread by the splashing of rain onto new tissue. Moisture is required for the germination of conidia on tissue. New tissue is the most vulnerable to infection. Overgrown vines also promote infection as they take longer to dry out after dew or rain, often due to decreased air flow in the canopy. The disease can become even more severe in areas of poorly drained soil or during years ofheavy rainfall or rain coupled with high temperatures.



Factors responsible for host resistance

A plant when attacked by a pathogen suffers little or no injury, this condition is known as disease resistance and susceptibility is the condition when the above quality is lacking in the plant. Being sessile, plants are attacked by wide range of pathogens under all natural environments. To defend itself from the pathogen attack, the host plant develops various inducible and constitutive mechanisms called defense responses. Several aspects of these responses are expressed even in non-infected parts of the host plant systemically (Heil and Ploss 2006). Without the use of induced antibiotics, plant relies on various structural and biochemical attributes to protect itself from the entire infection. The defense barriers in plants are a co-ordinated system of molecular, cellular and tissue-based responses to pathogen attack. Defense mechanisms in plant occur in the following order:

1.) The pathogen comes on the host and faces the pre-existing physical and chemical barriers which are heredity characteristics of the host plant, and

2.) The pathogen successfully overcomes the above barriers and then establishes parasitic relationship.

Then, the host system reacts with the foreign material which results in the formation of new physical and chemical barriers. These two conditions can be stated as resistance to penetration and disease development (Singh 1984). Various integrated and complex mechanisms of the host plant are involved in the prevention of infection. A large number of physiological, biochemical and molecular changes have been observed that can be correlated with the onset of pathogen attack (Klessing and Malamy 1994). These mechanisms can be local, constitutive or inducible. In some cases, the extents of these mechanisms, which affect disease progression, are poorly understood (Kessmann *et al* 1994). Considerable progress has been made in the past few years to understand the mechanism of disease resistance or susceptibility. The previous work done on physiological and biochemical defense mechanism of the host plant has been reviewed here.

Disease Incidence

In India, incidence of anthracnose was first recorded in 1903 near Pune (Butlar 1905). About 10-15% losses had been reported in Haryana. A remarkable reduction of 50% in the yield was reported during 1975 at Parbhani, Maharashtra. Gurme and Kore (1977) also reported this disease from Uttar Pradesh, Mysore (Karnataka), and Tamil Nadu. Similarly, 66-84% losses were reported by Thind and Jhooty (1985) due to anthracnose of chilli.

Different genotypes and different plant parts of grapevine behave distinctly with respect to susceptibility for anthracnose. Singh and Joshi (1971) evaluated 14 grape cultivars for resistance to *Gloeosporium ampelophagum*. Goyal *et al* (1971) reported anthracnose incidence on different varieties of grape at Jobner, Rajasthan. The results showed that the varieties Bharat Early and Hussaini remained free from infection and White Muscat was slightly affected. Yadav and Nirwan (1981) also screened grape cultivars against anthracnose. They found Delight, Himrod, Khalili and Gulabi as resistant and Beauty seedless and Thompson seedless as the most susceptible to the disease.

Many workers have also reported screening and identification of resistant sources in different crops. Bedi *et al* (1986) studied field reaction of different grapevine cultivars to anthracnose caused by *Sphaceloma ampelinum*. 35 cultivars were assessed for percentage of leaf infection, average foliar necrosis; shoot die-back, disease index, and varietal reaction. Patil *et al* (1990) observed incidence of anthracnose in 70 *Vitis vinifera* cultivars. Prasad *et al* (1992) assessed grape hybrids and found Bangalore Blue and its derivative E21/28 as highly resistant against anthracnose. Similarly screening for resistance to *Fusarium* crown rot of tomato caused by *Fusarium oxysporum*, a soil borne fungus was done by Jones *et al* (1992).

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. The results showed that Perlette and Beauty Seedless were most susceptible. Similarly, Shankar and Jindal (2002) screened 48 grape genotypes against anthracnose and categorized them into resistant, moderately resistant and susceptible. A high correlation of disease index on canes and leaf was observed (0.974) showing that the similar set of genes were controlling disease resistance which were expressed both on canes and leaves.Yuan *et al* (2006) assessed anthracnose resistance in grape germplasm. Disease incidence of white rust, downy mildew and *Alternaria* blight were studied by Sangeetha and Siddaramaiah (2007) by using 0-5 scale and then percent disease index was calculated.

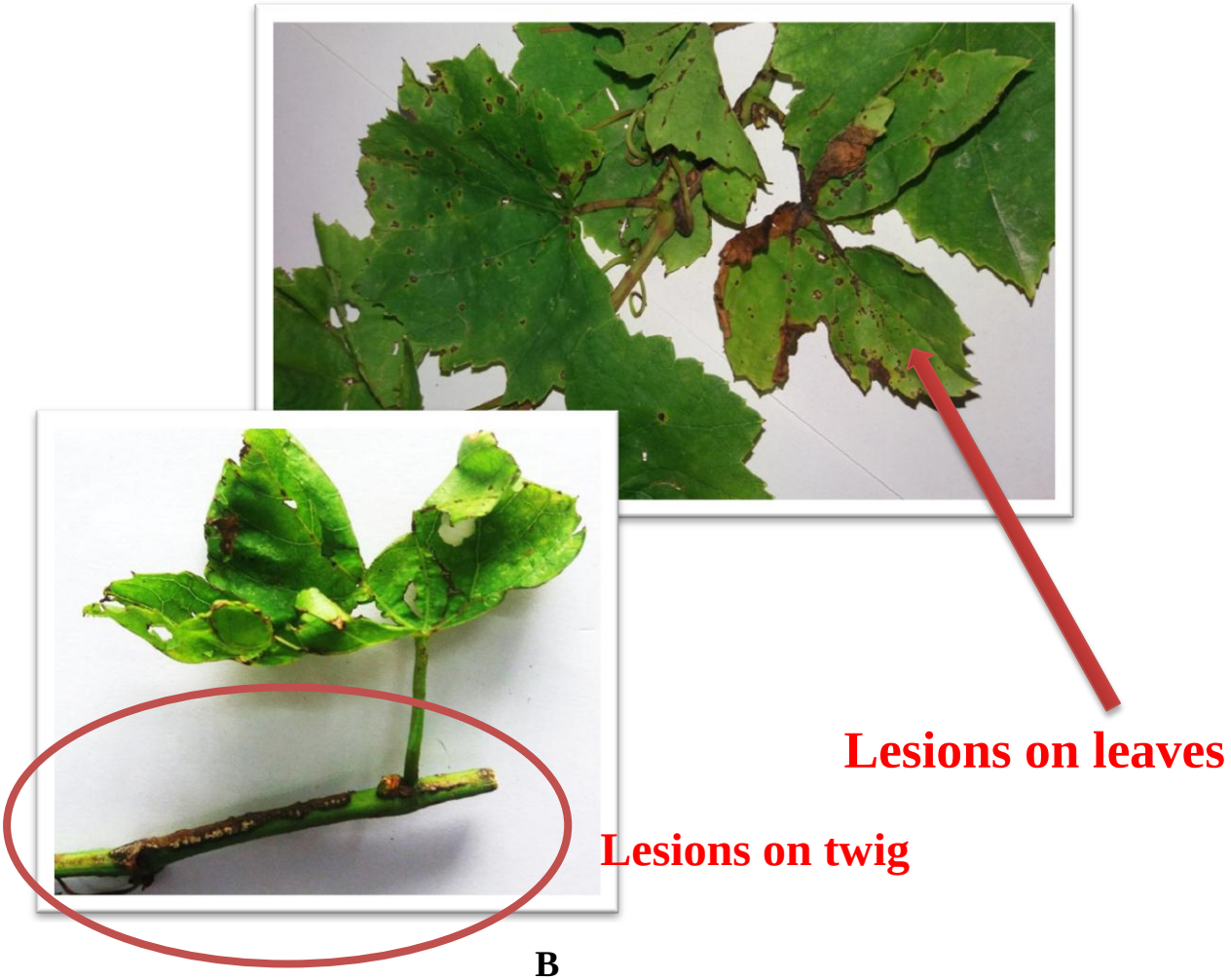
Similarly, 17 chilli genotypes (seven germplasm and 10 hybrids) were screened for anthracnose resistance (Prasath and Ponnuswami 2008). An observation by Li and Wan (2008) on 56 accessions and 13 wild Chinese *Vitis* sp. for anthracnose and white rot of grape

Plate I :

A: An overview of anthracnose infested vineyard

B: Symptoms of anthracnose infection

A



B

showed that all the accessions and wild species were resistant to anthracnose. Only two *Vitis vinifera* varieties Chardonnay and Caberanet Sauvignon were susceptible to anthracnose and white rot.

Investigation by Yanmin *et al* (2010) on five wine grape cultivars for resistance to ripe rot affected by *Colletotrichum* sp. by artificial inoculation was made in laboratory. Thind *et al* (2010) screened ten field grown date palm varieties against Graphiola Leaf Spot disease to evaluate the varietal susceptibility. Poolsawat *et al* (2012) evaluated grape genotypes for resistance against anthracnose. Resistance evaluations categorized NY65.0550.04, NY88.0507.01, Wilcox 321& Illinois 547-1 as resistant lines useful as parents for future breeding programmes. Kathe *et al* (2011) screened the reactions of different grape varieties for resistance and susceptibility to rust pathogen caused by *Physopella ampelopsidis* under artificial epiphytotic conditions in glasshouse and in field conditions. The observations on the development of symptoms were recorded 15 days after inoculation and then at regular interval of seven days.

Anthracnose and its relationship with Agro-meteorological factors

Anthracnose incidence has been reported to be greatly influenced by the epidemiological factors such as rainfall, relative humidity and temperature. Singh and Joshi (1971) associated the diseases of *Piper beetle* L. caused by *Colletotrichum capsici* to pre-monsoon showers, which increased the relative humidity and reduced temperature also. In Tamil Nadu, Jeyarajan*et al* (1970) observed severe incidence of anthracnose during high moisture usually in rainy seasons.Rao and Satyanarayana (1991) carried out step down regression analysis with anthracnose disease and meteorological parameters, which showed that rainfall (49.9 mm/week) distributed over 3.16 days per week with 39 h sunshine per week, almost 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 increased markedly in June and then showed increasing trend from July to September, afterwards it declined as observed by Thind *et al* (1997) while studying periodical development of grape anthracnose and its relationship with agro-meteorological factors. 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 (2000) surveyed grape anthracnose in the regions of Northern Karnataka. The results revealed that the anthracnose was severe in *Kharif* season (April pruning) than *Rabi* season (October pruning). Contrary to these observations, continuous rainfall in Hyderabad in 1962 was the cause for heavy infection in Anab-e-Shahi

variety due to anthracnose (after October pruning). Pampanagouda and Benagi (2005) observed that anthracnose fungus grows well at a maximum temperature of 30-34°C, when the minimum temperature was almost constant, maximum and minimum relative humidity were in increasing trend and rains were received throughout the period.

Anatomical and physiological characteristics

The first line of defense against pathogen is the surface of plants. The pathogen enters the host by penetrating the epidermis either by force or through natural openings or wounds and insect punctures. Many phylloplane characters function as barriers to penetration by the pathogen, which must be broken by the latter if it has to cause infection. Such characteristics include wax and cuticle that cover the epidermal cells. Epidermal tissues form a protective covering on both the surfaces of the leaf. Specialized cells in epidermis include leaf appendages like trichomes and natural openings like stomata are dispersed through the ground tissue of epidermis. The phylloplane barriers to pathogen penetration limit the rate of progress of infection (Campbell *et al* 1980).

Stomata are the openings on phylloplanes. Their major function is exchange of gases and regulation of transpiration. Stomata are turgor operated valves which interfere between the environments and plant whose opening and closing is controlled by a pair of guard cells. When fully opened, they are big enough for active penetration of germ tube or zoospores of fungi and passive entrance of bacterial cells and fungal spores present in the rain drops. The fungal pathogen could enter either through stoma directly by germ tube or hyphal tips, or by formation of appresoria and then penetrating by infection peg. In general, plants protect themselves against pathogens by pre-existing structural characteristics that act as physical barriers and prevent the pathogen to enter through the host tissues. Rich (1963) reviewed the role of stomata in disease resistance. Their importance was emphasized not only as sites of entry for the microorganisms but also as exits permitting their dissemination.

Mahajan and Dhillon (2000) while making a comparative study on the epidermal characteristics of 20 watermelon genotypes, reported that on adaxial surface, the average stomatal frequency was found to be significantly higher in susceptible genotypes (446.85 mm^{-2}) in comparison to resistant genotypes (267.22 mm^{-2}). Similar results were obtained with abaxial surface. The average stomatal size on adaxial and abaxial surfaces of the leaf was found to be significantly higher in susceptible genotypes (16.50µm, 16.58 µm) as compared with resistant genotypes (11.20 µmand 10.96 µm) respectively. Stomatal index on adaxial surface was also found to be significantly higher in susceptible genotypes as compared with resistant genotypes. Similar results were obtained from abaxial surface.

Yadav and Thakur (2001) reported lesser stomatal index in the highly resistant cultivar significantly when compared with highly susceptible cultivar of pearl millet against downy mildew. Similar observations were reported in potato genotypes in relation to the late

blight resistance caused by *Phytophthora infestans* (Mahajan *et al* 2003).Variable degree of resistance and susceptibility to *Colletotrichum falcatum* infection which causes the red rot disease in sugarcane was studied by Arora *et al* (2005). Here resistant genotypes exhibited significantly lower size, frequency and index of stomata which appears to be important in conferring structural resistance to red rot of pathogen. Disease resistance and susceptibility depends on the plant's physiology altering their metabolic activities. Normal physiological activities of the host cells are disturbed and morphological and anatomical changes appear as visible changes. Ghosh *et al* (2003) reported some changes in biochemical parameters of mulberry leaves after infection with leaf spot disease and observed that the moisture content was reduced by 24.52% after fungal infection. Benthod *et al* (2001) observed large reduction in foliage after leaf rust infection caused by *Puccinia recondita*. Vale *et al* (2001) in three field experiments in 1997, 1998 and 1999 reported reduction in leaf area due to defoliation caused by angular leaf spot of *Phaseolus vulgaris.*

Biochemical characteristics

Total chlorophyll and carotenoids

Photosynthesis is the ultimate source of energy used by all living cells, plants or animal whether directly or indirectly. The pathogen affects the photosynthesis by the destruction of the chlorophyll including chloroplast and decreases the efficiency of the process of photosynthesis per mole chlorophyll (Singh 1984). The composition of leaf pigments is sensitive to plant stress, with a range of biotic and abiotic factors responsible for loss of photosynthetic pigments like chlorophylls or the production of photo-protective pigments such as β- carotene or zeaxanthin (Demmig-Adams and Adams, 1992). Changes in the content of leaf pigment of grapevines were 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). Blanchfield *et al* (2006) investigated response of leaf pigments to phylloxera infestation in Cabernet Sauvignon and Pinot Noir grapevines grown under field conditions. A reduction in the leaf chlorophyll content and an increase in photo-protective pigment concentrations were observed in leaves of phylloxera infested grapevines compared to healthy vines. Kulkarni (2009) recorded higher amount of chlorophyll a and b in resistant and moderately resistant genotypes than susceptible genotypes in both healthy and infected green gram leaves. In all the varieties, decrease in chlorophyll a and chlorophyll b was observed in diseased leaves. Total chlorophyll also shows the same trend.

Atwal *et al* (2004) also reported decrease in chlorophyll content of leaves of *Brassica junea* in relation to *Alternaia* blight disease. Arora and Kaur (2004) reported the higher level of total chlorophyll in the chilli genotypes resistant to *Alternaria solani* as compared to

susceptible genotypes, which decreased upon infection. The decrease was high in susceptible genotypes. 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.

Total soluble sugars

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.

Total soluble proteins

Khan *et al* (2001) studied the biochemical changes in sorghum leaves after infection with leaf spot pathogen *Drechslera sorghicola.* A significant increase in total soluble proteins was observed with the progress of disease in infected sorghum leaves. Changes in proteins occur when pathogen penetrates the host cells which disturb protein and related metabolism.

A comparison of protein content was done between healthy and infected leaves of *Colocasia esculenta* cultivars resistant and susceptible to *Phytophthora* leaf blight. The results showed 81% increase in the protein content of resistant cultivars as compared to the susceptible cultivars.

Similarly, Arun *et al* (2010) compared the protein content in healthy and infected tissues of pearlmillet against *Sclerospora graminicola* infection. The protein content was higher in the healthy tissues of the susceptible variety as compared to the resistant. Following infection, there was increase in the protein content in resistant as well as susceptible cultivars, but the increase was more observed in resistant cultivars.

Free amino acids

Singh (2000) analysed biochemical parameters in *Brassica* against white rust and downy mildew. The results showed higher content of total proteins and free amino acids in susceptible cultivars as compared to the resistant. Biochemical contents like proteins, amino acids, carbohydrates and minerals etc. were analysed in the leaf blight infected mulberry

leaves showed decrease when compared to the healthy leaves (Shree and Nataraj 1993). The complete disappearance or decrease of certain amino acids might either be due to the utilization by the pathogen enzymatic degradation or had been utilized by the host plant for the defense mechanism (Bashalah *et al* 1984).

Total phenols

Phenolic compounds are present extensively in the plants. Many phenols are considered to be potential toxic substances associated with prevention of multiplication of pathogen. Many workers have correlated the higher content of phenols with the resistance to various pathogens. Phenols act as anti-fungal compounds and can prevent the infection by biotic agents (Sivaprakashan and Vidhyasakaran 1993).

Gupta *et al* (1995) 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. Saharan *et al* (2000) studied changes in phenolic 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 genotype74-10. 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 green gram genotypes 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.

Proline

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 and Cress 1997). Goicoechea *et al* (2000) reported modifications in the concentration of proline in foliar tissues of pepper against *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) observed the increased levels 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 and Heba (2011) reported significant increase in proline content in powdery mildew infected leaves of flax lines as compared with either resistant or susceptible parents.

Ascorbic acid and α-Tocopherol

Ascorbic acid is the most abundant and ubiquitous cellular antioxidant (Khan *et al* 2012, Turner *et al* 2002). Ascorbic acid can neutralize reactive oxygen species in plant cells and is considered as a major antioxidant compound among the plant antioxidant-defense system.

Hegde and Anahosur (2001) evaluated biochemical characters on the basis of resistance to fruit rot (*Colletotrichum capsici*) in chilli genotypes. Results showed higher ascorbic acid content in resistant genotypes than in susceptible ones.

Similarly, Arora and Kaur (2004) also observed that the chilli genotypes resistant to *Alternaria solani* contain higher levels of ascorbic acid as compared to the susceptible ones. The content gradually decreased after infection and this decrease was significantly higher in susceptible genotypes.

Tocopherols also known as vitamin E are lipophilic antioxidants, solely synthesized by photosynthetic organisms. α-tocopherol is the most produced form of vitamin E in plant tissues. It has the highest vitamin E activity (Caretto *et al* 2010).

Lipid peroxidation

Munne-Bosch and Alegre (2002) demonstrated that chlorosis can be a consequence of lipid peroxidation with an increase in malondialdehyde and subsequent reduction in the antioxidant defenses in chloroplast, which leads to decreased chlorophyll concentration and photosynthetic activity. Dhingra (2011) recorded data for lipid peroxidation at stage I and II in both healthy and diseased genotypes of *Brassica oleracea* L. against *Alternaria* blight. After the onset of disease, the levels of lipid peroxidation increased significantly in all the genotypes. There was maximum increase in highly susceptible genotypes.

Antioxidant enzymes: Peroxidase and Polyphenol oxidase

Enzymes are known to play decisive role in host pathogen interaction. The role of several oxidative enzymes and their metabolic products in defense mechanism in plants has been identified. The changes in phenolic compounds may depend upon alterations in the activity of enzymes responsible for their biosynthesis. Certain oxidative enzymes (polyphenol oxidase, peroxidase and phenylalanine lyase) of host pathogen interaction defend the host from being diseased. In the presence of oxygen, polyphenol oxidase oxidizes the phenolic compounds that are in the form of O-diphenol to O-quinone. Marutian *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.

The disease resistance in various host-parasite combinations were correlated by enhanced phenol synthesis and peroxidase activity (Ghosal *et al* 2004). 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.

Senthil *et al* (2010) observed the effect on some phytochemical properties in *Cucumis sativus* when infected by *Penicillium notatum*, a leaf spot disease. The activity of both POD and PPO was higher in diseased leaves than the healthy leaves and it increased considerably with progression of infection. This increase may be required for an additional deposition of lignin around the lesion court induced by pathogen. Peroxidase may be rapidly involved in the peroxidation of subtrate molecule, leading to accumulation of highly toxic properties. The oxidised phenols i.e. quinones are more reactive and more toxic to microorganisms than their non-oxidised forms.

The biochemical basis of disease resistance by using susceptible and resistant pearl millet cultivars infected with downy mildew was described by Arun *et al* (2010). The activity of PPO was higher in the infected tissues of the resistant cultivar as compared to the susceptible cultivar. The POD activity increased in resistant as well as susceptible cultivars following infection, but the resistant cultivars showed higher activity.

