Chapter 4

Biochemical basis of resistance and pathogenic variability of potential pathogens associated with melon vine decline diseases
Introduction

Melon, commercially important warm seasons crop is known for its medicinal properties. The fruit juice of melon is usually used for chronic and acute eczema lesions and also is a promising cure for dyspepsia. The seed oil of melon is used to treat many common infections both internally and externally. The root of melons is emetic in nature and has purgative action. All these medicinal properties of melon can be attributed for constituents of fruit. Fruits are known as good source of antioxidants including vitamins. Some of the components of the same fruit have been used as a therapeutic component to treat cancer (Wargovich, 2000). The melon crop loss is mainly due to vine decline diseases caused by *D. bryonieae*, *B. theobromae*, *F. oxysporum* and *F. solani*. General symptoms of this group include yellowing and death of the crown leaves with a gradual decline of the vine as the plant approaches maturity. Upon pathogenic infection and with associated disease progress major change in biochemical constituents mainly in total protein, total carbohydrates, phenols and Vitamins c have been studied in the previous chapters. But in depth knowledge with changes associated is not well supported in previous studies atleast with the host and pathogens the study is dealing with. Hence, the present study was taken up to understand the changes associated with proteins which might be induced or degraded during pathogenic infection and as there was distinct changes associated with total phenols and vitamin C. Peroxidase one of the key enzymes required for antioxidant activity and also well known as a pathogenesis related protein has been taken up in this part of the study.

According to Vanloon (1997) accumulation of pathogenic related proteins (PR-proteins) represent the major qualitative change in the total protein content of the tested plant parts and also defines PR-proteins as newly synthesized proteins associated with pathogen infection. Though not much of studies have been carried out on melon vine decline diseases studies on biochemical changes taking place in the tissue as a result of infection either by soft rot or decay fungi has been studied (Ram and Vir, 1996).
The same literatures were used as a basis to develop this study. The objectives of the study were,

i. Changes in protein banding pattern in the host plants after infection with vine decline pathogens.

ii. Differential activity of peroxidase, a pathogenesis related proteins in melon plants exhibited vine decline diseases.
Review of Literature

Association of proteins with plant disease resistance

The proteins associated with resistance acquired against powdery mildew were studied by Avila et al. (2002) in both resistant and susceptible cultivars of Cornus florida. Difference in protein banding pattern was detected in both the tested cultivars after inoculation. Study showed the detection of new protein bands in susceptible cultivars, which could be involved in disease establishment. Seven additional bands were detected in resistant cultivars after inoculation. The results suggest that the resistant mechanism in dogwood to powdery mildew may involve PR-protein synthesis.

Experiments conducted by Ramanathan and Vidyashekar (2002) revealed the induction of PR-proteins in black gram by biotic (fungal elicitor) elicitor isolated from Macrophomina phaseolina and abiotic elicitor (salicylic acid). In black gram leaves treated with biotic or abiotic elicitor a new 16kDa protein was shown to induce which was not detected in untreated leaves. Results indicated that induction of proteins is necessary for conferring resistance.

Increased in levels of total proteins, peroxidases, phenols and other enzymes were studied in tea plants with blister blight infection caused by Exobasidium vexans Massee by Chakraborty et al. (2002). A higher degree of peroxidase activity was noticed in infected leaves with the appearance of four isozymes in healthy and five in infected leaves in polyacrylamide gel electrophoresis. Protein showed a decline at the early stage of infection. Results showed the absence of many protein bands after infection.

Sharma et al. (2002) studied pathotype variation by analyzing the protein variation on SDS-PAGE. Electrophoresis shows variation in soluble protein fractions among the three virulent pathotypes of Neovossia indica (KB2) of wheat. Among the 23 bands resolved on electrophoresis 16 were common among the pathotypes.