Singh *et al* (1999) investigated defensive enzyme activity of *Brassica juncea* genotypes during pathogenesis of *Alternaria*. They observed higher PPO activity in infected leaves as compared to healthy one and the resistant genotypes expressed more PPO activity than the susceptible ones. Zhou *et al* (2012) studied correlation between resistance of eggplant and defense 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.

CHAPTER-III

MATERIALS AND METHODS

The present investigation on the "PHYSIOLOGICAL AND ANATOMICAL REACTION OF GRAPE (*Vitis vinifera* L.) GENOTYPES TO ANTHRACNOSE" was carried out in the Fruit Research Farm of Department of Fruit Science and in the Laboratories of Department of Botany, Punjab Agricultural University, Ludhiana during the year 2016 and 2017.

Vines from each of the five grape varieties, viz; Flame Seedless, Perlette, Pusa Navrang, Punjab MACS Purple (H 516), and Beauty Seedless grown in the Fruit Research Farm, Department of Fruit Science, PAU Ludhiana were selected. The vines selected were grown as per Package of Practices, PAU Ludhiana. The selected grapevines were visited regularly in order to monitor the disease incidence. In order to evaluate the disease severity, the leaves were collected and brought to the Laboratory, Department of Botany for analysis.

3.1 DISEASE INCIDENCE (Sridhar and Sohi 1970)

The grape varieties were screened for resistance and susceptibility against anthracnose on the basis of disease incidence. Three vines of each variety were selected for the evaluation of disease incidence on leaves. Data was recorded in 10 units per vine. The degree of severity was determined on the basis of visual observations using 0 to 5 scale.

Scale	Symptoms
0	Healthy foliage or minute lesions on leaves in traces
1	Upto 10% leaf area covered with lesions (> 1 mm in diameter)
2	Upto 25% leaf area covered with lesions, slight twig infection (1-3 cankers per twig)
3	Upto 50% leaf area covered with lesions, heavy twig infection (4-10 cankers per twig),>5% shoot damaged
4	Upto 75% leaf area covered with lesions, partly torn, very heavy twig infection, >25% shoot damaged
5	Above 75% leaf area covered with lesions, partly torn, very heavy twig infection, >50% shoot damaged

The formula for calculating Percent Disease Index (PDI) is as follows:

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

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

Plate II - B: Leaves of grape varieties showing variation in expression of disease score:

A. Scale 0 – Leaf showing no symptom of disease
B. Scale 1 – Upto 10% leaf area covered with lesions
C. Scale 2 – Upto 25% leaf area covered with lesions
D. Scale 3 – Upto 50% leaf area covered with lesions
E. Scale 4 – Upto 75% leaf area covered with lesions, partly torn
F. Scale 5 – More than 75% leaf area covered with lesions, partly torn

[According to the disease scale by Sridhar and Sohi (1970)]

A

B

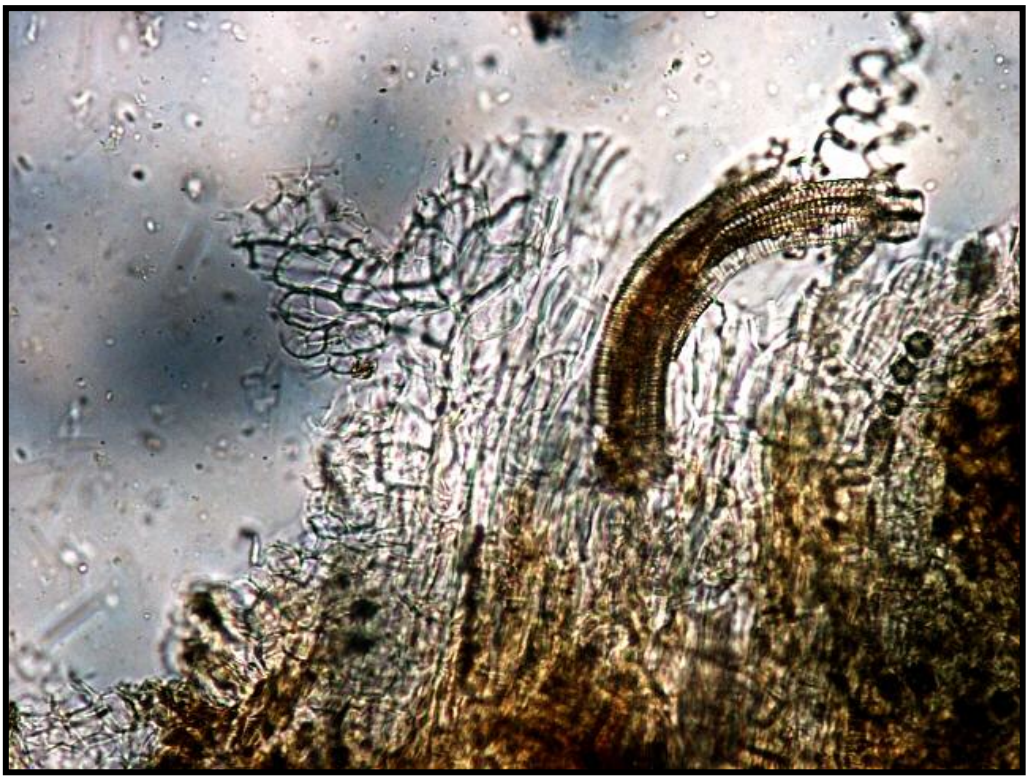
C

D

E

F

Plate III: *Elsinoe* hyphae as seen under the Leica Bright Field Research microscope (40 X)



Resistance level of each variety was rated on the basis of PDI, i.e. variety with highest PDI rated as extremely susceptible variety and the one with least PDI rated as resistant.

3.2 Phylloplane Morphology

The leaf samples of selected varieties collected and preserved in FAA (Formalin-acetic acid-ethyl alcohol) solution (prepared by mixing 85 ml of 50% ethyl alcohol, 5 ml of glacial acetic acid and 10 ml of 40% formaldehyde) (Johansen 1940) immediately after collection. The preserved leaves were washed thoroughly to remove preservative and dust. The surface of the leaf was dried between the folds of filter paper. Impressions were taken from middle abaxial and adaxial surface of the leaf by a standardized method by Dhillon *et al* (1997). A thin uniform layer of imprinting fluid was applied on both the surfaces of the leaf with the help of a spatula. The leaf surface was then dried at room temperature for 15-20 minutes. The imprinting film was peeled off with the help of forceps and mounted on microslides carefully. The coverglass was sealed with nail polish from all the sides. The following stomatal characteristics were studied on both adaxial and abaxial surfaces of the leaf with the help of Leica Bright Field Research Microscope.

Scanning Electron Microscopy (SEM)

For scanning electron microscopy (SEM), leaf tissues were cut into small pieces (0.5–1 cm^2), fixed in 2.5% (v/v) glutaraldehyde at 4°C overnight. Drained fixative and performed 3 washings with 0.1M Caco buffer after an interval of 15 minutes each. Then drained wash buffer and added 1% Osmium tetraoxide for 1-2 hours at 4°C. After draining OsO_4, 3 washings of 0.1 M Caco buffer were performed. After dehydration in a graded ethanol series (30, 50, 70, 80, 90, 100%, v/v), the samples were then critical-point dried, they were placed in vacuum desiccator overnight. They were coated with gold in a sputter coater, and examined with a scanning electron microscope available at EMN Lab, PAU.

3.2.1 Stomatal characteristics

a) Stomatal size

b) Stomatal Index

The number of stomata was counted in 10 randomly selected microscopic fields and the average number of stomata was expressed on mm^{-2} area of the microscopic field. Stomatal index was calculated by using following formula of Salisbury (1927).

$$\text{Stomatal Index} = \frac{S}{E+S} \times 100$$

S = No. of stomata

E = No. of epidermal cells

The size of stomata (i.e. the length of guard cell) was measured on Leica Bright Field Research Microscope fitted with a digital camera and computer imaging systems using

software NIS elements F 3.0 at 200X and pictomicrographs were taken.

3.3 Anatomical Studies

For anatomical studies, the healthy leaf samples of all the grape varieties evaluated were collected and preserved in FAA (Formalin-acetic acid-ethyl alcohol) solution (prepared by mixing 85 ml of 50 ethyl alcohol, 5ml of glacial acetic acid and 10 ml of 40 % formaldehyde) immediately after collection. Hand sections of leaves were cut by placing the leaves in the potato pith. The sections were stained by using a combination of safranin-fast green according to the following procedure:

Sections were stained in aqueous 2% safranin for 5 minutes. The sections were then washed with water and passed rapidly through upward series of alcohol. Counter staining was done by dipping the sections for few seconds in 2% fast green and then passed the sections through alcohol, alcohol : xylene (1:1) and finally to xylene. These sections were mounted in DPX under coverslip. The slides were observed under Lieca Bright Field Research Microscope fitted with digital camera and computing imaging systems using software NIS Elements F 3.0 at 200X.

3.4 Biochemical studies

For Biochemical studies, healthy and anthracnose infected leaves and berries were collected from the selected varieties. Three vines of each variety were selected for sample collection. The leaf samples were dried in the oven at 60°C and then ground into a fine powder to estimate different metabolites *viz.*, total soluble sugars, total phenols, total proteins and free amino acids. While fresh leaves were taken to estimate total chlorophyll, carotenoids, ascorbic acid, α-tocopherol, proline, lipid peroxidation and enzymatic antioxidants..

3.4.1 Total Chlorophyll content in leaves (Hiscox and Israelstam, 1979)

Chlorophyll content was estimated using Dimethyl Sulphoxide (DMSO) method. Freshly removed healthy and infected leaves of each variety were taken and 50 mg of the finely chopped portion was dipped in each test tube containing 10 ml of DMSO. The test tubes were then kept in an oven at 60°C for about 4 hours to facilitate the extraction of pigments. After a requisite period, the extract was allowed to cool down at the room temperature. Subsequently, the tubes were shaken to allow the pigment to distribute uniformly and the absorbance was read at 645 and 663 nm wavelengths on spectrophotometer (SL-171). The following formulae were used to determine the chlorophyll content:

$$\text{Chlorophyll a} = 12.7\,(A_{663}) - 2.69\,(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9\,(A_{645}) - 4.68\,(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total Chlorophyll} = 20.2\,(A_{645}) + 8.02\,(A_{663}) \times \frac{V}{1000 \times W}$$

Where,

A_{663} = Absorbance at 663 nm

A_{645} = Absorbance at 645 nm

V = Total volume of the extract (ml)

W = Weight of the sample (g)

The value of total chlorophyll was expressed as mg g^{-1} fresh weight.

3.4.2 Carotenoid content (Kirk and Allen, 1965)

100 mg of fresh healthy and infected leaves of each variety were collected and were homogenized thoroughly in pestle and mortar with 3 ml of 80% acetone and transferred to centrifuge tubes. Centrifuged at 3000 rpm for 10 minutes and a clear greenish supernatant was collected in the test tubes. Pellet was extracted again with 2 ml of 80% acetone and re-centrifuged. The two supernatants were pooled and the total volume was made upto 10 ml with acetone. The absorbance was taken at 480, 645 and 663 nm. The carotenoid content was determined using the following formula:

$$\text{Carotenoid} = A_{480} + (0.114\ A_{663} - 0.638\ A_{645}) \times \frac{V}{1000 \times W}$$

Where,

A_{480} = Absorbance at 480 nm

A_{663} = Absorbance at 663 nm

A_{645}= Absorbance at 645 nm

V = Total volume of the extract (ml)

W =Weight of the sample (g)

The value of carotenoid content was expressed as mg g^{-1} fresh weight.

3.4.3 Total soluble sugars (Dubois *et al* 1956)

Reagents

A : 5 % phenol

B : 80% ethanol

C : Chilled conc.H_2SO_4

D : Standard glucose = 10mg/100ml

Extraction

100mg of dried leaf material was homogenized in pestle and mortar with 5 ml of 80 % ethanol, followed by centrifugation at 3000 rpm. The extraction procedure was repeated with 3ml of 80 percent ethanol. The final volume of pooled supernatants was adjusted to 10 ml with distilled water.

Procedure

0.1ml of the extract was taken in a test tube and 0.9 ml of distilled water was added. Then 1 ml of 5% phenol was added to it. After 10 minutes, 5ml of reagent C was added slowly

with continuous stirring. The greenish brown colour was obtained. After rapid cooling, the absorbance was read at 470 nm in spectrophotometer against 1ml 5% phenol and 5 ml cold conc. H_2SO_4 as blank. The concentration of total soluble sugars/g (as glucose) of the sample was calculated against a standard curve prepared of pure glucose (10-100µg/ml) and expressed as mg g^{-1} dry weight.The following formula is used in calculating total soluble sugars:

$$\frac{\text{Conc. of Standard} \times \text{OD of test sample} \times \text{Total vol. of extract} \times 1000}{\text{OD of Standard} \times \text{Vol. of sample taken from extract} \times \text{amount of tissue/juice taken for extraction}}$$

3.4.4 Total Proteins (Lowry *et al* 1951)

Reagents:

A: 2% Sodium carbonate in 0.1 N Sodium Hydroxide

B: 0.5% $CuSO_4.5H_2O$ in 1% Sodium Potassium Tartarate

C: 15% Trichloroacetic acid (TCA)

D: 50 ml of reagent A mixed with 1 ml of reagent B just before use

E: Folin-Ciocalteau reagent diluted with water in 1:1 ratio at the time of use

Extraction:

100 mg of dried leaf material was homogenised in 5.0 ml of 0.1 N Sodium Hydroxide followed by centrifugation at 6000 rpm for 10 minutes and supernatant was collected. The residue was washed in 3 ml of 0.1 N Sodium Hydroxide and re-centrifuged. The two supernatants were pooled and the final volume was adjusted to 10 ml. From this, 2 ml of supernatant was treated with 15% Trichloroacetic acid (TCA) and kept for 24 hours at 4°C. The precipitates obtained were separated from aliquot by centrifugation at 6000 rpm for 15 minutes. The supernatant was recovered for estimating amino acids. The precipitates were dissolved in 10 ml of 0.1 N Sodium Hydroxide. The resultant solution was used as extract for the estimation of proteins.

Estimation:

0.2 ml of protein extract was diluted with 0.8 ml distilled water. To this, 5 ml of reagent D was added and mixture was constantly shaken and incubated at room temperature for 15 mins. Then 0.5 ml of reagent E was added rapidly with constant shaking. This was kept undisturbed at room temperature for 30 minutes. The absorbance of the resultant blue colour was read at 570 nm against a blank of replacing extract on spectrophotometer. The standard curve was prepared using different concentrations (20-100 µg ml^{-1}) of Bovine serum albumin (BSA). The total protein content was expressed as mg g^{-1} dry weight.

3.4.5 Free Amino Acids (Lee and Takahashi 1966)

Reagents:

A: 80% Ethanol

B: 1% Ninhydrin in 0.5 M citrate buffer

C: Glycerol

D: 0.5 M citrate buffer (pH 5.5)

E: Ninhydrin reagent (B: C: D :: 5 : 12 : 2)

Extraction:

500 mg of dry leaf material was homogenised in 5 ml of 80% ethanol followed by centrifugation. Collected the supernatant and repeated the extraction with 3 ml of 80% ethanol. The two supernatants were pooled and the total volume was adjusted to 10 ml.

Estimation:

To 0.2 ml of the extract, 5 ml of reagent E was added. The contents were shaken vigorously and boiled the reaction mixture for 12 minutes in water bath. Purple colour was obtained. The mixture was cooled to room temperature under running tap water. The absorbance was read at 570 nm against glycine as blank in spectrophotometer. The standard curve was prepared using different concentrations (0-100 µg ml^{-1}) of standard glycine solution. Standard glycine solution was made by dissolving 10 mg of glycine in 0.5 M citrate buffer and volume was made upto 100 ml (concentration 100 µg ml^{-1}).

3.4.6 Total Phenols (Swain and Hills 1959)

Reagents:

A: 0.3 N HCl in methanol

B: Folin-Ciocalteau reagent diluted 1:1 with distilled water

C: 35% solution of sodium carbonate

Extraction:

100 mg of dried leaf material was extracted twice with 5.0 ml of 0.3 N HCl in methanol followed by centrifugation at 5000 rpm for 10 minutes. Supernatants were pooled from two extractions in a china dish and evaporated to dryness on hot water bath. The dried residue was dissolved in 10 ml of distilled water.

Procedure:

0.5 ml of methanolic extract was diluted to 7 ml with distilled water. To this 0.5 ml of Folin-Ciocalteau reagent was added and shaken thoroughly. After 3 minutes, 1 ml of 35% solution of saturated sodium carbonate was added. These tubes were then placed in dark for 1 hour. A blue colour was developed in each tube because the phenols undergo a complex redox reaction with phosphorus-molybdic acid in Folin-Ciocalteau reagent in alkaline medium resulting in a blue coloured complex, molybdenum blue. Add 4 ml of distilled water to each test tube. The absorbance of blue coloured solution was read in spectrophotometer at 630 nm against the blank prepared from water and reagent only. The concentration of total phenol was determined by preparing a standard curve using Gallic acid (10-50 µg). The total phenol content was expressed as mg g^{-1} dry weight. The following formula is used in calculating total phenols:

$$\frac{\text{Conc. of Standard} \times \text{OD of test sample} \times \text{Total vol. of extract} \times 1000}{\text{OD of Standard} \times \text{Vol. of sample taken from extract} \times \text{amount of tissue/juice taken for extraction}}$$

3.4.7 Ascorbic Acid (Vitamin C) (Roe and Oesterling 1943)

Reagents:

A: Trichloroacetic Acid (TCA) solution: 0.5 mM of Na_2-EDTA IN 3% TCA

B: 2% Dinitrophenyl hydrazine solution (DNPH) : 2 gm of 2,4 DNPH + 100 ml of 9 N H_2SO_4

C: Thiourea solution: 10% thiourea in 70% ethanol

D: Sulphuric Acid (80%)

Extraction:

100 mg of fresh healthy and infected leaves were homogenized in 0.5 mM Na_2-EDTA in 3% TCA followed by centrifugation at 5000 rpm for 15 minutes. Extraction procedure was repeated twice and pooled supernatant was used for the assay.

Estimation:

To 0.2 ml extract added 1 ml of 2% Dinitrophenyl hydrazine solution, followed by the addition of one drop of thiourea reagent in the test tubes. Boiled the mixture in water bath for 15 minutes and then left the tubes to cool at room temperature and then at 0°C, to the reaction mixture, added dropwise 2.5 ml of 80% (v/v) H_2SO_4. The absorbance reading was taken at 540 nm against the water blank replacing the extract.

3.4.8 Lipid peroxidation (Heath & Packer 1968)

Reagents:

A: 0.1 % Trichloroacetic acid (TCA)

B: 0.5 % Thiobarbituric acid (TBA)

Extraction:

100 mg of fresh leaf tissue from each variety was homogenized by adding 5 ml of 0.1 % TCA. The homogenized tissue was centrifuged for 10 minutes at 10,000 rpm.

Estimation:

1 ml of supernatant was vortexed with 4 ml of 20 % TCA containing 0.5 % thiobarbituric acid and the solution was heated for 30 minutes at 90° C. Samples were cooled on ice for 5 minutes and re-centrifuged for 10 minutes at 10,000 rpm. The non-specific absorbance of supernatant at 600 nm was subtracted from the maximum absorbance at 532 nm for MDA measurement and was calculated using Lambert – Beer law with an extraction coefficient $\varepsilon M = 155\ mM^{-1}\ cm^{-1}$. Results were presented as n moles MDA gm^{-1} FW.

3.4.9 α-Tocopherol (Asthir *et al* 2009)

Reagents:

A: Absolute alcohol (Ethanol)

B: Xylene

C: α, α′-dipyridyl reagent: 120 mg of α,α′-dipyridyl in 100 ml of n-propanol

D: $FeCl_3$ reagent: 71.9 mg of $FeCl_3$ anhydrous in 100 ml of Absolute ethanol. Keep it in dark brown bottle

E: α-tocopherol acetate: 100 mg of DL-α-tocopherol acetate in 100 ml of absolute ethanol (used as reference standard)

Extraction:

0.5 g of fresh leaf sample was homogenised in 5 ml of absolute ethanol. It was then centrifuged at 8000 rpm for 20 minutes. The supernatant was used for estimation.

Estimation:

Blank 1 (at 460 nm): 1 ml distilled water + 1.5 ml absolute alcohol

Blank 2 (at 520 nm): 1 ml distilled water + 1.5 ml $FeCl_3$ reagent

2.5 ml of extract was taken in test tube. To it 1.5 ml of Xylene was added. The test tubes were shaken at vortex. The solution was poured in centrifuged tubes and was centrifuged at 5000 rpm for 10 minutes. Two layers were formed. 1 ml from upper xylene layer was pipetted out in test tube. To each test tube, added 1 ml of α, α′-dipyridyl reagent. Take readings at 460 nm against blank 1 and at 520 nm against blank 2.

3.4.10 Proline (Bates *et al* 1973)

Reagents

A: 3% Sulpho-salicylic Acid

B: Acid Ninhydrin reagent (1.86 g of Ninhydrin mixed in 45 ml of glacial acetic acid and 30 ml of 6M Orthophosphoric Acid, then kept in oven at 70°C till a clear solution was formed)

C: Toluene

D: 6M Orthophosphoric acid: prepared by adding 9.25 ml of orthophosphoric acid in 20.75 ml of distilled water

E: Standard: 0.01g/100 ml of distilled water

Proline = 0.02 to 0.1 µmoles

Extraction:

0.5 g of leaf sample was homogenized in 5 ml of 3% Sulpho-salicylic acid in pre-chilled mortar and pestle. The mixture was filtered through Whatman filter paper.

Estimation:

1 ml of filtrate was taken in a test tube. 1 ml of reagent B and 1 ml of acetic acid was added and it was boiled in a water bath at 100°C. The reaction was terminated by keeping the solution on an ice bath. Then 4 ml of toluene was added and mixed vigorously with the help of vortex for 30 seconds. The chromophore containing upper toluene layer (light pink) was collected and its absorbance was read at 520 nm on spectrophotometer using pure toluene as a blank.

The proline concentration in the samples was determined from a standard curve prepared by using analytical grade proline and calculated on fresh weight basis according to

the following formula.

$$\frac{\text{Conc. of Standard} \times \text{OD of test sample} \times \text{vol. after filtration}}{\text{OD of Standard } \mathbf{x} \text{ Vol. of sample taken for estimation } \mathbf{x} \text{ amount of fresh tissue/juice taken for extraction}}$$

3.4.11 Enzymatic Antioxidants

Assay of enzyme activities viz. peroxidase and polyphenol oxidase.

3.4.11.1 Peroxidase (PO) (EC 1.11.1.7)

Peroxidase activity was assayed by the method of **Thomas *et al* 1981**.

Extraction:

1 gm of fresh leaf sample was taken in a pre-chilled pestle and mortar and was homogenised thoroughly with 10 ml of ice-cold 0.1 M potassium phosphate buffer (pH = 7.5) containing 1% Polyvinylphosphate (PVP), 1mM EDTA, 10 mM β-mercaptoethanol. Extracted homogenate was transferred to ice-cold centrifuge tubes. The homogenate was centrifuged at 11,000 rpm for 20 minutes at 2°C. Floating particles present in the supernatant were removed by filtering through cotton wool and then it was used for estimation of enzymes.

Reagents:

A: 20 mM Guiacol

B: 20 mM of hydrogen peroxide

Estimation:

The assay utilized guiacol as the enzyme substrate. The reaction mixture was prepared by adding 3 ml of guiacol, 0.1 ml of H_2O_2 and at last 0.2 ml of enzyme extract. The absorbance was read at 470 nm for 3 minutes at every 30 second interval against water blank. The peroxidase activity was expressed as number of absorbance units per gram fresh weight of leaf/berries. The activity of the enzyme peroxidase can be calculated as:

Δ A = change in OD / minute

$$\text{Activity} = \frac{\Delta A \times \text{vol. after centrifuge (ml)}}{\text{Enzyme extract taken (ml) x fresh wt. (g)}}$$

$$= \text{units } min^{-1}\ g^{-1} \text{ of fresh weight.}$$

3.4.11.2 Polyphenol oxidase (PPO) (EC 1.10.3.1)

Polyphenol activity was assayed by the method of **Zaubermann *et al* 1991**.

Extraction:

0.2 g of fresh leaf tissue was homogenised in pre-chilled pestle and mortar with 1.5 ml ice-cold 0.1 M sodium phosphate buffer (pH = 6.8). It was then centrifuged at 10,000 rpm for 20 minutes in a cooling centrifuge at 4°C. The supernatant was used for enzyme estimation.

Reagents:

A: 0.1 M sodium phosphate buffer (pH = 6.8)

B: 100 mM 4-MethylCatechol

Estimation:

To 1.4 ml of 0.1 M sodium phosphate buffer (pH = 6.8), 0.5 ml of 100 mM 4-MethylCatechol was added. The reaction was initiated by adding enzyme extract as the last component. 0.1 ml of enzyme extract was added to the cuvette and the absorbance was read at 410 nm every 30 seconds upto 3 minutes. 1.5 ml of 0.1 M sodium phosphate buffer was taken as blank. The activity of PPO can be calculated as:

OD per minute = $OD_3 - OD_1 / 3 = a$

0.01 increase in absorbance = 1 unit

1 increase in absorbance = 1 / 0.01 unit

'a' increase in absorbance = 1 / 0.01 x a unit = b unit

$$\text{Activity} = \frac{\text{b unit} \times \text{vol. after centrifuge (ml)}}{\text{Enzyme extract taken (ml)} \times \text{fresh wt. (g)}}$$

= units min^{-1} g^{-1} of fresh weight

3.5 AGROMETEOROLOGICAL DATA:

The data of agrometrological parameters viz., maximum and minimum temperature, relative humidity, rainfall, evaporation and sunshine hours for the year 2016-17 was attained from School of Agricultural Meteorology, PAU. (Annexure)

3.6 STATISTICAL ANALYSIS:

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

CHAPTER-IV

RESULTS AND DISCUSSION

The present investigation on the "PHYSIOLOGICAL AND ANATOMICAL REACTION OF GRAPE (*Vitis vinifera* L.) GENOTYPES TO ANTHRACNOSE" was carried out in the year 2016-17 with the objective to evaluate grape varieties for susceptibility to grape anthracnose on the basis of disease severity and anatomical studies and an attempt was made to characterize physiological and biochemical parameters as indicator for identifying grape genotypes tolerant to anthracnose. The study was categorized in two experiments and the results are presented under the following headings:

4.1 Percent Disease Index

4.2 Physiological Characteristics

4.3 Biochemical Characteristics

4.1 Percent Disease Index

Screening of the grape varieties viz., Flame Seedless, Pusa Navrang, Punjab MACS Purple, Beauty Seedless and Perlette was done based on the field reactions of leaves and berries to anthracnose during the year 2016. The Percent Disease Index (PDI) of the grape varieties ranged from 0.66 to 73.00 in the field studies (Table 4.1). The variety Punjab MACS Purple having PDI = 0.66 is rated as resistant to anthracnose. The varieties Pusa Navrang and Flame Seedless with PDI of 27.66 and 36.33 are rated as moderately resistant varieties, whereas varieties Beauty Seedless and Perlette are rated as susceptible to the disease having PDI of 61.66 and 73.00 respectively. This deviation in the response of grape varieties to the incidence of *Elsinoe ampelina* shows that there is a specific genetic mechanism that confers the ability to become susceptible or resistant to a pathogen attack.

Table 4.1: Screening of grape varieties against Anthracnose (Percent Disease Index in leaves)

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

Similar studies were performed by Shankar and Jindal (2002) while screening the grape germplasm against anthracnose. They tested 48 genotypes, among which 11 were resistant, 19 moderately resistant and 18 susceptible. The variety Pusa Navrang has previously been rated as resistant under field conditions in a study undertaken on 32 grape

genotypes at IARI, New Delhi (Gurjar *et al* 2015).

The mean temperature and relative humidity was recorded to be 30.4°C and 85 percent respectively during the onset of anthracnose infection (Annexure).

4.2 Anatomical and physiological characteristics

The possible significance of anatomical characteristics contributing towards acquisition of resistance against *Elsinoe ampelina* in grape varieties is presented here. It had been observed that stomata were present only on the abaxial epidermis of the leaves and none of the varieties showed stomata on adaxial surface. Similar report had been made while reporting microscopic leaf characteristics of grapevine genotypes to downy mildew by Boso *et al* (2010).

4.2.1 Stomatal size

SEM studies were carried out in the healthy leaves of all the grapevine varieties evaluated and in the infected leaves of the most susceptible variety Perlette. The data for stomatal size (length and width) in the abaxial phylloplane of the grape varieties is presented in table 4.2. These stomatal length (22.93 µm) and width (10.23 µm) was significantly high in the most susceptible variety Perlette as compared to the rest of the varieties. The minimum stomatal width (5.66 µm) was in the variety Punjab MACS Purple which is the most resistant variety. There was a linear trend in the stomatal width being minimum in the most resistant variety and maximum in the most susceptible variety (Plate IV). Similar report regarding stomatal size in watermelon genotypes against *Alternaria* blight had been reported by Mahajan and Dhillon (2000), wherein they report significantly higher stomatal size in the susceptible genotypes as compared to the resistant genotypes. As the pathogen enters through the stomata, it may be inferred that the smaller stomatal size in Punjab MACS Purple is conferring resistance whereas bigger stomatal size is conferring susceptibility to variety Perlette.

Table 4.2: Variation in stomatal size (µm) in grape varieties (Abaxial surface of leaves)

VARIETY	PDI	Stomatal Length (µm)	Stomatal Width (µm)
Resistant			
Punjab MACS Purple	0.66	13.96[b]	5.66[b]
Moderately Resistant			
Pusa Navrang	27.66	13.70[b]	6.09[b]
Flame Seedless	36.33	16.13[b]	6.37[b]
Susceptible			
Beauty Seedless	61.66	14.90[b]	6.53[b]
Perlette	73.00	22.93[a]	10.23[a]
Mean	39.86	16.32	6.97

Least Squares-means with the same letter are not significantly different (P<0.05)

The most susceptible variety Perlette was further evaluated and the stomatal length and width of the healthy and infected leaves was compared (table 4.3). It was found that the stomatal length is significantly high in the healthy (22.93 µm) leaves as compared to their infected counterparts (10.40 µm). Similar trend with respect to stomatal width is observed i.e. stomatal width in healthy leaves (10.23 µm) is significantly higher than the infected leaves (3.72 µm) of the variety Perlette. It is possible that the pathogen *Elsinoe ampelina* reduced the stomatal size after causing infection (Plate IV)

Table 4.3: Variation in stomatal size (µm) in the most susceptible variety Perlette (Abaxial surface of leaves)

Perlette	Stomatal Length (µm)	Stomatal Width (µm)
Healthy	22.93[a]	10.23[a]
Infected	10.40[b]	3.72[b]
Mean	16.67	6.98

Least Squares-means with the same letter are not significantly different (P<0.05)

4.2.2 Stomatal index

The data on stomatal index for the grape varieties infested with *Elsinoe ampelina* is presented in table 4.4. The stomatal index was maximum (14.62 µm) on the abaxial surface of the leaves in the most susceptible variety Perlette which is significantly higher from the moderately resistant Pusa Navrang and Flame Seedless; and the resistant variety Punjab MACS Purple; whereas at par with the susceptible variety Beauty Seedless (Plate V).

Table 4.4: Variation in stomatal index in grape varieties

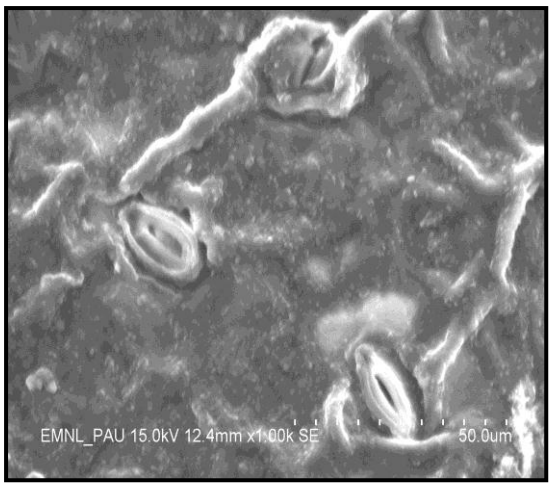
VARIETY	PDI	Stomatal Index
Resistant		
Punjab MACS Purple	0.66	8.44[b]
Moderately Resistant		
Pusa Navrang	27.66	7.17[b]
Flame Seedless	36.33	10.83[b]
Susceptible		
Beauty Seedless	61.66	14.26[a]
Perlette	73.00	14.62[a]
Mean	39.86	11.06

Least Squares-means with the same letter are not significantly different (P<0.05)

The stomatal openings provide an access to the pathogens which may be advantageous for the plant defense (Li *et al* 1999). The presence of maximum stomatal index and stomatal size in the most susceptible variety Perlette indicates the possible role of stomata towards susceptibility to *Elsinoe ampelina*. Similar report has been made by Gurjar *et al*

Plate IV: Scanning electron micrograph of leaf surface of all the varieties evaluated (Stomata observed)

A. Perlette Healthy
B. Perlette Diseased
C. Beauty Seedless
D. Flame Seedless
E. Pusa Navrang
F. Punjab MACS Purple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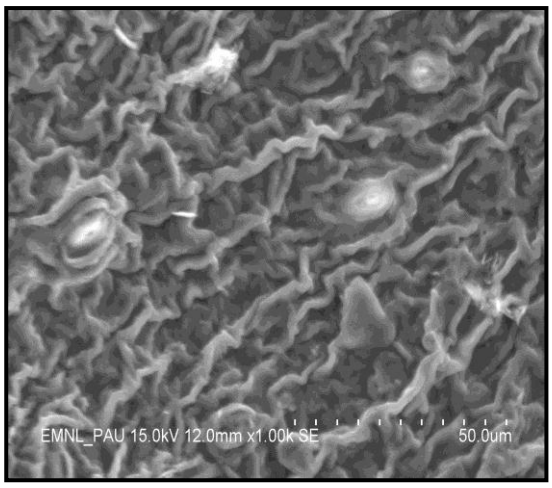
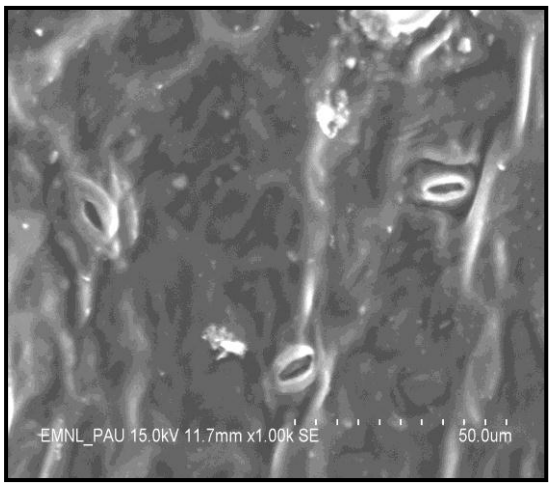
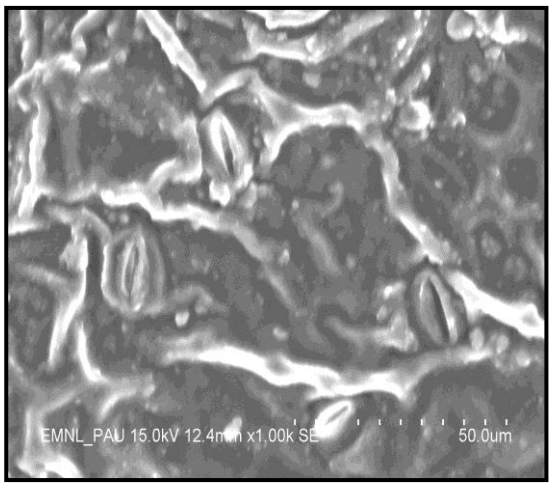
A


EMNL_PAU 15.0kV 12.0mm x1.00k SE
50.0um

B


EMNL_PAU 15.0kV 11.7mm x1.00k SE
50.0um

C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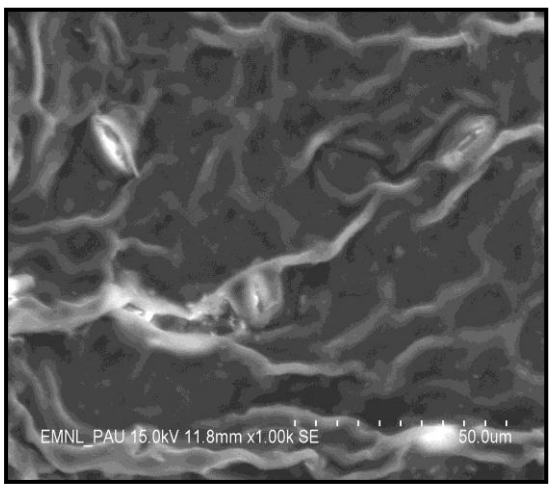
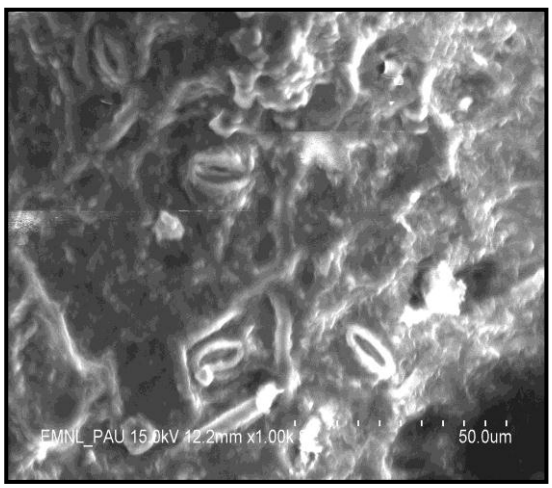
50.0um

D


EMNL_PAU 15.0kV 11.8mm x1.00k SE
50.0um

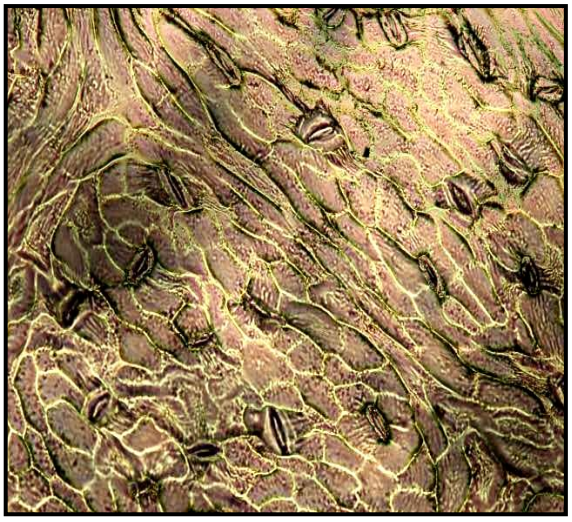
E


EMNL_PAU 15.0kV 12.2mm x1.00k
50.0um

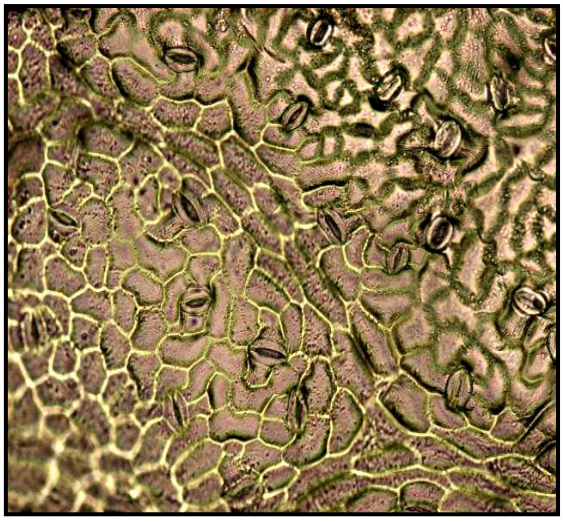
F

Plate V: Abaxial Surface view of leaves as observed under Leica Bright Field Research Microscope (20 X)

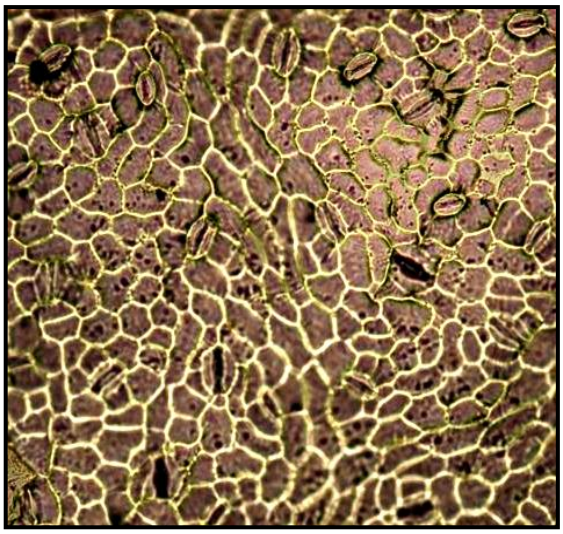
A. Perlette – Susceptible
B. Beauty Seedless – Susceptible
C. Flame Seedless – Moderately Resistant
D. Pusa Navrang – Moderately Resistant
E. Punjab MACS Purple – Resistant