Plant growth promoting microbes (PGPM) viz., T. harzianum and B. subtilis isolated from rhizosphere soil were tested for their ability to induce pathogenesis related proteins including peroxidase. Increase in PR proteins after application with biocontrol agents have been reported by several workers in different crops (Ji and Kuc, 1995; DeMeyer et al., 1998; Yedidia et al., 1999, Meena et al., 2000, Oostendorp et al., 2001). Enhanced accumulation of PR proteins and oxidative enzymes in the

**Association of peroxidases with disease resistance**

Class III plant peroxidase (POX), a plant-specific oxidoreductase was reported as one of the many types of peroxidases that are widely distributed in animals, plants and microorganisms (Hiraga *et al.*, 2001). POXs exist as isozymes in individual plant species and each isoenzyme has variable amino acid sequences and shows diverse expression profiles, suggesting their involvement in various physiological processes. Indeed, studies have provided evidence that POXs participate in lignification, suberization, auxin catabolism, wound healing and defense against pathogen infection.

The ability of fruit of muskmelon to resist disease was in part found related to compounds found in the tissues. One group of these is the peroxidases. The outer (exocarp) and middle (mesocarp) sections of the muskmelon fruit were assayed, at 5-day intervals, for peroxidases from 5-50 days post-pollination (DPP). Exocarp peroxidase increased through 30 DPP followed by a decrease. At all sampling times, peroxidase levels in the mesocarp were much lower than in the exocarp. Analysis found that up to eight isozymes, of peroxidase were present in the exocarp through 50DPP, and the number and location in the tissues changed during fruit development. Changes in peroxidase activity corresponded to fruit-net formation and mass associated with susceptibility to fruit rot (Juarez *et al.*, 2000).

Biles *et al.* (2000) characterized the muskmelon fruit peroxidases at different developmental stages. Muskmelon fruits are more susceptible to fungal attack as they reach maturity (40DPP). Peroxidase levels in the mesocarp were much lower than in the exocarp, which corresponds to tissue that is very susceptible to fungal colonization. Additional research will be required to determine the possible role of peroxidase enzymes in muskmelon fruit rot resistance.

A field experiment was conducted by Bose and Rajan (2000) using 24 genotypes of tomato with varying levels of resistance to bacterial wilt caused by *Ralstonia solanacearum*. The zymograms of peroxidase indicated the possibility of using this enzyme as markers for resistant and moderately resistant varieties of tomato.
Materials and methods

Characterization of potential vine decline pathogens through polyacrylamine gel electrophoresis:

Extraction of total proteins from mycelial mats of potential vine decline pathogens:

Mycelial mat (1.5g) of all potential vine decline fungal isolates (Db1, Bt2, Fo2 and CGN1) was used for extraction. Proteins were extracted using 2.5ml of 0.5M phosphate buffer at pH 7.0. The mycelial homogenate was then centrifuged at 10,000rpm for 10min. Protein content was measured in the supernatant collected from the homogenate as per the method of Bradford, 1976. The same supernatant was used for further studies.

Poly acrylamide gel electrophoresis (PAGE):

Reducing polyacrylamide gel electrophoresis (PAGE) was carried out using SDS as per the method of Lamelli (1970). For all the experiments, 12.5% of acrylamide gel containing 1%- w/v SDS was used. Supernatants of mycelial mats containing 10 - 20μg of total protein was loaded into gels after the addition of equal amount of denaturing sample buffer containing 0.1% β mercaptoethanol. Samples were boiled for 10min, cooled on ice before loading. Electrophoresis run was carried out at constant voltage of 100V for 90min along with appropriate molecular weight standards.

The gel composition is as follows:

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Silver nitrate staining:

After electrophoresis the gel was fixed in the fixative [12.5ml of 50% alcohol +3ml of 12% acetic acid + 12.5ml of 5% formaldehyde] for 1h. The solution was drained off and the gel was incubated twice in 50% alcohol for 30 minutes. The gel was further incubated in sodium thiosulphate (0.005g/25ml) solution for exactly one minute, then was washed thrice in distilled water for 20 seconds and incubated in silver nitrate (AgNO₃) solution (0.05g/25ml) containing formaldehyde (40µl) for 30 minutes. Gel was subsequently washed couple of time to wash away the extra silver on the gel before developing. Thoroughly washed gel was placed in developer solution containing sodium carbonate (6%) and formaldehyde (2%). Reaction was stopped by the addition of 70% acetic acid to visualize reddish brown bands of protein and was documented using bioprofile image analysis system (Vilbert Lourmat, France).