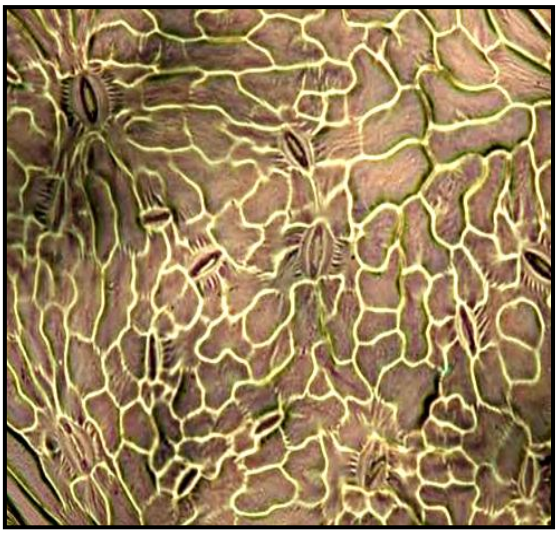
A



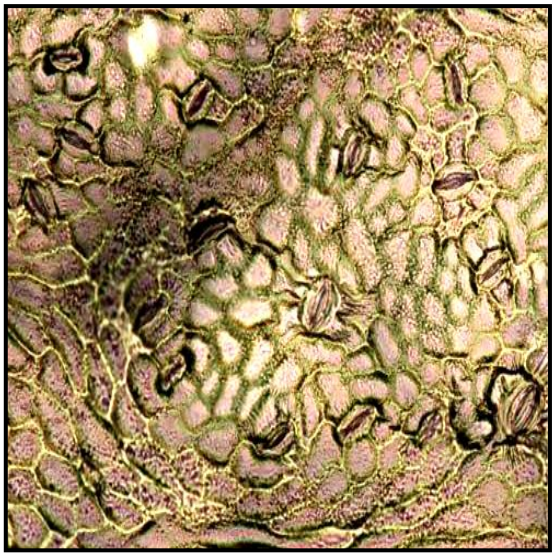
B



C



D



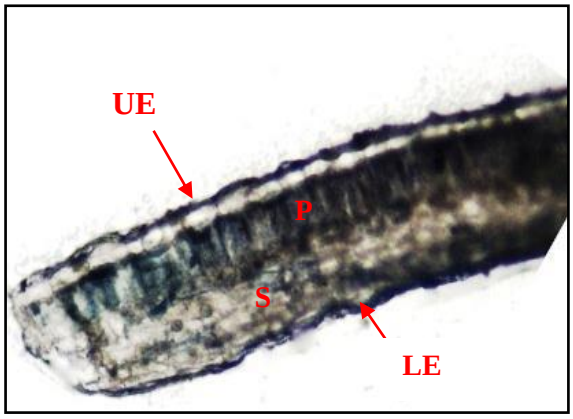
E

Plate VI : Transverse section of leaves of all the grape varieties evaluated

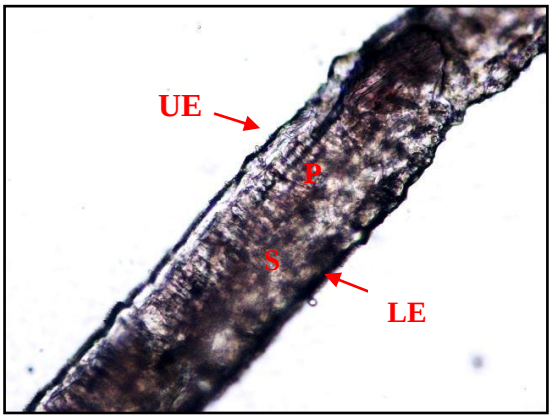
A. Perlette – Susceptible - intercellular spaces in spongy mesophyll
B. Beauty Seedless – Susceptible – lesser intercellular spaces in spongy mesophyll
C. Flame Seedless – Moderately Resistant intercellular spaces in spongy mesophyll
D. Pusa Navrang – Moderately Resistant – compactly arranged cells in spongy mesophyll
E. Punjab MACS Purple – Resistant - compactly arranged cells in spongy mesophyll

(P = Palisade mesophyll, S = Spongy mesophyll, UE = Upper epidermis,

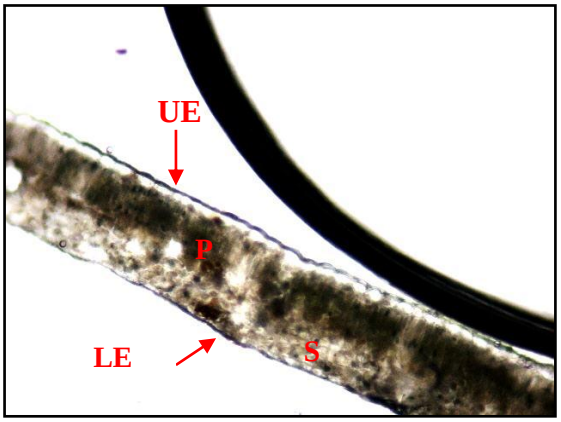
LE = Lower epidermis)



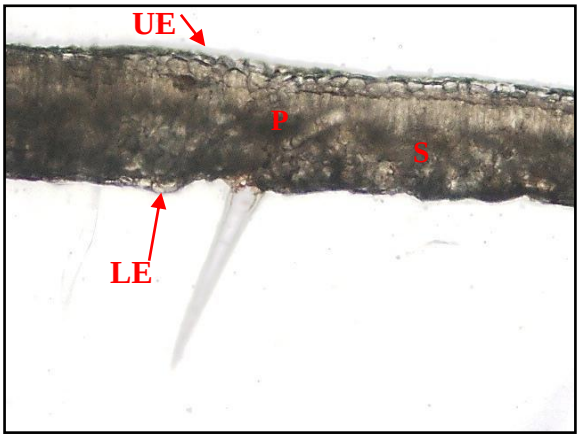
A



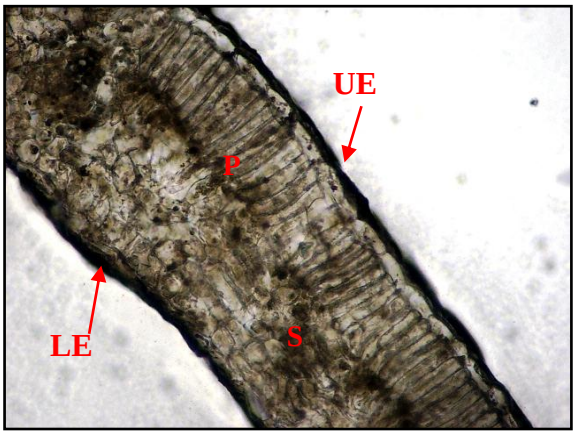
B



C



D

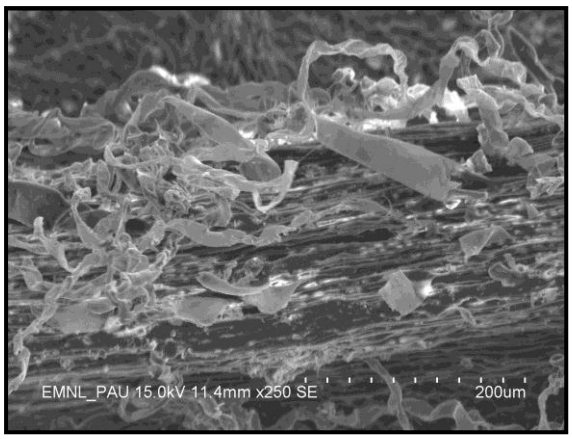


E

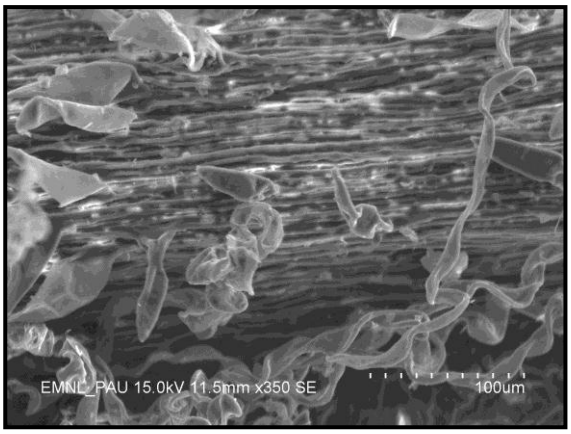
Plate VII: Scanning electron micrograph of leaf surface of all the varieties evaluated

A & B: Trichomes in healthy leaf of Pusa Navrang- moderately resistant

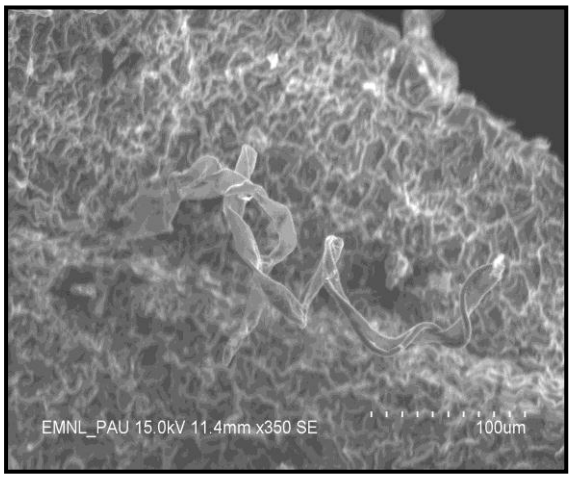
C & D: Trichomes in healthy leaf of Punjab Purple- resistant


EMNL_PAU 15.0kV 11.4mm x250 SE
200um

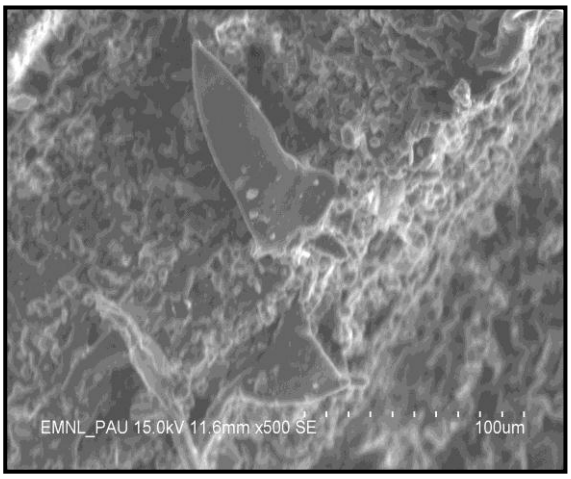
A


EMNL_PAU 15.0kV 11.5mm x350 SE
100um

`B


EMNL_PAU 15.0kV 11.4mm x350 SE
100um

C

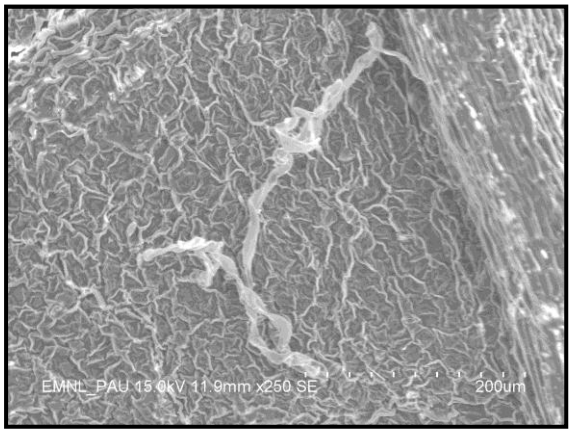

EMNL_PAU 15.0kV 11.6mm x500 SE
100um

D

Plate VIII: Scanning electron micrograph of leaf surface of all the varieties evaluated

A & B: Trichomes in anthracnose infected leaf of Flame Seedless- moderately resistant

C & D: Trichomes in anthracnose infected leaf of Perlette- susceptible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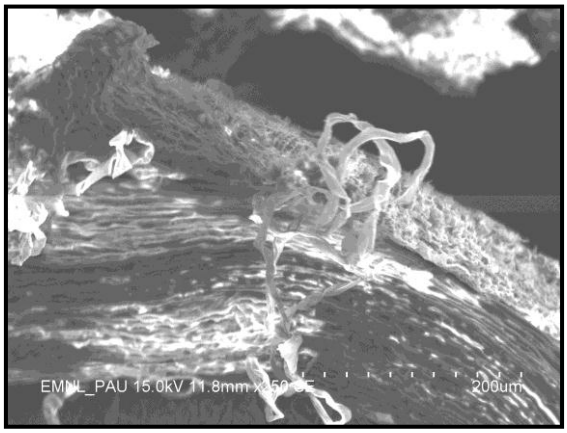
200um

A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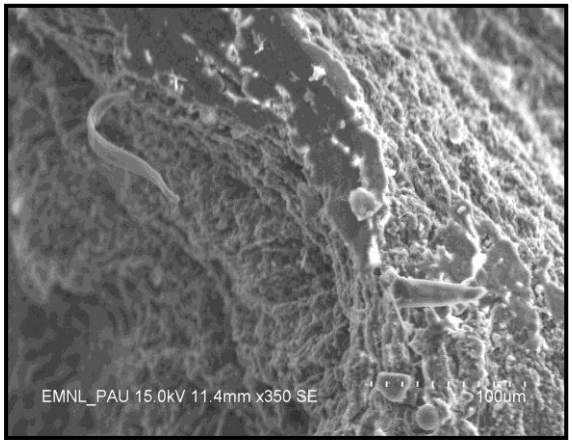
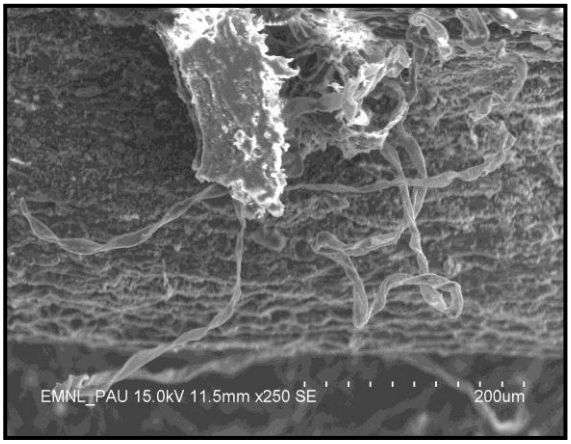
200um

B


EMNL_PAU 15.0kV 11.4mm x350 SE
100um

C


EMNL_PAU 15.0kV 11.5mm x250 SE
200um

D

(2015) on grape genotypes against anthracnose incidence.

4.2.3 Structure of mesophyll

Plate VI shows the size of mesophyll cells in the lamina of the grape varieties. The transverse sections of the leaves of all the genotypes show the upper epidermis, the lower epidermis, the mesophyll cells and the cuticle. The cuticle was visibly thickest in the variety Punjab MACS Purple which is the most resistant variety with minimum PDI. The upper epidermal cells were uniform in all the varieties. The palisade mesophyll in all the varieties was only one cell layer cell with elongated cells that were arranged in a compact manner. There were 4-5 layers of cells constituting the spongy mesophyll in all the varieties. There was a possible existence of a positive correlation between the thickness of cuticle with the resistance to anthracnose in Punjab MACS Purple. The cuticle may be playing an important role for the adhesion of sporangia around the stomata. The highly compact spongy mesophyll in Punjab MACS Purple (Plate VI-E) and Pusa Navrang (Plate VI-D) may hamper the growth of the mycelium of the host tissue. These findings are in agreement with those of Ribereau-Gayon and Peynaud (1982) that the mesophyll structure may influence the spread of pathogen in the grapevine.

4.2.4 Trichome characteristics

SEM studies were carried out in the healthy leaves of all the varieties and the infected leaves of Perlette and Flame Seedless. Trichomes were observed in healthy leaves of Pusa Navrang (Plate VII-A, B), in the healthy leaves of Punjab MACS Purple (Plate VII-C, D) and in the anthracnose infected leaves of Flame Seedless (Plate VIII-A, B) and Perlette (Plate VIII-C, D). It is likely that the trichomes in grape varieties Pusa Navrang and Punjab MACS Purple confer resistance against the pathogen *Elsinoe ampelina*. In the variety Perlette no trichomes were observed in the healthy leaves but the appearance of trichomes post infection is probably the in-built mechanism of the susceptible variety to avoid the further infestation of the pathogen. It is evident from the Plate VII (A, B) that the trichome density is very high in Pusa Navrang which was conferring resistance against the pathogen. This dense layer of trichomes in the resistant varieties may act as a constitutive barrier against infections by grapevine as they prevent proper leaf wetness required for the release of zoospores and targeting stomata (Kiefu *et al* 2002).

4.2.5 Pearson correlation coefficient between Percent disease index (PDI) and stomatal characteristics

Pearson correlation coefficients were computed between PDI and stomatal length, width and index. A significantly positive correlation of PDI is being reported with stomatal length (0.685^{**}), stomatal width (0.584^{*}) and stomatal index (0.883^{**}) and these are significant at $P<0.05$ (Table 4.5).

Table 4.5: Correlations among Percent disease index (PDI) and stomatal characteristics

S.No.	Characteristics	1	2	3	4
1.	PDI	1.000	0.685**	0.584*	0.883**
2.	Stomatal length on abaxial phylloplane		1.000	0.637*	0.661**
3.	Stomatal width on abaxial phylloplane			1.000	0.522**
4.	Stomatal index on abaxial phylloplane				1.000

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

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

4.3 Physiological characteristics

4.3.1 Chlorophylls and Carotenoids

It has been observed in the current experiment that there was a reduction in content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids in disease infected leaves in comparison to the healthy leaves in all the varieties of grape (Table 4.6). The maximum chlorophyll a (1.36 mg/g FW), chlorophyll b (1.69 mg/g FW) and total chlorophyll (3.04 mg/g FW) has been observed in the healthy leaves of the resistant grape variety i.e. Punjab MACS Purple which is significantly higher as compared to the rest of the varieties evaluated. However, the carotenoids in this variety were the lowest (2.5×10^{-3} mg/g FW). There was a linear decrease in the chlorophyll as the rating of varieties change from resistant to moderately resistant to susceptible. Henceforth, the minimum chlorophyll a (0.84 mg/g FW), chlorophyll b (0.86 mg/g FW) and total chlorophyll (1.68 mg/g FW) has been recorded in the non-infected Perlette leaves which scored maximum PDI. Similar linear relation in the chlorophyll a, chlorophyll b and total chlorophyll has been observed for the infected leaves of the grape varieties. The response of the grape varieties with respect to the contents of the carotenoids does not show a linear trend in the current experiment as the varietal response of carotenoid content to grape anthracnose may vary.

Our results are in agreement in respect of chlorophyll content with the reports of Lobatto and Goncalves (2009) who also reported low in chlorophyll in the susceptible varieties infected by *Colletotrichum lindmuthianum* causing anthracnose of bean. Siddaramaiah and Hegde (1988) while observing mulberry leaves infected by *Cercospora moricola* made similar observations as reported here. They reported that the infection resulted in decrease in chlorophyll a and chlorophyll b as compared to their healthy counterparts.

Carotenoids are the pigments other than the chlorophyll which have important function of protecting chlorophyll against oxidative destruction. The fungal pathogen affects

Table 4.6: Chlorophyll and carotenoid content in healthy and anthracnose infected leaves of grape varieties

Variety	PDI	Chlorophyll a (mg/g FW)				Chlorophyll b (mg/g FW)				Total Chlorophyll (mg/g FW)				Carotenoids (mg/g FW)			
		Healthy	Infected	Mean	% decrease over healthy	Healthy	Infected	Mean	% decrease over healthy	Healthy	Infected	Mean	% decrease over healthy	Healthy	Infected	Mean	% decrease over healthy
Resistant																	
Punjab MACS Purple	0.66	1.36^{a}	1.02^{d}	1.19^{a}	-25.00	1.69^{a}	1.48^{c}	1.58^{a}	-12.42	3.04^{a}	2.50^{d}	2.77^{a}	-17.76	$2.5x10^{-3\ f}$	$1.4x10^{-3\ h}$	$2.0x10^{-3\ a}$	-44.00
Moderately Resistant																	
Pusa Navrang	27.66	1.17^{b}	1.04^{cd}	1.10^{b}	-11.50	1.57^{b}	1.54^{b}	1.55^{b}	-1.91	2.74^{b}	2.61^{c}	2.67^{b}	-4.74	$2.7x10^{-3\ f}$	$2.1x10^{-3\ g}$	$2.4x10^{-3\ d}$	-22.22
Flame Seedless	36.33	1.06^{c}	0.89^{e}	0.97^{c}	-16.03	1.45^{c}	1.15^{e}	1.30^{c}	-20.68	2.51^{d}	2.04^{f}	2.27^{c}	-18.72	$9.5x10^{-3\ b}$	$6.8x10^{-3\ d}$	$8.1x10^{-3\ b}$	-28.42
Susceptible																	
Beauty Seedless	61.66	1.04^{cd}	0.88^{e}	0.95^{c}	-16.34	1.22^{d}	1.08^{f}	1.15^{d}	-11.47	2.26^{e}	1.95^{g}	2.10^{d}	-13.71	$8.5x10^{-3\ c}$	$6.1x10^{-3\ e}$	$7.3x10^{-3\ c}$	-28.23
Perlette	73.00	0.84^{f}	0.72^{g}	0.78^{d}	-14.28	0.86^{g}	0.83^{g}	0.84^{e}	-3.48	1.68^{h}	1.54^{i}	1.61^{e}	-8.33	$12.6x10^{-3\ a}$	$8.6x10^{-3\ c}$	$10.6x10^{-3\ a}$	-31.74
Mean	39.86	1.09^{a}	0.91^{b}	1.00	-16.51	1.36^{a}	1.21^{b}	1.28	-11.02	2.44^{a}	2.12^{b}	2.28	-13.11	$7.2x10^{-3\ a}$	$5.0x10^{-3\ b}$	$6.1x10^{-3}$	-29.57

the pigment levels by affecting the chloroplast structure. Ghose *et al* (2010) reported a drastic reduction in carotene in blight infected mulberry leaves over the healthy ones which corroborates our results. Abnormalities in the form and destruction of chloroplasts are common features of diseased tissues of plants infected with pathogens, which usually exhibit reduced photosynthetic rate, phosphorylation, hill reaction and carbon assimilation (Bawden 1999). The reduction in chlorophyll may be attributed to toxic metabolites produced by pathogen which may destroy the chloroplast (Senthil *et al* 2010, Halloin *et al* 1970) or to the inhibition of synthesis of chlorophyll rather than the degradation of pre-existing pigments (Ammajamma and Patil 2008). When a foliar pathogen establishes infection on host tissue, the chlorophyll content is usually decreased which is accompanied by yellowing of the infected leaf (Senthil *et al* 2010).