Post-infectional variation in the protein banding patterns of melon seedlings:

**Extraction of total proteins**

Seeds of melon samples DB1, DB2, MCGH2 and CGN1 were soaked in suspension of Db1 (1 X 10⁸ spores/ml), Bot2 (1 X 10⁸ spores/ml), Fo2 (5X10⁶ spores/ml) and Fs1 (5 X 10⁶ spores/ml) respectively for 16h at RT. Seeds were then blotted and plated onto petriplates containing pre-wetted blotters. Care was taken to ensure that each petriplate contained only ten seed to provide proper spacing. Seedlings were collected 8 and 15 days after plating and stored at -20°C till use.

Collected seedling samples were grinded in prechilled mortar containing sterile sand. Proteins were extracted by the addition of 2.5 ml of potassium phosphate buffer (10mM) pH at 7.0. Homogenate was collected and centrifuged at 10000rpm for 10 min. Clear supernatant was collected to measure total protein as per the method of Bradford (1976). These protein samples were used for all further studies.

**Accumulation of peroxidase in melon fruit samples after 30DPPP and 40DPP from obtained from plants raised from seeds inoculated with spore suspension of potential vine decline pathogens along with untreated control:**

Seeds of melon samples DB1, DB2, MCGH2 and CGN1 were soaked in suspension of Db1 (1 X 10⁸ spores/ml), Bot2 (1 X 10⁸ spores/ml), Fo2 (5X10⁶ spores/ml) and Fs1 (5 X 10⁶ spores/ml) respectively for 16h at RT. Seeds were blotted and sown into field microplots described elsewhere in the study. Plants were
maintained with routine cultural practices and measured according to schedules. Fruits were collected from these plants after 30 and 40DPP. Collected fruits were washed and cut to collect the mesocarp -edible portion of the melon fruit. Mesocarp collected was weighed and was used for enzyme extraction.

**Enzyme extraction in mesocarp of melon fruits:**

All the enzyme extraction procedures were carried out at 4°C to maintain the stability of enzyme extracted. Mesocarp (one gram) of each sample was homogenized in a prechilled mortar with addition of 10mM potassium buffer at pH 6.8. The homogenate was centrifuged in refrigerated high speed centrifuge at 10000rpm for 15min. Supernatant of the homogenate served as enzyme source. Before doing the assay total protein extent was measured in the supernatant as per the method of Bradford, (1976).

**Spectrophotometric estimation of peroxidase activity in melon fruit samples:**

Peroxidase assay reaction mixture constituted 3ml of 10mM potassium phosphate buffer pH 6.8 containing 0.25% guaiacol. The reaction was initiated by adding crude enzyme extract (25μl) and measuring the absorbance at 470nm to note the absorbance of initial reaction mixture. Reaction of peroxidase was initiated by addition of 10mM-H₂O₂ - a substrate of peroxidase. Reaction rate was measured by noting the absorbance at 470nm/min. Enzyme activity is expressed as absorbance at 470nm/min/mgprotein (Hammerschmidt et al., 1982).

**Analysis of in situ peroxidase activity by native PAGE analysis:**

Peroxidase activity was analysed on 8% polyacrylamide (non-reducing) gel. Crude extracts were loaded onto the gel after mixing them with non-denaturing sample buffer. Electrophoresis was carried out at constant voltage of 100V for 90min. Gel was stained as per the protocol of Schrauwen (1966). As per the protocol, 40mg of benzidine was dissolved in 0.5ml of 95% ethanol, and made upto 40ml by adding distilled water. Acetic acid was added (~ 400μl) to clear the solution. Clarified benzidine solution was then filtered through cotton. Hydrogen peroxide was added just prior to staining. Gel was soaked in the stain till bluish bands are visualized and transferring gel to distilled water stopped the reaction. Stained gel was documented using bioprofile image analysis system (Vilbert Lourmat, France).
Results

Characterization of potential vine decline pathogens by polyacrylamide gel electrophoresis:

Protein banding pattern of isolates *D. bryoniae* showed almost general pattern, but the intensity of protein banding varied from isolate to isolate. Isolate Db5 and Db6 showed the additional protein banding approximately ~45 and ~39kDa in molecular weight, which were prominently absent in other isolates. The common protein band of ~26 and ~21kDa were the major bands observed in all the isolates analysed. Isolate Db4 showed that higher intensity in 21kDa band though isolate Db5 recorded increased bandwidth for the same protein. Isolates Db2, Db4 and Db7 recorded higher intensity bands with ~14 and ~12 kDa, whereas higher intensity of ~16kDa was observed only in isolate Db2 of *D. bryoniae* (Fig - 28).