4.4 Biochemical Characteristics

4.4.1 Total soluble sugars

Sugar content of the leaves is one of the most important parameters to categorize any variety to a particular biotic agent as resistant or susceptible. The anthracnose of grapevine has been reported to be a high sugar disease (Mohanraj *et al* 1972). The data on total soluble sugars of healthy and anthracnose infected leaves of the grape varieties is presented in Figure 4.1. It has been observed that the total sugar content was more in the infected leaves as compared to the healthy ones in all the varieties. On the basis of the mean of total soluble sugar content of healthy and infected samples, it is inferred that the infected leaves have higher (6.92 mg g^{-1} of DW) activity as compared to healthy (6.24 mg g^{-1} of DW). The infected leaves recorded higher total soluble sugar as compared to the healthy leaves. Similar observations were made by Mohanraj *et al* (1972) in grapes in response to anthracnose.

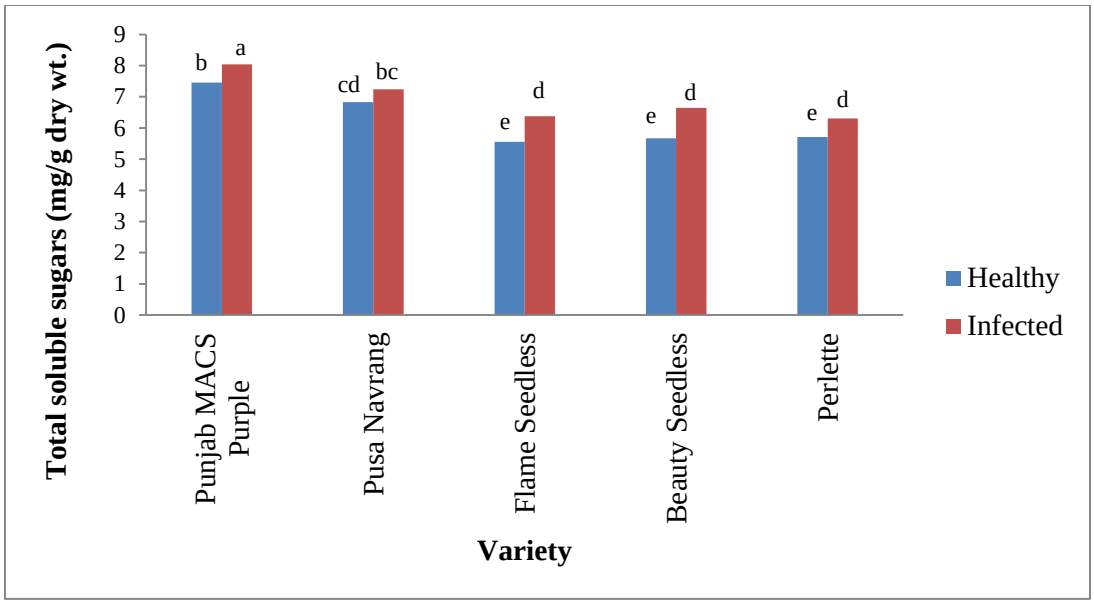


Fig. 4.1: Total soluble sugar content in healthy and anthracnose infected leaves of grape varieties

Bindra and Kapoor (1979) also found higher sugar content in infected leaves than healthy leaves. It has been observed that the percent increase in sugar levels was higher in susceptible genotypes than in resistant ones. Gurjar *et al* (2015) however reported that anthracnose affected leaves of susceptible grape genotypes had higher content of total sugars as compared to the resistant ones.

4.4.2 Total soluble proteins

The total soluble protein content of the healthy and anthracnose infected leaves of grape varieties is presented in Figure 4.2. The results indicate that the total soluble protein content of the grape leaves was higher in the infected samples as compared to their healthy counterparts in the resistant (Punjab MACS Purple) and moderately resistant (Pusa Navrang and Flame Seedless) whereas, in the susceptible varieties (Beauty Seedless and Perlette) the healthy leaves had higher total soluble protein content as compared to their infected counterparts. It may be argued that it is the higher total soluble protein content that confers susceptibility towards anthracnose. Saud *et al* (2000) reported a decrease in leaf protein content in guava after infection with *Aspergillus niger*. On the analysis of protein content of healthy and powdery mildew infected leaves of six mulberry varieties Shree *et al* (1986) reported that mildew infection reduced the levels of proteins in four varieties, whereas the infection increased the protein level in the infected leaves as compared to the healthy ones in two varieties. It could be that proteins are synthesised at a faster rate and remain more stable in the infected leaves, or may be less utilized by the anthrone causing pathogen in the resistant and moderately resistant varieties, whereas in the susceptible varieties which show lower soluble protein content in the infected leaves may be because the proteins are degraded at a

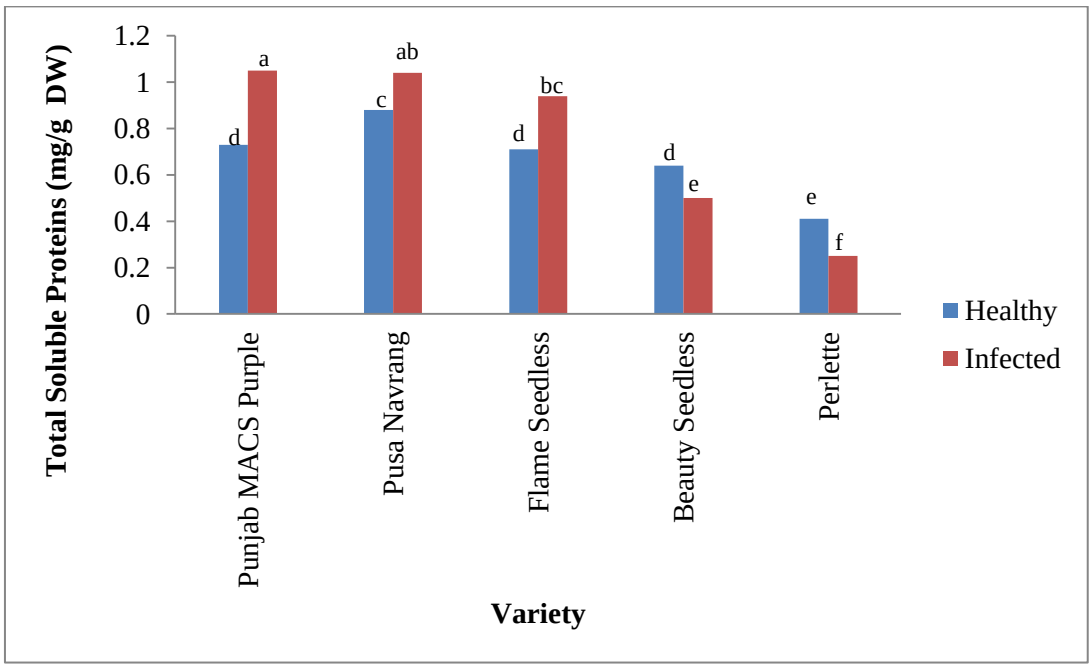


Fig. 4.2: Total soluble protein content in healthy and anthracnose infected leaves of grape varieties

faster rate due to the infection, or more of proteins were being utilized by the pathogen resulting in the lower total soluble protein content in the infected leaves (Manner and Leath 1978)

The increase in total soluble protein content due to infection may be because of accumulation of pathogenesis related proteins (PRs) which are proteins coded for by the host plant and are associated with the development of systemic acquired resistance against further infection by the disease. Induction of PRs suggests their role in adaptation to biotic stress (Van Loon and Van Strien 1999).

4.4.3 Free amino acids

It is evident from the Figure 4.3 that anthracnose infection led to reduced free amino acid level in the infected leaves of grape varieties as compared to their healthy counterparts in all the varieties evaluated. The mean free amino acid content was significantly higher (0.112 mg/g DW) in the resistant variety Punjab MACS Purple at par with Perlette (0.122 mg/g DW) followed by moderately resistant varieties i.e. Pusa Navrang (0.089 mg/g DW) and Flame Seedless (0.059 mg/g DW). The susceptible variety Perlette though regarded as susceptible on the basis of PDI show higher free amino acid content. Generally, the reduction in the free amino acid content due to the infection may be due to the utilization of amino acids by the pathogen metabolism, or these may be utilized by the host plant as a defense mechanism (Bashalah *et al* 1984). It may be added that due to variable response of amino acid content by the grape varieties, the free amino acid content may not be adopted as a selective trait in selection for tolerance to anthracnose. Singh (2000) reported that higher content of free amino acid in the cultivars of *Brassica* which were susceptible to downy mildew as compared to the resistant cultivars. This is consonance with our report indicating that the susceptible variety Perlette had maximum free amino acid (0.122 mg/g DW).

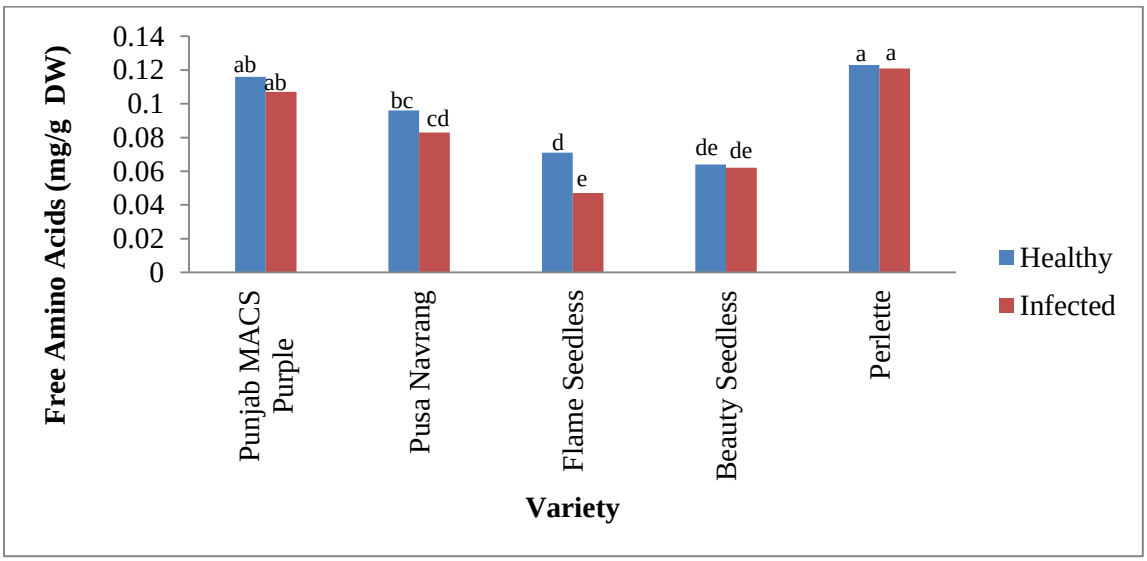


Fig. 4. 3: Free Amino Acid content in healthy and anthracnose infected leaves of grape varieties

4.4.4 Total Phenols

The data on total phenol content of healthy and anthracnose infected leaves of the grape varieties is presented in Figure 4.4. It is observed that the total phenol content in all the varieties evaluated was more in the infected leaves as compared to the healthy ones. On the basis of the mean healthy and mean infected leaf phenolic content, it was inferred that the infected leaves had higher (4.36 mg g^{-1} DW) phenolic content as compared to healthy (3.73 mg g^{-1} DW). The variety regarded as resistant to grape anthracnose i.e. Punjab MACS Purple had a maximum mean leaf phenolic content (5.22 mg g^{-1} DW) which was significantly more as compared to the other varieties. The susceptible variety Perlette with maximum PDI (73.00) recorded the minimum mean total phenolic content (3.13 mg g^{-1} DW). The mean total phenolic content was significantly different in different in Punjab MACS Purple, Pusa Navrang and Perlette. The maximum accumulation of total phenolic content has earlier been reported in severely infected powdery mildew leaves of grapes variety Thompson seedless by Taware *et al* (2010). Similar observations in phenolic content were reported by Abusaleha *et al* (1989) in pea leaves in response to rust infection (*Uromyces fabae*). Higher phenolic content was found in *Colletotrichum* resistant genotypes of *Capsicum annum* L. as reported by Kaur *et al* (2011).

Phenols are antifungal compounds when oxidized to quinones are more toxic to pathogens (Sivaprakashan and Vidhyasakaran 1993). Higher level of both constitutive and inducible phenols were observed by Madhavi *et al* (2005) in the resistant genotypes of wild sunflower as compared to susceptible genotypes upon infection by *Alternaria helianthi*. Contrastingly, significant reduction in phenolic content was reported in the chilli genotypes resistant and susceptible to wilt (Jabeen *et al* 2009) Phenolic compounds are widely distributed in healthy and infected plants and increase in phenolics can be observed after infection (Ruelas *et al* 2006).

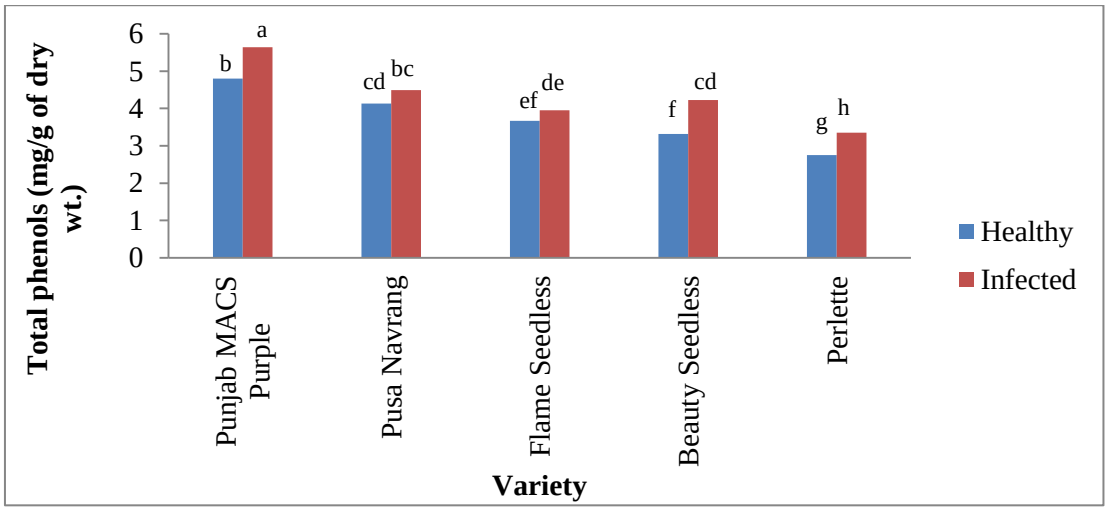


Fig. 4.4: Total Phenol content in healthy and anthracnose infected leaves of grape varieties

The accumulation of phenolic compounds in infected tissues is thought to be related to their release from glycosidic esters by the enzymatic activity of pathogen or host (Noveroske *et al* 1964). There is an increase in synthesis by host through the shikimic acid pathway (Neish 1964) or due to the phenolic migration from the healthy tissues (Farkas and Kiraly 1962). The activity of polyphenol oxidase enzyme can be correlated with the levels of phenols upon infection. But Jabeen *et al* (2009) remarked reduction in the total phenol content to increase in the polyphenol oxidase activity. The phenols get oxidise to quinones by higher PPO activity, thereby lowering the phenolic content

4.4.5 Ascorbic Acid

The data for variation in ascorbic acid content of healthy and anthracnose infected grape leaves is presented in Figure 4.5. Ascorbic acid provides resistance and is an anti-oxidant found in all the cells and acts as a scavenger of reactive oxygen species. It helps in balancing the redox system. The content of ascorbic acid of the leaf plays a significant role in susceptibility and resistance to the disease.

It is evident from the figure that the variety with maximum mean ascorbic acid (6.16 mg/g FW) had minimum PDI (0.66) and was regarded as resistant and the same was significantly higher than other varieties. The varieties categorised as moderately resistant i.e. Pusa Navrang and Flame Seedless with (PDI range 25-50) recorded ascorbic acid content of 5.50 mg/g FW and 3.94 mg/g FW respectively. However, the variety categorised as susceptible to anthracnose with maximum PDI (73.00) scored minimum mean ascorbic acid (2.84 mg/g FW).

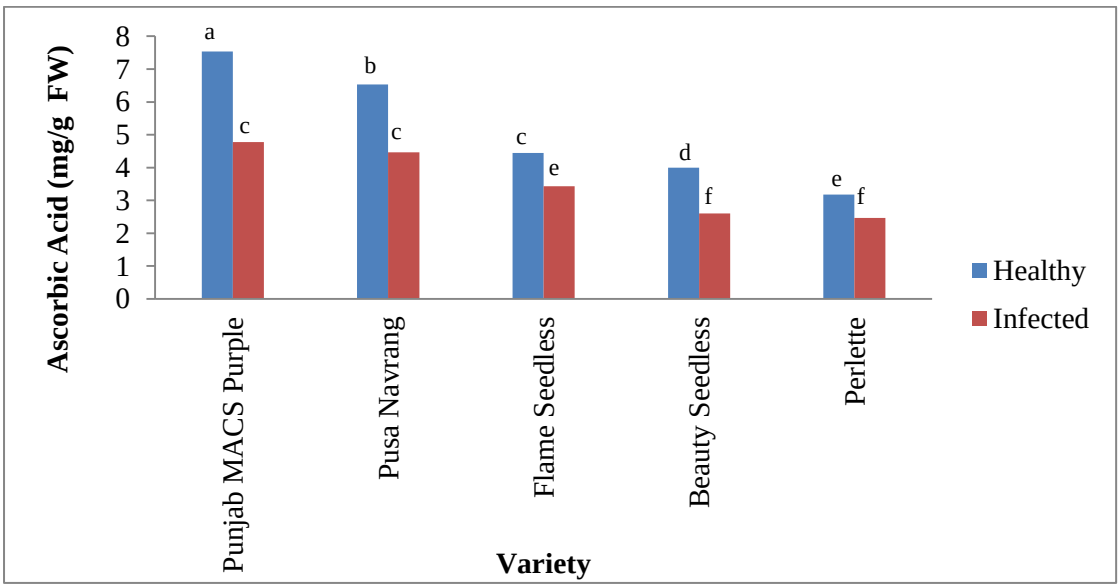


Fig. 4.5: Ascorbic Acid content in healthy and anthracnose infected leaves of grape varieties

The increase in ascorbic acid provoked by activators of disease resistance might induce changes in the redox balance of the environment of the cell, thereby stimulating PR

gene expression (Boubakri *et al* 2016).

Arora (2004) studied role of phytoanticipins in resistance against red rot of sugarcane and observed that resistant genotypes exhibited high level of ascorbic acid as compared to susceptible genotypes which decreased further after pathogenesis. Guleria *et al* (1997) also observed higher abscorbic acid levels in resistant pea cultivars than the susceptible ones, which exhibited 10.82% reduction following powdery mildew infection.

4.4.6 Proline

The proline content of the leaves infected with anthracnose is presented in Figure 4.6. The data presented in the figure reveals that there was a linear relationship between the PDI and the mean proline content. As the PDI increased from a minimum of 0.66 (Punjab MACS Purple) to a maximum of 73.00 (Perlette), the mean proline content recorded was maximum in Punjab MACS Purple (39.46 µg/g FW) and minimum in Perlette (22.09 µg/g FW). Therefore it is evident that maximum proline accumulation is in the resistant variety (Punjab MACS Purple). Further, the infected leaves accumulated higher proline content as compared to the healthy ones and this is true for all the varieties evaluated.

It may be inferred that signal of pathogen infection leads to synthesis of proline which confers resistance to the host against the pathogen. The moderately resistant varieties (Pusa Navrang and Flame Seedless) exhibited 34.78 µg/g FW and 26.95 µg/g FW mean proline content respectively. Proline accumulation is a plant resistant mechanism to abiotic as well as biotic stress. Similar reports depicting that proline accumulation was higher in infected conditions as compared to non-infected conditions in bread wheat genotypes has been made by Sahhafi *et al* (2012). Sivritepe *et al* (2009) observed that level of proline accumulation increased in response to two spotted spider mite and mite infection caused an increase in proline content in the leaves of muscle and Sultana grapevines respectively.