With the culture filtrates of all the isolates of *D. bryoniae* were analysed, most of the protein bands of cultured filtrates are strikingly similar with the protein-banding pattern of mycelial mats of same isolates. A protein band of ~45 kDa was the most predominantly observed one. The intensity of ~45kDa protein band didn’t show much variation among the isolates except for isolate Db6, which showed weaker intensity when compared to other isolates (Fig - 29). In totality, from the results it seems like low molecular weight proteins are more in all isolates of *D. bryoniae*.

Unlike the banding pattern of *D. brynoiae* all isolates of *B. theobromae* showed predominant accumulation of high molecular weight protein bands compared to low molecular weight protein bands. Though there was variation in the intensity of these protein bands viz., ~66, ~48, ~45, ~39 and ~26kDa, only isolate Bt1 and Bt5 showed just detectable amount of intensity of all bands except for ~26kDa (Fig-30). Whereas, protein-banding pattern of culture filtrate of same isolates showed that these were not secretory proteins, In spite of the fact that very faint band of ~26 and ~21kDa bands were observed. The accumulation of ~26 and ~21kDa bands in culture filtrate of all isolates might be due to some of the dead spores in the culture filtrate (Fig-31).

Among the seven isolates of *F. oxysporum*, isolate Fo1-3 accumulated ~28kDa protein, which was completely, absent in other isolate. The bands of molecular weight ~45 and ~39kDa were commonly observed in all the isolates. Isolate Fo1, Fo3 & Fo4 didn’t show ~ 21kDa protein band though it was distinctly visible in other isolates
Culture filtrates of these isolates showed distinct accumulation of low molecular weight proteins (~21 to ~12kDa), which was not observed in the protein-banding pattern of mycelial mat of same isolates. The intensity of these molecular weight proteins was very weak in isolate Fo4, Fo6 & Fo7 when compared to other isolates. Irrespective of isolates uniform accumulation of ~39kDa band was observed (Fig-33).

Though, F. solani is also one of the Fusarium species protein banding pattern when compared to F. oxysporum was entirely different. Accumulation of ~45, ~31, ~21 & ~14kDa appeared to be common in both the Fusarium species, whereas ~29, ~26, ~16 & ~12kDa proteins accumulated only in F. solani. Isolate Fs1, Fs5 & Fs6 of F. solani showed higher intensity in ~45kDa band. Isolate Fs3, Fs4 & Fs7 recorded very weak banding pattern when compared to other isolates (Fig-34). All these comparisons are made keeping in mind the fact that equal amount of protein was loaded into each lane of the gel. Unlike other culture filtrates F. solani protein banding pattern showed that this species has more amount of secretory proteins when compared to other fungi tested. Culture filtrates of isolate Fs1 & Fs3 showed the accumulation of ~39kDa protein band very prominently when compared to other isolates. Among the culture filtrates of all the isolates banding pattern of isolate Fs3 showed prominent accumulation of secretory proteins (Fig-35).

Post-infectional variation in the protein banding pattern of melon seed samples soaked in spore suspension of potential vine decline pathogens:

Melon seed samples, DB1, DB2, MCGH2 and CGN1 when soaked with spore suspension of Db1 (1 X 10^8 spores/ml), Bot2 (1 X 10^8 spores/ml), Fo2 (5X10^6 spores/ml) and Fs1 (5 X 10^6 spores/ml) respectively for 16h at RT showed specific banding patterns. Samples of DB1 & DB2 showed almost common banding patterns with uniform intensities. The reason behind this unmistakable uniform accumulation couldn’t be deciphered, whereas the same was not the case of Fusarium species. Although most of the bands seen were uniform the difference in accumulation pattern of low molecular weight proteins was distinctly observed. F. oxysporum infected sample MCGH2 showed the accumulation of ~16 & ~12 kDa in contrast with F. solani which showed only ~14 kDa band. The same uniform banding pattern was also observed when seedlings raised from seeds soaked in spore suspension were used as
samples for analysis. Though the seedlings used for the experiment were at two different phases of growth viz., 8 and 15-day-old seedlings. The results given here were repeated to confirm nearly uniform pattern between the fungi tested (Fig-36 & 37).