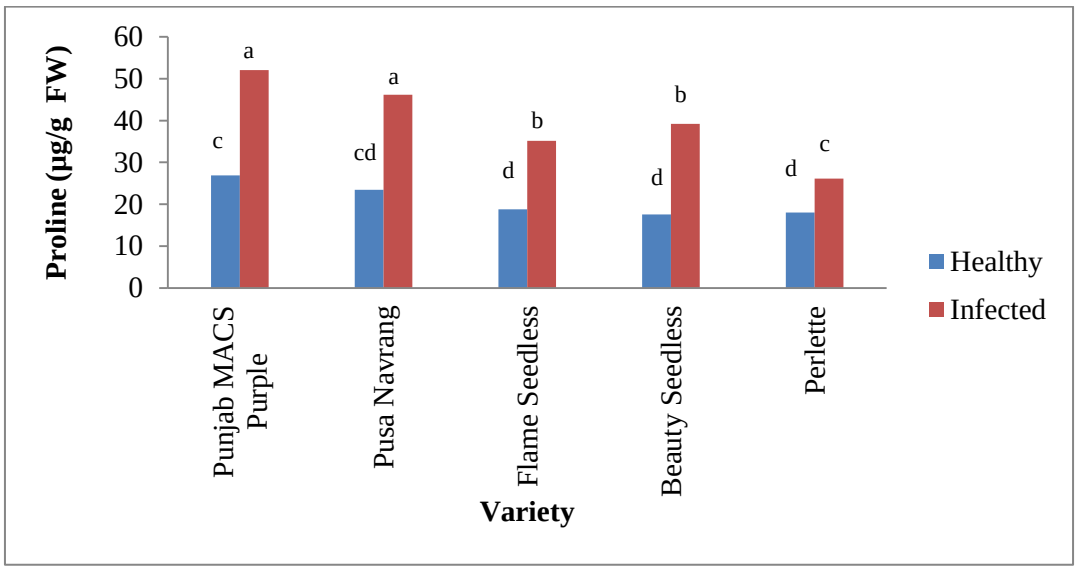


Fig. 4.6: Proline content in healthy and anthracnose infected leaves of grape varieties

4.4.7 α-tocopherol

Tocopherols collectively called Vitamin E are of four forms i.e. α, β, γ and δ and among these α-tocopherol is a form of Vitamin E and has highest Vitamin E activity. Tocopherols quench the reactive oxygen species. The inducers of disease resistance in plants like jasmonic acid modulate the endogenous level of tocopherols in plants (Antognoni *et al* 2009). It is reported that amount of tocopherol is negatively correlated with the amount of ascorbic acid. However in some cases, high level of tocopherol is positively correlated with ascorbic acid.

In our findings (Figure 4.7) we report that the most susceptible variety Perlette with maximum PDI recorded minimum mean α-tocopherol (0.90 mg/g FW) and the resistant variety that is Punjab MACS Purple with minimum PDI recorded minimum mean α-tocopherol (2.16 mg/g FW) in the anthracnose infected samples and these are significantly different from each other. However, the infected leaves of all the varieties had higher α-tocopherol as compared to the healthy leaves of the varieties evaluated. Higher tocopherol accumulation in the infected leaves might be implicated as a specific signalling mechanism that contributes in the modulation of cell function. Our studies reveal a negative correlation between tocopherol and amount of ascorbic acid (Table 4.9). This has been concluded on the basis of the mean ascorbic acid and the mean α-tocopherol content of the grape varieties. The resistant variety Punjab MACS Purple has maximum mean ascorbic acid (6.16 mg/g FW) and minimum α-tocopherol (0.90 mg/g FW) and the most susceptible variety Perlette has the minimum mean ascorbic acid (2.82 mg/g FW) and the maximum α-tocopherol (2.16 mg/g FW) {Figure 4.5 and 4.7}.

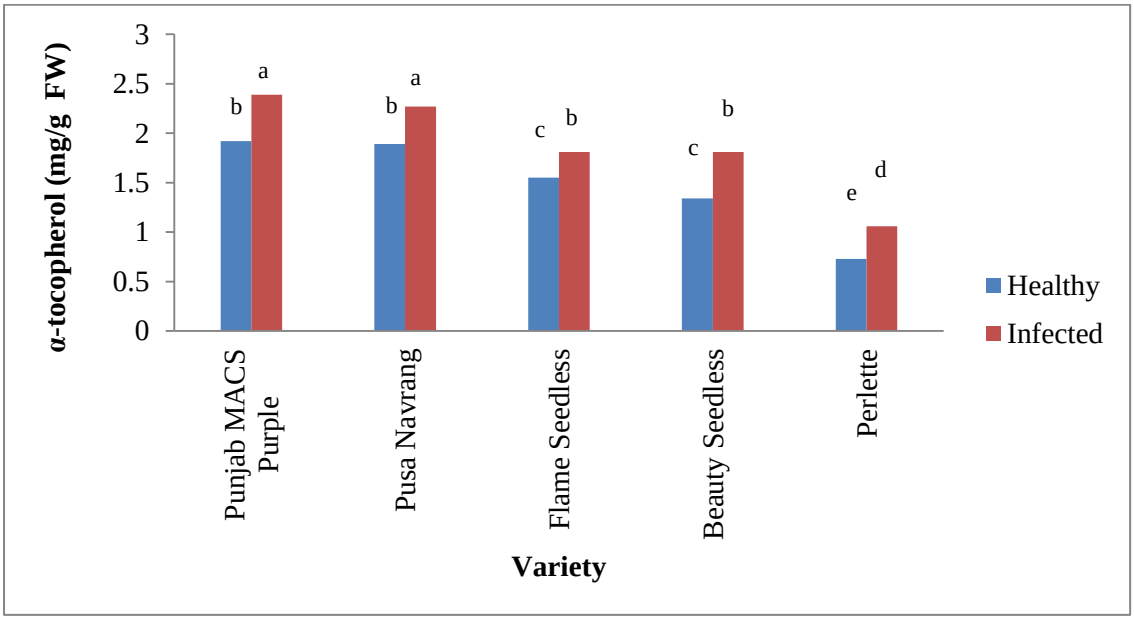


Fig. 4.7: α-tocopherol content in healthy and anthracnose infected leaves of grape varieties

4.4.8 Lipid peroxidation (MDA content)

The MDA content of the healthy and anthracnose infected leaves of grape varieties in presented in Figure 4.8. It is evident from the data presented in the figure that the anthracnose infection resulted in an elevated MDA content in all the grape varieties evaluated. It has also been observed that the susceptible variety Perlette had maximum mean MDA content (3.64 MDA/g FW) and the most resistant variety Punjab MACS Purple among the varieties evaluated had minimum mean MDA content (2.13 MDA/g FW) and the differences were significant at P = 0.05. It has been reported that on encountering stress due to infection with a disease, the plant cells accumulate ROS that result in cell membrane lipid peroxidation and metabolic disorders that lead to stress (Sheokand *et al* 2008). Hence, the infected leaves and the susceptible varieties have higher MDA content compared to the healthy and resistant varieties respectively. Wu *et al* (2014) reported that higher lesion length (infection) due to sheath blight infestation resulted in higher MDA content indicating that more the infestation more the MDA content. The MDA content is the initial product of membrane lipid peroxidation and is index of the degree of injury to plant cells (Demiral and Turkan 2005).

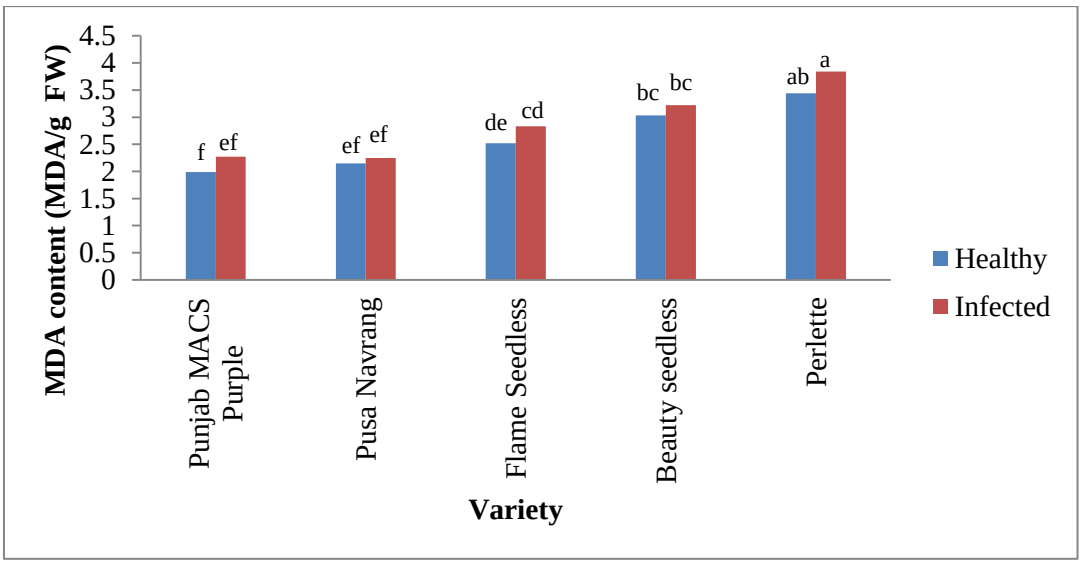


Fig. 4.8: MDA content in healthy and anthracnose infected leaves of grape varieties

4.4.9 Enzymatic activity: Peroxidase and Polyphenol oxidase

The data on peroxidase activity (POD) of healthy and anthracnose infected leaves of the grape varieties is presented in Table 4.7. It is observed that the peroxidase activity was more in the infected leaves in all the varieties as compared to the healthy ones. On the basis of the data on mean healthy and mean infected peroxidase activity, it is inferred that the infected leaves have higher (2.42 OD/min./g FW) activity as compared to healthy (2.02 OD/min/g FW). The variety regarded as resistant to grape anthracnose i.e. Punjab MACS Purple had maximum mean peroxidase activity (3.24 OD/min./g) which was significantly higher than the rest of the varieties. However, the infected leaves recorded higher peroxidase activity as

compared to the healthy leaves. The susceptible variety Perlette with maximum PDI (73.00) recorded the minimum mean total of activity of POD (1.57 min/g FW).

Table 4.7: Peroxidase activity in healthy and anthracnose infected leaves of grape varieties

VARIETY	PDI	Peroxidase activity (OD/min/g FW)			% increase over healthy
		Healthy	Infected	Mean	
Resistant					
Punjab MACS Purple	0.66	2.98 [b]	3.51 [a]	3.24 [a]	+17.78
Moderately Resistant					
Pusa Navrang	27.66	2.55 [c]	3.33 [ab]	2.94 [b]	+30.58
Flame seedless	36.33	1.61 [d]	1.81 [d]	1.71 [c]	+12.42
Susceptible					
Beauty seedless	61.66	1.55 [d]	1.76 [d]	1.66 [c]	+13.54
Perlette	73.00	1.43 [d]	1.71 [d]	1.57 [c]	+19.58
Mean	39.86	2.02 [b]	2.42 [a]	2.22	+19.80

The data presented in Table 4.8 reveals the polyphenol oxidase activity (PPO) of the healthy and anthracnose infected leaves of the experimental grape varieties. It is evident from the table that there was a linear relationship between the varieties and activity of PPO, i.e. it was lowest (0.0125 OD/min/g FW) in the susceptible variety and maximum (0.0224 OD/min/g FW) in the resistant variety .

Within varieties the activity of PPO was significantly higher in the infected leaves as compared to the healthy ones. In the resistant variety Punjab MACS Purple, there was 20.43% increase in PPO activity over healthy and in the most susceptible variety Perlette there was an increase of 11.61% of PPO activity in the infected leaves.

Enzymes act as defensive agents for the plants under adverse conditions. These are among the most powerful and ubiquitous proteins in the plants. Enhanced PPO and POD activities were reported in plants exposed to various biotic and abiotic inducer activities (Raghvendra *et al* 2007). They reported that in disease resistant varieties, the activities of PPO and POD alleviated in comparison with highly susceptible genotypes as in our experimental observations. Shankar and Jindal (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 higher in comparison with highly susceptible cultivars. Singh

et al (1999) also reported increased enzymatic activity upon disease infection in *Alternaria* leaf spot in resistant *Brassica* genotypes.

Table 4.8: Polyphenol oxidase activity in healthy and anthracnose infected leaves of grape varieties

VARIETY	PDI	Polyphenol oxidase activity (OD/min/g FW)			% increase over healthy
		Healthy	Infected	Mean	
Resistant					
Punjab MACS Purple	0.66	0.0186^{c}	0.0224^{a}	0.0205^{a}	+20.43
Moderately Resistant					
Pusa Navrang	27.66	0.0156^{d}	0.0206^{b}	0.0181^{b}	+32.05
Flame seedless	36.33	0.0123^{g}	0.0136^{ef}	0.0129^{d}	+10.56
Susceptible					
Beauty seedless	61.66	0.0129^{fg}	0.0144^{e}	0.0136^{c}	+11.62
Perlette	73.00	0.0112^{h}	0.0125^{g}	0.0118^{e}	+11.61
Mean	39.86	0.0141^{b}	0.0167^{a}	0.0154	+18.43

It may be concluded that the activity of enzymes Peroxidase and Polyphenol oxidase is directly related to the resistance of the host and the enzymatic activity in infected leaves has been found to be higher than that in the corresponding healthy leaves. This may be due to biotic stress created by pathogen attack. Increase in peroxidase activity could be correlated with infection in plants as polymerisation of cinnamyl alcohols to ligninis catabolised by peroxidase lignification leading to disease resistance (Gurjar *et al* 2015). Peroxidase may be directly involved in stopping pathogen development, preventing the advancing of infection or affect on the synthesis of compounds responsible for conferring resistance to the disease. Hence, the change in the activity of POD and PPO could be a good trait for selection of grape varieties resistant to anthracnose.

Campos *et al* (2004) also 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.

4.5 Correlation Studies

Pearson correlation coefficients were generated for establishing the correlation between the PDI and different physiological (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids), biochemical parameters (total soluble sugars, total soluble proteins, free amino acids, total phenols, ascorbic acid, MDA content, α-tocopherol & proline) and enzymes (peroxidase and polyphenol oxidase). It is evident from the Table 4.9 that there is a significant positive correlation of PDI with carotenoids ($r = 0.816^{**}$), total soluble sugars ($r = 0.766^{**}$)

Table 4.9: Pearson Correlation among Percent disease index (PDI), physiological and biochemical parameters

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	PDI	1.00	-0.762**	-0.886**	-0.859**	0.816**	0.766**	-0.792**	-0.075	-0.846**	-0.789**	0.900**	-0.821**	-0.476**	-0.832**	-0.814**
2	Chlorophyll a		1.00	0.909**	0.958**	-0.586**	-0.816**	0.521**	0.047	0.463*	0.952**	-0.852**	0.510**	-0.052	0.540**	0.485**
3	Chlorophyll b			1.00	0.990**	-0.768**	-0.860**	0.756**	-0.053	0.668**	0.872**	-0.953**	0.766**	0.248	0.730**	0.690**
4	Total chlorophyll				1.00	-0.721**	-0.861**	0.693**	-0.026	0.608**	0.915**	-0.941**	0.694**	0.152	0.680**	0.635**
5	Carotenoids					1.00	0.470**	-0.720**	-0.028	-0.892**	-0.620**	0.748**	-0.913**	-0.679**	-0.905**	-0.901**
6	Total soluble sugars						1.00	-0.665**	0.205	-0.435*	-0.788**	0.876**	-0.550**	0.009	-0.403*	-0.355
7	Total soluble proteins							1.00	-0.308	0.668**	0.500**	-0.799**	0.839**	0.556**	0.709**	0.717**
8	Free amino acids								1.00	0.063	0.233	0.061	-0.240	-0.096	0.249	0.172
9	Total phenols									1.00	0.472**	-0.668**	0.891**	0.778**	0.856**	0.901**
10	Ascorbic acid										1.00	-0.839**	0.475**	-0.052	0.602**	0.505**
11	MDA											1.00	-0.771**	-0.309	-0.732**	-0.698**
12	α-tocopherol												1.00	0.764**	0.823**	0.865**
13	Proline													1.00	0.689**	0.779**
14	Peroxidase (POD)														1.00	0.968**
15	Polyphenol oxidase (PPO)															1.00

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

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

and MDA content (0.900**). A significant negative correlation has been established between the PDI and the antioxidants α- tocopherol (r = -0.821**) and ascorbic acid (r = -0.789**). Negative significant correlation of PDI with the activity of POD (r = -0.832**) and PPO (r = -0.814**) has also been observed. Similar report on the correlation of enzyme activity to disease severity has been reported by Zhou *et al* (2012) by working on *Verticillium* wilt resistance. In our studies, we report that the highest Pearson correlation coefficient is observed between PDI and chlorophyll b (r = -0.886**) followed by phenols (r = -0.846**). Total phenol content is probably an important trait for using as an indicator of disease severity. The negative correlation of PDI with total phenols is because the resistant variety Punjab MACS Purple with minimum PDI (0.66) has the maximum total phenols in healthy (4.80 mg/g DW) and infected (5.64 mg/g DW) leaves. The total phenols are significantly positively related to PPO, thus suggesting the role of PPO thus suggesting the role of PPO in diseased tolerance.

CHAPTER-V

SUMMARY

The present investigation on the "PHYSIOLOGICAL AND ANATOMICAL REACTION OF GRAPE (*Vitis vinifera* L.) GENOTYPES TO ANTHRACNOSE" was carried out at the Fruit Research Farm of Department of Fruit Science and in the Laboratories of Department of Botany, Punjab Agricultural University, Ludhiana during the year 2016 and 2017. Five grape varieties, viz., Flame Seedless, Perlette, Punjab MACS Purple, Pusa Navrang and Beauty Seedless were selected to evaluate their reaction to anthracnose caused by *Elsinoe ampelina* (de Bary) Shear on the basis of physiological and biochemical parameters. Diseases caused by fungi or any other microorganism are known to alter the biochemical and physiological machinery of the host. Hence, efforts were made to evaluate five varieties for their reaction to anthracnose on the basis of Percent disease incidence. The viticulture orchard was visited regularly to observe the incidence of anthracnose on the plant organs. The data on agrometrological parameters viz., maximum and minimum temperature, relative humidity, rainfall, evaporation and sunshine hours was attained from School of Agricultural Meteorology, PAU. The quantification of the chlorophylls, carotenoid content, total soluble sugars, total soluble proteins, free amino acids, total phenols, proline, ascorbic acid, α-tocopherol, lipid peroxidation and enzymatic activity: peroxidase and polyphenol oxidase in the healthy and anthracnose infected leaves of the selected varieties was made, in order to establish a correlation between the percent disease index (PDI) and the biochemical and physiological parameters, if any.

On the basis of the field reaction of the grape varieties to anthracnose by employing 0-5 scale, the varieties were rated as resistant, moderately resistant and susceptible. The basis of rating was the PDI of the anthracnose infected leaves. Among the five varieties, Punjab MACS Purple with minimum PDI (0.66) was rated as resistant, Pusa Navrang and Flame Seedless with moderate PDI were rated as moderately resistant whereas, Beauty Seedless and Perlette were rated as susceptible on the basis of a high score of PDI.

Stomata are the openings on phylloplanes which provide chances of fungal spores to enter the leaf tissue. Scanning electron microscopic studies revealed differences in the stomatal characteristics of all the grape varieties evaluated. The stomatal length and width was significantly high in the most susceptible variety Perlette as compared to the rest of the varieties. Narrower the stomatal aperture, lower are the chances and instances of the spore to enter the host. The infected leaves of the most susceptible variety Perlette were further evaluated and it was found that the stomatal length was significantly high in the healthy leaves as compared to their infected counterparts. It is possible that the pathogen *Elsinoe ampelina* reduced the stomatal size after causing infection. The stomatal index was maximum

on the abaxial surface of the leaves in the most susceptible variety Perlette which is significantly higher from the moderately resistant Pusa Navrang and Flame Seedless; and the resistant variety Punjab MACS Purple; whereas at par with the susceptible variety Beauty Seedless.

The cuticle was visibly thickest in the variety Punjab MACS Purple which is the most resistant variety with minimum PDI. There is a possible existence of a positive correlation between the thickness of cuticle with the resistance to anthracnose in Punjab MACS Purple. The cuticle plays an important role for the adhesion of sporangia around the stomata. The highly compact spongy mesophyll in Punjab MACS Purple and Pusa Navrang hampers the growth of the mycelium of the host tissue.

Trichomes were observed in healthy leaves of Pusa Navrang .in the healthy leaves of Punjab MACS Purple and in the anthracnose infected leaves of Perlette and Flame Seedless. It is likely that the trichomes in grape varieties Pusa Navrang and Punjab MACS Purple confers resistance against the pathogen *Elsinoe ampelina*. In the variety Perlette no trichomes were observed in the healthy leaves but the appearance of trichomes post infection is the in-built mechanism of the susceptible variety to avoid the further infestation of the pathogen.

Pearson correlation coefficients were computed between PDI and stomatal length, width and index. A significantly positive correlation of PDI is being reported with stomatal length, stomatal width and stomatal index and these are significant at $P<0.05$.

Maximum content of pigments viz., chlorophyll a, chlorophyll b and total chlorophyll content was recorded in the resistant variety Punjab MACS Purple which was rated as resistant based on the PDI, whereas the most susceptible variety on the basis of PDI, Perlette exhibited minimum chlorophylls in the anthracnose infected leaves. However, the carotenoids were maximum in the susceptible variety Perlette and minimum in the resistant variety Punjab MACS Purple. The total chlorophylls and carotenoids were low in the infected leaves as compared to their healthy counterparts. The fungal infection damages the chloroplasts which reduce the photosynthetic activity and then lead to the reduction of chlorophyll content.

The total soluble sugar content in the leaves is one of the most important parameters to categorize any variety to a particular reaction, viz., resistant or susceptible to any biotic stress. The low total soluble sugar content in susceptible varieties as compared to the resistant varieties in the present investigation might be due to the increase in the rate of respiration or utilization by the pathogen as respiratory substrate during pathogenesis. Low sugar content favours the growth of the pathogen. It is observed that the total soluble sugar content is more in the anthracnose infected leaves as compared to the healthy ones in all the varieties. On the basis of the mean of healthy and infected total sugar content, it is inferred that the infected leaves have higher total soluble sugar activity as compared to their healthy counterparts.

The total soluble protein content of the grape leaves were higher in the infected samples as compared to their healthy counterparts in the resistant (Punjab MACS Purple) and moderately resistant (Pusa Navrang and Flame Seedless) whereas, in the susceptible varieties the healthy leaves had higher total soluble protein content as compared to their infected counterparts. It may be argued that it is the higher total soluble proteins in the healthy leaves that confers susceptibility towards anthracnose. It could be that proteins are synthesized at a faster rate and remain more stable in the infected leaves, or may be less utilized by the anthrone causing pathogen in the resistant and moderately resistant varieties whereas in the susceptible varieties which show lower soluble protein content in the infected leaves may be because the proteins are degraded at a faster rate due to the infection, or more of proteins were being utilized by the pathogen resulting in the lower total soluble protein content in the infected leaves. The increase in total soluble protein content due to infection may be because of accumulation of pathogenesis related proteins (PRs) which are the proteins that are coded by the host and are generally associated with development of systemic acquired resistance against any further infection by the disease. Induction of PRs suggests their role in adaptation to biotic stress.

The mean free amino acid content is higher in the resistant variety Punjab MACS Purple followed by moderately resistant varieties i.e. Pusa Navrang and Flame Seedless. However, the susceptible varieties Beauty Seedless and Perlette though regarded as susceptible on the basis of PDI show higher free amino acid content. Generally, the reduction in the free amino acid content due to the infection may be due to the utilization of amino acids by the pathogen metabolism, or these may be utilized by the host plant as a defense mechanism. It may be added that due to variable response of amino acid content by the grape varieties, the free amino acids may not be assumed as a selective trait in selection for tolerance to anthracnose.

The total phenol content in all the varieties is more in the infected leaves as compared to the healthy ones. On the basis of the mean of healthy and infected leaf phenolic content, it is inferred that the infected leaves have higher phenolic content as compared to healthy leaves. The variety regarded as resistant to grape anthracnose i.e. Punjab MACS Purple had maximum mean leaf phenolic content. However, the infected leaves recorded higher phenolic content as compared to the healthy leaves. The susceptible variety Perlette with maximum PDI (73.00) recorded the minimum mean total phenolic content. The accumulation of phenolic compounds in infected tissues is thought to be due to break down of glycosidic esters by the enzymatic activity of the pathogen or the host. There is an increase in synthesis by host through the shikimic acid pathway or due to the phenolic migration from the healthy tissues. The plants' defense mechanism involves the rapid accumulation of phenolics at the infection site that slowdowns the growth of the pathogen.

Ascorbic acid provides resistance and is an anti-oxidant found in all the cells and acts as a scavenger of reactive oxygen species. It helps in balancing the redox system. The content of ascorbic acid of the leaf plays a significant role in susceptibility and resistance to the disease. The variety with maximum mean ascorbic acid has minimum PDI i.e. Punjab MACS Purple and is regarded as resistant and the same is significantly higher than other varieties. However, the variety categorized as susceptible to anthracnose with maximum PDI (73.00) scored minimum mean ascorbic acid. The increase in ascorbic acid evoked by the activators of disease resistance might instigate changes in the redox balance of the cellular environment, stimulating expression of PR genes.

There is a negative relationship between the PDI and the mean proline content. As the PDI increases from a minimum of 0.66 (Punjab MACS Purple) to a maximum of 73.00 (Perlette), the mean proline content decreases from Punjab MACS Purple towards Perlette. The accumulation of proline is a plant resistance mechanism to abiotic as well as biotic stress.

Tocopherols collectively called Vitamin E are of four forms i.e. α, β, γ and δ and among this α-tocopherol is a form of Vitamin E and bears highest Vitamin E activity. Tocopherols quench the reactive oxygen species. The most susceptible variety Perlette with maximum PDI recorded minimum mean α-tocopherol and the resistant variety that is Punjab MACS Purple with minimum PDI recorded maximum mean α-tocopherol. Higher tocopherol accumulation in the infected leaves might be implicated as a specific signaling mechanism that contributes in the modulation of cell function.

The anthracnose infection resulted in an elevated MDA content in all the grape varieties evaluated. It has also been observed that the susceptible variety Perlette has maximum mean MDA content and the resistant variety Punjab MACS Purple among the varieties evaluated has minimum mean MDA content and their differences are significant at P=0.05. It has been reported that on encountering stress due to infection with a disease, the plant cells accumulate reactive oxygen species that result in cell membrane lipid peroxidation and metabolic disorders that lead to stress. Hence the infected leaves and the susceptible varieties have higher MDA content compared to the healthy and resistant varieties respectively.

The peroxidase (POD) activity is higher in all the varieties in the infected leaves as compared to the healthy ones. On the basis of the mean of healthy and infected peroxidase activity, it is inferred that the infected leaves have higher activity as compared to healthy. The varieties regarded as resistant to grape anthracnose i.e. Punjab MACS Purple and Pusa Navrang had maximum mean peroxidase activity. However, the infected leaves recorded higher peroxidase activity as compared to the healthy leaves. The susceptible variety Perlette with maximum PDI (73.00) recorded the minimum mean total of activity of POD. The polyphenol oxidase activity (PPO) is higher in infected leaves of the experimental grape

varieties than the susceptible ones. There is a linear relationship between the varieties and activity of PPO, i.e. it was lowest in the most susceptible (Perlette) variety and maximum in the resisitant (Punjab MACS Purple) variety. Enzymes act as defensive agents for the plants under adverse conditions and are the most powerful as well as ubiquitous proteins. Enhanced PPO and peroxidase activities have been reported in plants exposed to biotic and abiotic inducer activities. It may be inferred that the activity of enzymes peroxidase and polyphenol oxidase is directly related to the resistance of the host and the enzymatic activity in infected leaves has been found to be higher than that in the corresponding healthy leaves. This may be due to biotic stress created by pathogen attack. Increase in the activity of peroxidase could be correlated with infection in plants as polymerization of cinnamyl alcohols to lignin is catabolised by peroxidase lignifications leading to disease resistance. Peroxidase may be directly involved in arresting the development of the pathogen, preventing the advance of infection or affect the synthesis of compounds responsible for conferring resistance to the disease. Hence, the change in the activity of POD and PPO could be a good trait for selection of grape varieties resistant to anthracnose.

Pearson correlation coefficients were generated for establishing the correlation between the PDI and different physiological (chlorophylls and carotenoids), biochemical parameters (total soluble sugars, total soluble proteins, free amino acids, total phenols, ascorbic acid, MDA content, α-tocopherol & proline) and enzymes (peroxidase and polyphenol oxidase). There is a significant positive correlation of PDI with carotenoids, total soluble sugars and MDA content. A significant negative correlation has also been established between the PDI and the antioxidants α- tocopherol and ascorbic acid. Negative significant correlation of PDI with the activity of POD and PPO has also been observed.

Our study thus provides information for elucidating the events leading to resistance of grape varieties to anthracnose and for future breeding of the variety resistant to the disease.

REFERENCES

Abusaleha, Pal A B and Vani A (1989) Changes in sugars and phenols in pea leaves in response to rust (*Uromyces fabae*) infection. *Ind J Hort* **46**(2): 222-24.

Ammajamma R and Patil P V (2008) Biochemical factors imparting rust (*Phakospora pachyrhizi)* resistance in soybean. *Karnataka J Agri Sci* **21**:65-69.

Anonymous (2016) Area and Production of fruit crops in India. *National Horticulture Board.* P4.

Antognoni F, Faudale M, Poli F and Biondi S (2009) Methyl jasmonate differentially affects tocopherol content and tyrosine amino transferase activity in cultured cells of *Amaranthus caudatus* and *Chenopodium quinoa. Pl Biol* (Stuttgart, Germany) **11**(2):161-69.

Arora N (2004) Role of phytoanticipins in resistance against red rot of sugarcane (*Saccharum officinarum* L.) *Env Eco* **32**(2): 154-56.

Arora N and Kaur R (2004) Biochemical factors influencing mechanism of resistance in chilli leaf during *Alternaria solani* infection. *Env and Eco* **22**: 853-55.

Arora N, Madhumeeta and Kumar B (2005) Phyloplane in defense mechanism of sugarcane to red rot pathogen. *Ind J Ecol* **32**(2): 154-56.

Arun K, Mali P C and Manga V K (2010) Changes in some phenolic compounds and enzyme activities of infected pearlmillet caused by *Sclerospora graminicola*. *Int J Pl Physiol Biochem* **2**: 6-10.

Asthir B, Kaur S and Mann S K (2009) Effect of salicylic and abscisic acid administered through detached tillers on antioxidant system in developing wheat grains under heat stress. *Acta Physiol Pl* **31**(5): 1091–96.

Atwal A K, Ramandeep, Munshi S K and Mann A P S (2004) Biochemical changes in relation to Alternaria leaf blight in Indian mustard. *Pl Dis Res* **19**: 57-59.

Balabaa S I, Zake A Y and Elshamy A M (1974) Total flavonoid and rutin content of the different organs of *Sophora japonica* L. *J Assoc Anal Chem* **57**: 752-55.

Bashalah M O, Sulaiman A AA and Mohammad S (1984) Metabolic changes in the leaves of *Solanum melongia* affected with *Alternaria tenuissima. Ind Phytopathol* **37**: 46-51.

Bates L S, Waldren R P and Tears I D (1973) Rapid determination of free proline for water stress studies. *Plant and Soil* **39**: 205-07.

Bawden F C (1999) *Plant Diseases.* Green World Publishers, Lucknow.

Bedi J S, Jhooty J S and Cheema S S (1986) Field reaction of different grapevine cultivars to anthracnose caused by *Sphaceloma ampelinum*. *Punjab Hort J* **26**(1): 74-77.

Benthod O, Huber L and Slimi H (2001) Photosynthetic responses of wheat to stress induced by *Puccinia recondita* and post-infectional drought. *Photosynthetica* **39**: 581-90.

Bertamini M and Nedunchezhian N (2004) Photosynthetic responses for *Vitis vinifera* plants grown at different photon flux densities under field conditions. *Biologia Plantarum* **48**: 149-52.

Bertamini M, Muthuchelian K and Nedunchezhian N (2004) Effect of grapevine leafroll on the photosynthesis of field grown grapevine plants (*Vitis vinifera* L. Cv. Lagrein). *J Phytopath* **152**: 145-52.

Bertamini M, Muthuchelian K, Rubinigg M, Zorer R and Nedunchezhian N (2005) Low-night temperature (LNT) induced changes of photosynthesis in grapevine (*Vitis vinifera* L.) plants. *Pl Physiol Biochem* **43**: 693-99.

Bindra A S and Kapoor S (1979) Biochemical differences in grapevines in relation to their infestation with anthracnose. *Research Bulletin of Marathwada Agricultural University* **3**(4): 53-55.

Blanchfield A L, Robinson S A, Renzullo L and Powell K S (2006) Phylloxera infested grapevines have reduced chlorophyll and increased photoprotective pigment contentcan leaf pigment composition aid pest detection. *Func Pl Biol* **33**: 507- 17.

Boso S, Alonso-Villaverde V, Santiago J L, Gago P, Durrenberger M, Duggelin M, Kassemeyer H H and Martinez M C (2010) Macro- and microscopic leaf characteristics of six grapevine genotypes (*Vitis spp.*) with different susceptibilities to grapevine downy mildew. *Vitis* **49**(1): 43-50.

Boubakri H, Gargouri M, Mlik, Brini F, Chong J and Jbara M (2016) Vitamins for enhancing plant resistance. *Planta* **244**: 529-43.

Caimi G, Cardlo C and LoPrest R (2003) Diabetes mellitus: Oxidative stress and Wine. *Curr Med Res Opin* **19**(7): 581-86.

Campbell C J, Huang J S and Payne G A (1980) Defense at the perimeter: the outer walls and the gates. In: Horsfall J G and Cowling E B (eds) *Plant Disease-An advanced treatise* pp 103 Academic Press, New York.

Campos A D, Ferreira A G, Vozari Hampe M M, Antunes I F, Brancao Nely, Paulo da Silveira, Expedito Osorio V A and Augustin l E (2004) Peroxidase and polyphenol oxidase activity in bean anthracnose resistance. *Pesquisa Agropecuaria Brasileira* **39**(7): 637-43.

Caretto S, Nisi R, Paradiso A and De Gara L (2010) Tocopherol production in plant cell cultures. *Mol Nutr Food Res* **54**(5):726–30.

Chandermohan, Singh J and Singh T S (2002) Prevalence of grape anthracnose and carbendazim resistance in *Gloeosporium ampelophagum* in Punjab. *Plant Dis Res* **23**: 147-50.

Chaumont M, Osorio M L, Chaves M M, Vanacker H, Morot-Gaudry J F and Foyer C H (1997) The absence of photoinhibition during the mid-morning depression of photosynthesis in *Vitis vinifera* grown in semi-arid and temperate climates. *J Pl Physiol* **150**(6): 743-51.

Chen S, Sun X Z, Kao C and Zhon D (1998) Suppression of breast cancer cells growth with grape juice. *Pharma Biol* **36**: 53-61.

Chohan J S (1956) Diseases of grapes in Punjab and their control. *Punjab Hort* **5**:181-84.

Chowdappa P, Reddy G S, Kumar A, Rao B M and Rawal R D (2009) Morphological and molecular characterisation of *Colletotrichum* species causing anthracnose disease of

grapes. *The Asian and Aus J Pl Sci & Biotech* **3**:71-77.

Clifford L, Jiang L , Oghenekome O , Hemanth K N Vasanthaiah, Devaiah K, Sheikh M B and Hae K Y (2011) Resistance to *Elsinoe ampelina* and expression of related resistant genes in *Vitis rotundifolia* Michx. Grapes *Int J Mol Sci* **12**: 3473–88.

Demiral T and Turkan I (2005) Comparative lipid peroxidation, antioxidant defense systems and proline content in roots oftwo rice cultivars differing in salt tolerance. *Env Exp Bot* **53**(3):247–57.

Demmig-Adams B and Adams W W (1992) Carotenoid composition in sun and shade leaves of plants with different life forms. *Pl Cell & Env* **15**: 411-19.

Deshmukh R B (2006) Grape Research in Maharashtra. Proc Int Symp Grape Prod Process Feb 6-11, Nation Res Centre Grapes, Baramati, Maharashtra (India) Pp.116.

Dhillon M, Kaur R and Basra R K (1997) A rapid method for obtaining leaf impression for studying leaf epidermal system.*J Pl Sci Res* **13**: 7-10.

Dhingra M (2011) *Screening of Morpho-physiological and biochemical characteristics imparting resistance against Alternaria blight in cauliflower (Brassica oleracea L.) genotypes.* Ph.D Thesis submitted to Punjab Agricultural University, Ludhiana. Pp 80.

Dubois M, Giles K A, Hamilton J K, Reters P A and Smith F (1956) Caorimetric method for the determination of sugars and related substances. *Anal Chem* **28**: 350-56.

Farkas J and Kiraly Z (1962) Role of phenolic compounds in the physiology of plant diseases and disease resistance. *Phytopath Z* **44**:105-50.

Flexas J, Briantais J M, Cerovic Z, Medrano H and Moya I (2000) Steady-state and maximum chlorophyll fluorescence responses to water stress in grapevine leaves: A new remote sensing system. *Remote Sensing of Environment* **73**(3): 283-297.

Frankel E and Meyer A (1998) Antioxidants in grape juices and their potential health effects. *Pharma Biol***36**: 14-20.

Gandhi S R (1956) Grape culture in India. *Farm Bulletin* ICAR No. 12, pp 56.

Ghosal T K, Dutta S, Senapati S K and Deb D C (2004) Role of phenol contents in legume seeds and its effect on the biology of *Collosobruchus chinensis. Ann Pl Protec Sci* **12**: 442-44.

Ghose L, Neela F A, Chakravorty T C, Ali M R and Alam M S (2010) Incidence of leaf blight disease of mulberry plant and assessment of changes in amino acids and photosynthetic pigments of infected leaf. *Plant Pathol J* **95**: 140-43.

Ghosh L, Alam M S, Ali M R, Shoheal A M, Alam F and Islam R (2003) Changes in some biochemical parameters of mulberry (*Morus* spp) leaves after infection with leaf spot disease. *Online J Biol Sci* **3**: 508-14.

Ghosh S N, Tarai R andPal P P (2008) Performance of eight grape cultivars in laterite soil of west bengal. Proceedings of the International symposium on grape production and processing. *Acta Hort* **785**: 73-77.

Ginter E (1979) Decline of coronary mortality in the United States and vitamin C. *Am J Clin Nutr* **32**: 511-12.

Goicoechea N, Aguirreolea J, Cenoz S and Garc´ıa-Mina J M (2000) *Verticillium dahliae* modifies the concentrations of proline, soluble sugars, starch, soluble protein and abscisic acid in pepper plants. *Eur J Pl Pathol* **106**: 19-25.

Goyal J P, Pathak V N and Sharma H C (1971) Incidence of anthracnose on different varieties of grapes in Jobner, Rajasthan (India). *Tropical Agriculturist* **127**(2): 105-106.

Guleria S, Paul B and Bajaj K L (1997) Biochemical changes in powdery mildew (*Erysiphe polygoni* D.C) resistant and susceptible cultivars of Pea (*Pisum sativum* L.) *Pl Dis Res* **12**: 185-88.

Gupta S K, Gupta P P and Kaushik C D (1995) Changes in leaf peroxidase, polyphenol oxidase, catalase and total phenols due to *Alternaria* leaf blight in *Brassica* species. *Indian J Mycol Pl Pathol* **25**: 175-80.

Gurjar P S, Singh S K, Singh A K and Verma M K (2015) Field reaction and biochemical response of grape genotypes to anthracnose incidence under sub-tropical conditions. *Int J Sci Natu* **6**(2): 248-55.

Gurme P N and Kore S S (1977) Incidence of anthracnose and varietal resistance in grape in Marathawada, Maharashtra State. *J Maharashtra Agric Univ* **2**: 177.

Halloin J M, De Zoeten G A, Gaard G and Walker J C (1970) The effects of tentoxin on chlorophyll synthesis and plastid structure in cucumber and cabbage. *Plant Physiol* **45**: 310-14.

Hare P D and Cress W A (1997) Metabolic implications of stress-induced proline accumulation. *Plant Growth Regulation* **21**: 79-102.

Hartman J R and Kaiser C A (2008) Fruit Rots of Grape. *Plant Pathology Fact Sheet*, University of Kentucky College of Agriculture.

Heath R L and Packer L (1968) Photooxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch BiochemBiophys***125**: 189-98.

Hegde G M and Anahosur K H (2001) Biochemical basis of resistance to fruit rot (*Colletotrichum capsici*) in chilli genotypes. *Karnataka J Agric Sci* **14**: 686-90.

Heil and Ploss K (2006) Induced resistance enzymes in wild plants- do early birds escape from pathogen attack? *Naturwissenschaften* **93**: 455-60.

Hendrickson L, Chow W S, Forster B and Furbank R T (2004) Processes contributing to photoprotection of grapevine leaves illuminated at low temperature. *Physiologia Plantarum* **121**(2): 272-81.

Hiscox J D and Israelstam G F (1979) A method for the extraction of chlorophyll from leaf tissue without maceration using dimethyl sulphoxide. *Can J Bot* **57**: 1332-34.

Jabeen N, Ahmed N, Ghani M Y and Sofi P A (2009) Role of phenolic compounds in resistance to chilli wilt. *Communications in Biometry and Crop Sci* **4**: 52-61.

Jamadar M M and Lingaraju S (2011) *In vitro* evaluation of fungicides, botanicals and bioagents against *Elsinoe ampelina*- An incitant of anthracnose of grapevine. *Karnataka J Agric Sci* **24**(2):146-48.

Jeyarajan R, Singh R and Singh R (1970) Incidence of anthracnose and Cercospora leaf spot diseases of grape varieties. *Madras Agric J* **57**:160-64.

Johansen D A (1940) *Plant Microtechnique.*Mc Graw Hill Book Co. Inc, New York, USA.

Jones P J, Woltz S S and Scott J W (1992) Screening for resistance to fusarium crown rot of tomato. *Proc Fla State Hort Soc* **105**: 345-47.

Karibasappa GS, Adsule PG, Sawant S D and Banerjee K (2006) Present scenario of wine industry in India. http://indianwine.com.

Kathe D D, Barhate B G and Bade S J (2011) Reactions of different grape varieties to rust caused by *Physopella ampelopsidis. J Pl Dis Sci* **6**(1): 35-38.