**Accumulation of peroxidase in melon seed sample after 30DPP and 40DPP obtained from plants raised from inoculated seeds:**

Spectrophotometric analysis of peroxidase accumulation in melon fruit samples of different maturity from plants raised from inoculated seeds was compared with fruits from healthy plants and uninoculated plants. Peroxidase accumulation increased with maturity of fruits when plants were free of infection. There was tremendous decrease in activity with increase in maturity of fruits when the plants showed the incidence of vine decline disease. Surprisingly there was increase in peroxidase activity in the infected samples, which were provided with artificial infection. The increase in activity ranged between 10 to 50%, which varied with test samples. If the peroxidase activity is compared between fruit samples collected at 30 and 40DPP approximately 50% reduction in peroxidase activity was observed in plants provided with artificial infection. Among the sample CGN1 showed the maximum reduction followed by DB2, DB1 & MCGH2. With increase in maturity of the fruits, the increase in peroxidase levels in healthy samples varied between 15-35% (G - 17).

*In situ* peroxidase activity showed five isoforms of peroxidase. Experiment was carried out with mesocarp of fruit samples obtained from artificially inoculated plants after 30 & 40DPP (Fig-38).

Overall peroxidase activity was reduced with increased fruit maturity though the number of isoforms didn’t change. In samples of 30DPP, MCGH2 showed higher intensity of banding pattern in all peroxidase isoforms. All other showed almost uniform accumulation of all isoforms, but isoform 2 appeared as a faint band in DB1, DB2 & CGN1 samples. Isoform 1 of *F. solani* showed to have different molecular weight compared to isoform 1 of other samples.

Peroxidase accumulation pattern in 40DPP fruit sample was very less compared to 30DPP sample. The important change noticed that in this experiment was the absence of isoform 3 and 4 in sample DB1. Sample DB1 also exhibited weaker intensity of isoform 1 along with sample MCGH2. No other distinct characteristics were seen in accumulation of isoforms between the samples (Fig - 39).
Discussion

One of the major problems concerning the production of food crops is the difficulty in controlling plant disease to maintain high quality yield accepted by both producer and consumer. Control of plant diseases is a high priority in many crops as early infection by pathogens may kill the plant leading to poor crop establishment or even complete crop loss. Melon is one of the important commercial crops, which often succumbs to vine decline diseases, which contributes to major crop loss. Lack of literature on study of biochemical basis of resistance in melon crop against vine decline pathogens proves that not much work has been carried out on this aspect. In the present study an attempt has been made to study the same. As the first incidence, protein profiles of the vine decline pathogens were studied to rule out the possibility of ignoring any new proteins associated with infections. Culture filtrates of the pathogens were also tested in the present study. For all the experiments inoculation of sample DB1 was always done with spore suspension of isolate 1 of *D. bryoniae*. The isolate selections were based on pathogenicity test and also time and extent of symptoms expressed in melon plants upon inoculation. But protein profile pattern of Db1 didn’t differ much from isolate Db3. Both the isolates showed the protein band of ~26kDa band was more in isolate 3 of *D. bryoniae*. Though the results don’t give much of conclusion on the virulence of the isolate, as per the results it is evident that the amount of protein expressed may also act as a factor to exhibit virulence.

Extracellular accumulation of 45kDa protein band denotes that this might be a secretory protein of *D. bryoniae*. The same faint band was also observed in the protein profiles obtained by the mycelial mat of the same fungi. Virulent isolate Db1 recorded the faintest band at this molecular weight. In *B. theobromae* profile we could see that a total number and the amount of secretory protein was less indicating the dominance of intracellular proteins. Isolate Bt2 was found to be more virulent as the results obtained from pathogenicity test. Protein banding pattern of Bt2 showed distinct and clear bands of proteins, which compared to other isolates with the molecular weight ~66, ~45, ~39, ~31 & ~26kDa. Though other isolates had the same banding pattern there were also other additional proteins present in them. The prominent absence of ~48 kDa protein was distinctly observed in this isolate compared to other isolates. Comparing *D. bryoniae* and *B. theobromae* we could see that the absence of protein bands was
correlating with the virulence factor of that particular isolate.

Among the *Fusarium* isolates, though the same fungal mycelial mat was used for protein extraction, *F. solani* showed higher protein accumulation with distinctive pattern. Isolate Fo2 was used in all the experiments showed five distinctive bands, which was almost similar with isolate Fo1, and Fo3. But the presence of ~21kDa band was uniquely present in isolate Fo2, though the same band could be observed in isolate Fo5, Fo6 & Fo7. Though there was similar banding pattern in isolates Fo4, Fo5, Fo6 & Fo7, which resembled isolate Fo2 with respect to ~21kDa the predominant absence of ~31 & ~21kDa was observed. As per the results of protein banding pattern the virulence of isolate Fo2 was the result of cumulative accumulation of ~31, ~28 & ~21kDa bands. Protein banding pattern of culture filtrates of these isolates showed that the ~21kDa protein band also had secretory nature.

From the pathogenicity test it was confirmed that isolate 1 of *F. solani* was the most virulent of all the isolates. Protein banding patterns showed that there was prominent accumulation of ~29kDa band in isolate 1 along with accumulation of all three low molecular weight protein bands *viz.*, ~16, ~14 & ~12kDa. Comparing both the *Fusarium* banding patterns the results inferred that the virulence of the *Fusarium* species is due to the co-accumulation of multiple proteins than due to single protein. Protein banding pattern of culture filtrates of same species showed the presence of ~39kDa band along with ~16, ~14 & ~12kDa. All these proteins were distinctly observed in isolate 1 whereas, other isolates showed addition or deletion of some of these bands.

The study followed the method of Dristig and Dianese (1990), who compared 65 isolates representing three biovars 1, 2 and 3 of *Pseudomonas solanacearum* isolated from seven host plants and five different regions of Brazil were compared. Protein bands of ~37 and ~39 kDa were common in several strains of biovars 1and 3 and were not found in biovar 2. *Pseudomonas* isolates of 10 plant species showed marked variations among themselves and between fluorescent and non-fluorescent groups, when comparatively analysed for their total proteins separated through SDS-PAGE (Nandakumar *et al.*, 2002). Results of this study shows the difference in protein banding pattern among the species correlated well with the results of the present study where we could see the difference in banding pattern among the species of *Fusarium*. 
Protein banding pattern of melon seed samples soaked in spore suspension of *D. bryoniae* and *B. theobromae* for 16h didn’t show any difference in the banding pattern. The strikingly similar pattern of these bands could be due to non-entry of the pathogen due to the presence of thicker seed coat or might be due to the fact that these pathogens can proliferate well once they find the point of entry into the host. As the melon seeds take longer time for germination the possibility is the pathogen couldn’t find point of entry into the host. Though the same goes with *Fusarium* species, the absence of ~16 and ~12kDa bands and the presence of ~14kDa band was observed only in *F. solani*. The reason for this couldn’t be deciphered. But the work carried out by Chakraborty et al. (2002) in tea leaves infected with blister blight pathogen *Exobasidium vexans* correlated by showing no new protein synthesis after infection which might be the same with melon seeds infected with potential vine decline pathogens.

Differential protein pattern was observed in 8 and 15 day old seedlings of different samples *viz.*, DB1, DB2, MCGH2 & CGN1. Though there was difference in banding pattern between the samples there was not much difference within the sample irrespective of age of the seedlings. The possible reason for the similar pattern might be as described above. We could also think that there was a possibility that fungus stayed subdues up to certain stage of plant growth without affecting any factors. This is also one of the main criteria to record vine decline diseases, where plants seem to be healthy up to certain stage of growth and all of a sudden symptoms start appearing eventually leading to vine decline and death of plant.