Kaur N, Dhiman J S and Khurana D S (2011) Physiological and biochemical traits analysis of *Capsicum annum* L. germplasm for Resistance to *Colletotrichum capsici*. *J Cell Pl Sci* **2**(3): 12-21.

Kavitha R and Umesha S (2008) Regulation of defence related enzymes associated with bacterial spot resistance in tomato. *Phytoparasitica* **36**(2): 144-59.

Kessman H, Staub T, Hofmann C, Maetzke T, Herzog J, Ward E, Uknes S and Ryala J (1994) Induction of systemic acquired acquired resistance in plants by chemicals. *Ann Rev Phytopath* **32**:439-59.

Khan A J, Deadman M L, Srikandakumar A, Al-Maqbali Y M, Rizvi S G and Al-Sabahi (2001) Biochemical changes in sorghum leaves infected with leaf spot pathogen *Drechslera sorghicola*. *Pl Pathol J* **17**: 342-46.

Khan TA, Mazid M and Mohammad F (2012) Potential of ascorbic acid against oxidative burst in plants under biotic stress: a review. *J Ind Res Technol* **2**(2):72–80

Kiefer B, Riemann M, Buche C, Kassemeyer H H and Nick P (2002) The host guides morphogenesis and stomatal targeting in the grape-vine pathogen *Plasmodium viticola*. *Planta* **215**: 387-93.

Kirk J T O and Allen R L (1965) Dependence of chloroplast pigment synthesis on protein synthesis: effects of actidione. *Biochem Biophys Res Commun* **22**: 523-30.

Klessing D F and Malamy J (1994) The salicylic acid signal in plants. *Pl Mol Biol* **26**: 1439-58.

Kulkarni S (2009) Sources of resistance to anthracnose in green gram and biochemical parameters for resistance. *Karnataka J Agric Sci* **22**(5): 1123- 125.

Kumar S and Thind T S (1992) Reduced sensitivity in *Gloeosporium ampelophagum* to carbendazim in Punjab. *Pl Dis Res* **7**: 103- 05.

Kumar S, Thind T S and Mohan C (1994) Occurrence of *Gloeosporium ampelophagum* and *Colletotrichum gloeosporioides,* the incitants of grape anthracnose, during different months in Punjab. *Pl Dis Res* **9**(2): 222-24.

Lee Y P and Takahashi T (1966) An improved calorimetric determination of amino-acids with the use of ninhydrin. *Ann Biochem* **14**: 71-77.

Li D and Wan Y (2008) Relatedness of resistance to anthracnose and white rot in Chinese wild grapes. *Vitis* **47**(4): 213-15.

Li J, Li R Q, Zang Z S and Wag J B (1991) Microscopic and ultrastructural studies on resistance to cucumber to *Pseudopronospora cubensis. J Wuhann Bot Res* **9**: 212-14.

Lobato A K S and Goncalves M C (2009) Changes in the photosynthetic pigment and carbohydrate content in common bean cultivars infected by *Colletotrichum lindemuthianum*. *Plant Soil Env* **55**(2): 58-61.

Lowry O H, Rosenbrough N J, Farr A L and Randell R J (1951) Protein measurement with folin-phenol reagent. *J Biol Chem* **193**: 265-75.

Lu J (1997) Oriental *Vitis* species-A new germplasm source for Florida grape breeding. *Proc Fla State Hort Soc* **110**:166–68.

Madhavi K J, Sujatha M, Raja Ram Reddy D and Rao C (2005) Biochemical characterization of resistance against *Alternaria helianthi*in cultivated and wild sunflowers. *Helia* **28**: 13-24.

Mahajan M and Dhillon M (2000) Role of epidermal system in downy mildew resistance in muskmelon (*Cucumis melo* L.). *J Maharashtra Agric Uni* **23**: 302-04.

Mahajan M, Thind T S and Dhillon M (2003) Structural variability amongst potato cultivars possessing varying grades of resistance to late blight. *Indian Phytopath* **56**: 443-45.

Manner A and Leath K T (1978) *Phytopathology* **68**: 1252-55.

Maroco J P, Rodrigues M L, Lopes C and Chaves M M (2002) Limitations to leaf photosynthesis in field-grown grapevine under drought-Metabolic and modelling approaches. *Func Pl Biol* **29**(4): 451-59.

Marutian S A, Antonyan A S, Abadzhyan R A, Petrosyan Z H A, Margaryan A A, Snkchyan C L, Kazaryan L V and Nagapeiyan Z H A (1979) Metabolic changes in leaves of new and elite forms of grapevine during infection by mildew. *Biologicheskii Zhurnal Armenii* **32**(8): 801-06.

Medrano H, Bota J, Abadla A, Sampol B, Escalona J M and Flexas J (2002) Effects of drought on light-energy dissipation mechanisms in high-light-acclimated, fieldgrown grapevines. *Func Pl Biol* **29**(10): 1197-1207.

Michael A E and Erincik O (2008) Anthracnose of Grape.*Fact Sheet Agriculture and Natural Resources*. Department of Plant Pathology.

Mirica II (1998) Anthracnose In: P(ed) Pearson R C and Goheen A C (eds.) *Compendium of Grape Diseases, American Phytopathology Society* pp 18-19,St.Paul MN, USA.

Mohan Chander and Thind T S (1995) Development of carbendazim resistance in *Gloeosporium ampelophagum* and strategies for its management. *Ind J Mycol Pl Pathol* **25**: 26-33.

Mohanraj D, Vidhyasekaran P and Kandaswamy T K (1972) Possible role of sugars in the anthracnose disease resistance mechanisms in grapevine varieties. *Ind Phytopath* **25**: 138-139.

Munne-Bosch S and Alegre L (2002) Plant ageing increase oxidative stress in chloroplasts. *Planta* **214**: 608-15.

Naglaa A A and Heba I M (2011) Impact of secondary metabolites and related enzymes in flax resistance and or susceptibility to powdery mildew. *W J Agric Sci* **7**(1): 78-85.

Naik S T, Satry M N L and Lingaraju S (1987) Host parasite interaction of *Sphaceloma ampelinum. Indian Phytopathol* **40**(4): 547-48.

NeishA C (1964) Major pathways in biosynthesis of phenols, In: J.B. HarboneP(ed) *Biochemistry of phenolic compounds.* pp 295-359, Academic Press, New York, USA.

Noveroske R L, Williams E B and Kuc J (1964) 7-Glucosidase and polyphenol oxidase in apple leaves and their possible relation to *Venturiaina equallis. Phytopath* **54**:98-103.

Pampanagouda B (2000) Studies on anthracnose of grape caused by *Sphaceloma ampelinum*. M.Sc. (Agri.) Thesis submitted to the University of Agricultural Sciences, Dharwad. Pp. 113.

Pampanagouda B and Benagi V I (2005) Role of weather factors in the disease development and spread of anthracnose of grape. *Agri Sci Digest* **25**(4): 311-12.

Park P and Ikeda Ken-inchi (2008) Ultrastructural analysis of responses of host and fungal cells during plant infection. *J Gen Plant Pathol* **74**: 2-14.

Patil S G, Honrao B K, Rao V G and Patil V P (1990) Field evaluation of grape germplasm for resistance against anthracnose. *Biovigyanam* **16**(2): 69-72.

Pearson R C and Goheen A C (1988) Eds. *Compendium of grape disease.*APS Press, USA. Pp 93.

Poolsawat O, Tharapreuksapong A, Wongkeaw S, Chaowiset W and Tantasawat P (2012) Laboratory and field evaluations of resistance to *Sphaceloma ampelium* causing anthracnose in grapevine. *Aust Plant Pathol* **41**: 263-69.

Prakash Om and Khirbhat S K (2011) Biochemical basis of resistance to fruit rot (*Colletotricum capsici*) in chilli genotypes. *Plant Dis Res* **26**(2): 180.

Prasad A and Nirvan R S (1965) Grape anthracnose and its control.*Punjab Hort J* **5**:186-90.

Prasad S V R, Rawal R D, Singh R and Murthy B N S (1992) Reaction of grape hybrids and their parents to anthracnose disease. *Proceedings of the International Symposium on Recent Advances in Viticulture and Oenology, held from 17 – 19th February* pp. 129-32.

Prasath D and Ponnuswami V (2008) Screening of chilli genotypes against *Colletotrichum capsici* and analysis of biochemical and enzymatic activities in inducing resistance.*Ind J Gen Pl Breeding* **68**(3): 344-46.

Raghvendra V B, Lokesh S, Govindappa M and Vasanth K T (2007) Dravyaas, an organic agent for the management of seed borne fungi of sorghym and its role in the induction

of defense enzumes.*Pest J Physol & Biochem.***89**: 190-97.

Ramakrishnan T S and Sundaram N V (1955) Grapevine diseases and their control.*Madras Agric J* **1**:220-27.

Rao K C and Satyanarayana C (1991) Epidemiology of anthracnose of grape (*Vitis vinifera* L.) caused by *Elsinoe ampelina* around Hyderabad. *Ind Phytopath* **44**: 501-03.

Rapport L and Jockwood B (2001) Proanthocyanidins and grape seed extract. *Pharma J* **266**: 581- 84.

Ribereau-Gayon J and Peynaud E (1982) traite d Ampelologie. Sciences et Techniques de la Vigne. Tome 1: Biologie de la Vigne, Sols de Vignobles. Tomes 2: Culture, Pathologie, Defense Sanitaire de la Vigne. Ed Dunod, Paris.

Rich S (1963) The role of stomata in plant disease. In: Zelitch I (ed) *Stomata and water relation in plants. Conn Agric Expt Sta Bull* **664**:102-16.

Roe J H and Oesterling M J (1943) The determination of dehydro- ascorbic acid and ascorbic acid in plant tissue by the 2,4 dinitrophenyl hydrazine method. *J Biol Chem* **152**: 511-17.

Ruelas C, Tiznado-Hernandez M E, Sanchez-Estrada A, Robles-Burgueno M R and Troncoso-Rojas R (2006) Changes in phenolic acid content during *Alternaria alternate* infection in tomato fruit. *J Phytopath* **154**(4): 236-44.

Saharan G S, Joshi, U N and Saharan M S (2000) Phenolic compounds and oxidative enzymes in healthy and *Alternaria* blight infected leaves of cluster bean. *ActaPhytopathologica* **34**: 299-06.

Sahhafi S R, Bagheri F and Assad M T (2012) Evaluation of some biochemical responses in resistance of fifteen bread wheat (*Triticum aestivum*L.) genotypes to wheat streak mosaic virus. *J Agric Sci* **4**:5.

Salisbury E J (1927) On the cause and ecological significance of stomatal frequency with special reference to woodland flora. *Phil Trans Royl Sac London* **241**: 1-65.

Sampol B, Bota J, Riera D, Medrano H and Flexas J (2003) Analysis of the virus induced inhibition of photosynthesis in malmsey grapevines. *New Phytologist* **160**(2): 403-12.

Sangeetha C G and Siddaramaiah A L (2007) Epidemiological studies of white rust, downy mildew and *Alternaria* blight of Indian mustard [*Brassica juncea* (Linn.) Czern and Coss] *African J Agric Res* **2**: 305-08.

Saud Z A, Razzaque S, Zaman S and Absar N (2000) Changes in some biochemical parameters and enzyme content of guava after infection with fruit rot disease. *Bangladesh J Genet Biotechnol* **1**: 85-90.

Senthil V, Ramasamy P, Elaiyaraja C and Ramola Elizabeth A (2010) Some phtochemical properties affected by the infection of leaf spot disease of *Cucumis sativus* L. caused by *Penicillum notatum. African J Basic & Appl Sci* **2**: 64-70.

Shankar B and Jindal P C (2001) Biochemical resistance of grape genotypes against anthracnose. *Indian J Agric Res* **35**(1): 44-47.

Shankar B and Jindal P C (2002) Screening of grape germplasm against anthracnose (*Sphaceloma ampelinam* de Bary). *Ind J Agri Res* **36**(2): 145-48.

Shanmugavelu K G (2003) Grape cultivation and processing. *Agrobios (India)*, pp. 289.

Shelke R B, Ingle S T, Patil S R and Sonalkar U V (1997) Susceptibility to grape varieties to major disease in Vidarbha region. *J Soil Crops* **7**:150-52.

Sheokand S, Kumari A and Sawhney V (2008) Effect of nitric oxide and putrescine on antioxidative responses under NaCl stress inchickpea plants. *Physiol Mol Biol Pl* **14**(4): 355–62.

Shobharani T and Ravikumar R L (2003) Reaction of interspecific lines to *Alternaria* leaf and stem blight in sunflower. *Helia* **26**: 115-20.

Shree M P and Natraja S (1993) Post-infectional biochemical and physiological changes in mulberry. *Curr Sci* **65**(4): 337-41.

Shree M P, Boraiah G and NarayanaGowda S N (1986) *J Plant Sci Res* **2**: 129-32.

Siddaramaiah A L and Hegde R K (1988) *Mysore J Agri Sci* **22**: 498-504.

Singh B P and Joshi L K (1971) Studies on the diseases of pan (*Piper beetle* L.) in Jabalapur (MP) assessment of losses due to *Colletotrichum capsici*. *Science and Culture* **37**: 581- 82.

Singh D N, Singh N K and Srivastava S (1999) Biochemical and morphological characters in relation to *Alternaria blight* resistance in rapeseed mustard. *Annual Agri Res* **20**: 472-77.

Singh H V (2000) Biochemical transformation in *Brassica* species against downy mildew and white rust of mustard. *Pl Dis Res* **5**: 154-56.

Singh R S (1984) *Introduction to principles of plant pathology.*Oxford and IBH publishing Co. Pvt Ltd, New Delhi.

Singh R S (2000) Diseases of fruit crops. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, pp 311.

Singhrot R S, Singh S P and Suhag L S (1982) Effects of anthracnose disease on productiveness of thompson seedless cultivar of grape. *Indian J Mycol Pl Pathol* **12**: 13-15.

Sivaprakasan K and Vidhyasakaran P (1993) Phenylalanine ammonia lyase gene for crop disease management. In: Vidhyasakaran P (ed) *Genetic Engineering, Molecular Biology and Tissue Culture for Crop Pest and Disease Management.* pp 113-22, Daya Publishing, Delhi.

Sivritepe N, Kumral N A, Erturk U, Yerlikaya C and Kumral A (2009) Responses of grapevines to two-spotted spider mite mediated biotic stress. *J Biol Sci* **9**: 311-18.

Sridhar T S and Sohi H S (1970) Relative resistance and susceptibility of different grape varieties against *Gloeosporium ampelophagum* Sacc. *Indian J Hort* **27**: 161-64.

Suhag L S and Grover R K (1972) Distribution of grape anthracnose in Haryana and estimation of losses in term of yield. *Haryana J Hort Sci* **1**: 83–86.

Swain T and Hills W E (1959) The phenolic constituents of *Prunusdomestica*- The qualitative analysis of phenolic constituents. *J Sci Fd Agric***10**: 63-68.

Taware P B, Kondiram N D, Dasharath P O, Sangram H P and Banerjee K (2010) Phenolic alterations in grape leaves, berries and wines due to foliar and cluster powdery mildew infections. *Int J Pharm Bio Sci* **1**(1).

Thind S K, Kaur N and Kumar A (2010) Reaction of date palm varieties to leaf spot disease of Graphiola. *J Res Punjab Agric Univ* **47**(1 & 2): 39-41.

Thind S K, Monga P K, Kaur N and Arora J K (1998) Effect of anthracnose disease on fruit quality of grapes. *J Mycol Pl Pathol* **31**: 253-54.

Thind S K, Monga P K, Kaur N and Kumar H (1997) Evaluation of grape varieties against anthracnose and its fungicidal control. *Pl Dis Res* **12**: 99-100.

Thind T S and Arora J K (2004) Epidemiology of powdery mildew, downy mildew and anthracnose diseases of grapevine. *Dis Fruits Veges***1**: 621-38.

Thind T S, Munshi G D, Sohi S S, Chandermohan and Grewal R K (1992) Seasonal periodicity in grapevine diseases in Punjab and their management. *Proc Of Intern Symp On Recent Advances in Viticulture and Oenology*,held from 14-17 February, 1992 at Hyderabad, India. 303-10 pp.

Thomas R L, Jen J J and Morr C V (1981) Changes in soluble and bound peroxidase-IAA oxidase during tomato fruit development. *J Food Sci* **47**: 158-61.

Turner J G, Ellis C and Devoto A (2002) The jasmonate signal pathway. *Plant Cell* 14 (suppl **1**): S153–64.

Vale De X, de Jesus Junior W C, Loelho R R, Hau B, Zambolin I, Costa L C and Filho A B (2001) Effect of angular leaf spot and rust on yield loss of *Phaseolus vulgaris. Phytopathology* **91**: 1045-53.

Van Loon L C and Van Strein E A (1999) The families of pathogenesis-related proteins, their activities and comparative analysis of PR-1 type proteins. *PhysiolMol Plant Pathol* **55**(2): 85-97.

Watson L and Dallwitz M J (1992) The families of flowering plants: descriptions, illustrations, identification and information retrieval. http://delta-intkey.com.

Whitehead TP, Robinson D, Allaway S, Synses I and Halle A (1995) Effect of red wine ingestion on the antioxidant capacity of serum. *Clin Chem* **41**:32-35.

Wu W, Wan X, Shah F, Fahad S and Huang J (2014) The role of antioxidant enzymes in adaptive responses to sheath blight infestation under different fertilization rates and hill densities. *The Scientific World J* **2014** pp 1-8.

Yadav G R and Nirwan R S (1981) Evaluation of grape varieties for their resistance to anthracnose disease. *Progressive Hort* **12**:69-72.

Yadav N K and Thankur D P (2001) Anatomical defense mechanism in pearl millet leaves against downy mildew infection. *J Mycol Pl Pathol* **31**: 185-87.

Yamada T, Ichinose S, Shiraishi T and Toyoda K (1997) Host specificity and the control of the defense genes. In: Yamada T, Shimamoto K and Watanabe Y (eds) A new frontier in plant science. *Plant Cell Technology Series* vol **8** Shujunsya Pub, Tokyo, pp 65-74.

Yanmin W, Zhongyue W, Jianbo C, Fanfang K, Jingjing S and Xinghong L (2010) Study on the anthracnose resistance of the main wine grape cultivars in Yantai. *SinoOverseas Grapevine and Wine* **1**: 15-17.

Yilmaz Y and Toledo R T (2004) Major flavonoids in grape seeds and skins: antioxidant capacity of catechin, epicatechin and gallic acid. *J Agril Food Chem* **52**(2): 255-60.

Yuan H K, Park K S, Rho J H and Jo H M (2006) Evaluating the resistance against anthracnose by bioassay using culture filtrates from *Elsinoe ampelina. Hort Sci* **40**(4): 1070.

Zauberman G R, Ronen M, Akerman A, Weksler I Rot and Fuchs Y (1991) Post-harvest retention of the red colour of litchi fruit pericarp. *Sci Hort* **47**: 89-97.

Zhou B, Chen Z, Du L, Ye X and Li N (2012) Correlation between resistance of eggplant and defense-related enzymes and biochemical substances of leaves. *Afr J Biotech* **11**(74): 13896-902.

ANNEXURE

Monthly agrometrological data for the year 2016

MONTH	TEMPERATURE (°C)			RELATIVE HUMIDITY(%)			RAINFALL (mm)	EVAPORATION	SUNSHINE HOURS
	MAX	MIN	MEAN	MORNING	EVENING	MEAN			
January	17.2	7.4	12.3	95	64	80	19.4	32.6	2.4
February	23	9	16	91	46	69	8.8	72.5	7.7
March	28	14.6	21.3	87	43	66	41.5	124.1	8.2
April	36.6	19.6	28.1	62	22	42	3	250.4	9.7
May	39.6	24.6	32.1	57	27	42	25.2	306.6	9.1
June	39.8	28.5	33.7	65	42	53	86	270.5	7.8
July	33.5	27.3	30.4	85	66	75	305.5	146.3	5.8
August	33.3	26.2	29.7	85	65	75	87.6	115.8	5.5
September	34	25.5	29.7	86	55	71	15	136.9	7.5
October	32.7	19	25.9	89	38	63	0	107	6.1
November	27.7	12	19.9	89	32	60	2	74.4	5.6
December	22.2	8.6	15.4	95	49	72	0	38.9	4.7

(attained from School of Agricultural Meteorology, PAU)

VITA

Name of the student	:	Sarah Murria
Father's name	:	Mr. Yuvraj Murria
Mother's name	:	Mrs. Kanchan Murria
Nationality	:	Indian
Date of Birth	:	31.05.1994
Permanent home address	:	M-44, Basant Vihar Colony Noorwala Road, Ludhiana - 141 008

EDUCATIONAL QUALIFICATIONS

Bachelor's degree	:	B.Sc. Medical (Hons.)
University	:	Panjab University, Chandigarh
Year of Award	:	2015
% age of marks	:	71.90%, 78.00%
Master's degree	:	M.Sc. Botany
University and Year of Award	:	Punjab Agricultural University, Ludhiana 2017
OCPA	:	7.01/10.00
Title of Master's Thesis	:	Physiological and anatomical reaction of grape (*Vitis vinifera* L.) genotypes to anthracnose