Melon fruits are known to have rich antioxidant property, which made us to study the level of peroxidases in fruit samples of different maturity. Spectrophotometric analysis showed a drastic reduction of peroxidase level, which was inversely, proportional to age of the plant. Peroxidase levels in fruits of 40DPP that was not provided with additional inoculum showed very less peroxidase activity when compared to samples, which was provided with additional inoculum. This might be due to the partial resistance developed in samples, which was pre-exposed to the pathogens. Decrease in peroxidase with increase in maturity correlates well with very poor fruit quality as a result of vine decline diseases. Whereas in the natural conditions the fruit shows increase peroxidase levels with increase in maturity. These results of specific
assay were supported by in situ gel staining activity of peroxidase.

Total of five isoforms of peroxidase was observed in all the samples irrespective of the samples infected. *Fusarium* species recorded higher intensity of bands when compared to other two pathogens in fruits of 30DPP. This may be due to the fact that the plants infected with *D. bryoniae* and *B. theobromae* show complete fruit loss due to rotting whereas, fruits from plants infected with *Fusarium* lose the nutritional quality of the fruit. Though the fruits obtained from *Fusarium* infected plants seem to be healthy the results in previous chapter shows biodeterioration, which can be correlated to this results. Increased peroxidase levels in fruits of *Fusarium* infected plants can be related to the healthy fruit production upto 30DPP without the loss in nutritional quality. But the peroxidase levels at 40DPP shows tremendous decrease in the intensity of bands, which indicates the biodeterioration. The overall increase in peroxidase activity at 30Dpp shows that during vine decline diseases there is a normal fruit-setting period, but with increased progression of the disease fruit quality decreases. The results of 40DPP show very weak banding pattern with fruits obtained from *D. bryoniae* and *B. theobromae* infected plants, which might be due to fruit rotting during this infection. The results of this chapter correlate well with the delayed symptom expression of vine decline symptom inspite of existence of pathogen within the host. The same decrease in peroxidase activity in mesocarp of melon fruits with increased maturity has been demonstrated by several workers (Juarez et al., 2000; Biles et al. 1996).

The nutritional quality of seeds has to be maintained in order to maintain the quality of plant stand in the field conditions. Above all these, accumulation of total phenols was known to be in higher amount in resistant genotypes as compared to susceptible ones. Hence, phenolic compounds and related oxidative enzymes are mostly considered as one of the biochemical parameters for development of disease resistance (Pradeep and Jambale, 2000).
G - 17. Peroxidase accumulation in relation to disease severity due to natural and artificial infection of vine decline fungi at 30 and 40 days post pollination (DPP) melon fruit samples. Bars indicate SE (n=3).
Differential accumulation of peroxidases (OD at 470nm/total amount of protein)

- Natural infection 40 DPP
- Artificial infection 30 DPP
- Artificial infection 40 DPP
- Healthy 30 DPP
- Healthy 40 DPP

Fruit samples collected from plants after 30 DPP and 40 DPP

G - 17.
Fig - 27.  Gelatin hydrolysis
a.  *F. oxysporum*
b.  *F. solani*
c.  *B. theobromae*
d.  *D. bryoniae*
e.  *D. bryoniae*
Fig - 28. **Protein profile of total proteins extracted from mycelial mat of D. bryoniae**
   Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
   Lane 2-8: Isolates of *D. bryoniae* viz., Db1, Db2, Db3, Db4, Db5, Db6 & Db7 respectively.

Fig - 29. **Protein profile of total proteins extracted from culture filtrate of D. bryoniae**
   Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
   Lane 2-8: Isolates of *D. bryoniae* viz., Db1, Db2, Db3, Db4, Db5, Db6 & Db7 respectively.

Fig - 30. **Protein profile of total proteins extracted from mycelial mat of B. theobromae**
   Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
   Lane 2-8: Isolates of *B. theobromae* viz., Bot1, Bot2, Bot3, Bot4, Bot5, Bot6 & Bot7 respectively.

Fig - 31. **Protein profile of total proteins extracted from culture filtrate of B. theobromae**
   Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
   Lane 2-8: Isolates of *B. theobromae* viz., Bot1, Bot2, Bot3, Bot4, Bot5, Bot6 & Bot7 respectively.
Fig - 32. **Protein profile of total proteins extracted from mycelial mat of *F. oxysporum***
Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
Lane 2-8: Isolates of *F. oxysporum* viz., Fo1, Fo2, Fo3, Fo4, Fo5, Fo6 & Fo7 respectively.

Fig - 33. **Protein profile of total proteins extracted from culture filtrate of *F. oxysporum***
Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
Lane 2-8: Isolates of *F. oxysporum* viz., Fo1, Fo2, Fo3, Fo4, Fo5, Fo6 & Fo7 respectively.

Fig - 34. **Protein profile of total proteins extracted from mycelial mat of *F. solani***
Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
Lane 2-8: Isolates of *F. solani* viz., Fs1, Fs2, Fs3, Fs4, Fs5, Fs6 & Fs7 respectively.

Fig - 35. **Protein profile of total proteins extracted from culture filtrate of *F. solani***
Lane 1: Protein molecular weight standards (range: 10 - 120kDa)
Lane 2-8: Isolates of *F. solani* viz., Fs1, Fs2, Fs3, Fs4, Fs5, Fs6 & Fs7 respectively.
Fig-36. Protein profile of total proteins extracted from melon seed samples soaked in spore suspension of potential vine decline pathogens
Lane M: Protein molecular weight standards
Lane 1: Seed sample DB1 soaked in spore suspension of *D. bryoniae* (1X 10^6 spores/ml) for 16h
Lane 2: Seed sample DB2 soaked in spore suspension of *B. theobromae* (1X10^6 spores/ml) for 16h
Lane 3: Seed sample MCGH2 soaked in spore suspension of *F. oxysporum* (5x10^6 spores/ml) for 16h
Lane 4: Seed sample CGN1 soaked in spore suspension of *F. solani* (5x10^8 spores/ml) for 16h

Fig-37. Protein profile of total proteins extracted from 8 and 15 day old melon seedlings raised after soaking in potential vine decline pathogens
Lane M: Protein molecular weight standards
Lane 1 and 2: Seedlings of sample DB1 raised from seeds soaked in spore suspension of *D. bryoniae* (1X 10^6 spores/ml for 16h) collected 8 & 15 days post inoculation period.
Lane 3 and 4: Seedlings of sample DB2 raised from seeds soaked in spore suspension of *B. theobromae* (1X10^6 spores/ml for 16h) collected 8 &15 days post inoculation period.
Lane 5 and 6: Seedlings of sample MCGH2 raised from seeds soaked in spore suspension of *F. oxysporum* (5X10^5 spores/ml for 16h) collected 8 &15 days post inoculation period.
Lane 7 and 8: Seedlings of sample CGN1 raised from seeds soaked in spore suspension *F. solani* (5X10^8 spores/ml) collected 8 &15 days post inoculation period.
Fig - 38. Accumulation of peroxidases in fruits collected after 30 days post pollination (DPP) from plants raised from seeds of sample Arkajeet soaked with spore suspension of potential vine decline pathogens.
Lane 1: Fruits collected after 30DPP plants raised from seeds soaked with spore suspension of *D. bryoniae* at a concentration of 1X10^6 spores/ml for 16h.
Lane 2: Fruits collected after 30DPP plants raised from seeds soaked with spore suspension of *B. theobromae* at a concentration of 1X10^6 spores/ml for 16h.
Lane 3: Fruits collected after 30DPP plants raised from seeds soaked with spore suspension of *F. oxysporum* at a concentration of 5X10^6 spores/ml for 16h.
Lane 4: Fruits collected after 30DPP plants raised from seeds soaked with spore suspension of *F. solani* at a concentration of 5X10^6 spores/ml for 16h.

Fig - 39. Accumulation of peroxidases in fruits collected after 40 days post pollination (DPP) from plants raised from seeds of sample Arkajeet soaked with spore suspension of potential vine decline pathogens.
Lane 1: Fruits collected after 40DPP plants raised from seeds soaked with spore suspension of *D. bryoniae* at a concentration of 1X10^6 spores/ml for 16h.
Lane 2: Fruits collected after 40DPP plants raised from seeds soaked with spore suspension of *B. theobromae* at a concentration of 1X10^6 spores/ml for 16h.
Lane 3: Fruits collected after 40DPP plants raised from seeds soaked with spore suspension of *F. oxysporum* at a concentration of 5X10^6 spores/ml for 16h.
Lane 4: Fruits collected after 40DPP plants raised from seeds soaked with spore suspension of *F. solani* at a concentration of 5X10^6 spores/ml for 16h.