Chapter VII

Summary and Conclusion

Summary

Establishment and identification of Vesicular Arbuscular Mycorrhizal fungi

Indigenous VAM found in Vallabhb Vidyapith was established in tomato from nut grass roots via Common Mycorrhizal Networks, was identified and was grown in vitro. Presence of VAM was checked in Cyperus rotundus roots from six different localities of Vallabh Vidyapith. Out of six localities; five localities were VAM positive amongst which locality 1 (BRD school of Bioscience) showed maximum % colonization, plant root length and mycorrhizal root length. VAM isolated from selected locality (locality 1) was successfully established in Lycopersicon esculentum Mill via Common Mycorrhizal Networks using two different methods: static and continuous flow method. Continuous flow method proved to be more efficient in establishment of VAM in tomato roots. Starter culture of six weeks old tomato root colonized with VAM was developed in the laboratory. Presence of VAM in starter culture roots was confirmed by appearance of VAM specific structures vesicles and arbuscules (trypan blue staining, acid fuschin staining, fluorescence microscopy and SEM), formation of formazon in Succinate Dehydrogenase staining. Statistical comparison (t-test, P < 0.05) of growth parameters between colonized and uncolonized plants showed that there was significant difference between mean values of fresh weight, root length and shoot length (colonized plants having higher values); while mean values of dry weight, plant height and no. of leaves were insignificant at 95% confidence interval. Molecular identification performed using ITS sequence data; which was
found to be *Glomus geosporum* MBAL (GenBank entry: KJ830770). *Glomus geosporum* MBAL was successfully cultured *in vitro* on Potato Dextrose medium.

**Optimization and Characterization of Enzymes**

Peroxidase, polyphenol oxidase, catalase and chitinase enzymes from 2 weeks old tomato seedlings were partially purified via ammonium sulphate fractionation and were characterized for their physico-kinetic parameters. Peroxidase showed maximum activity in 70% ammonium fraction with 13.23% yield and with 1.42 fold purification. Out of three substrates - o-dianisidine, TMB, pyrogallol selected peroxidase showed maximum Km and Vmax with o-dianisidine. Optimum pH and stable pH range for peroxidase was 7.8 and 6.0 to 9.0 respectively. Optimum temperature and stable temperature range for peroxidase was 50°C and 25°C to 40°C respectively. Optimum molarity in potassium phosphate buffer (pH, 6.0) was found to be 0.1 M. Polyphenol oxidase (substrate: catechol) showed maximum activity in 50% ammonium fraction with 22.55% yield and 2.61 fold purification. Optimum pH and stable pH range for polyphenol oxidase was 7.0 and 8.0 to 10 respectively. Optimum temperature and stable temperature range for polyphenol oxidase was 40°C and 10°C to 30°C respectively. Optimum molarity in sodium phosphate buffer (pH, 6.5) was found to be 1 M. Catalase (substrate: hydrogen peroxide) showed maximum activity in 60% ammonium fraction with 63.308% yield and 2.11 fold purification. Optimum pH and stable pH range for catalase was 7.0 and 7.0 to 9.0 respectively. Optimum temperature and stable temperature range for catalase was 70°C and 25°C to 40°C respectively. Optimum molarity in potassium phosphate buffer (pH, 7.0) was found to be 0.2 M. Chitinase (substrate: colloidal chitin) showed maximum activity in 75% ammonium fraction with 54.2% yield and 1.1 fold purification. Optimum pH and stable pH range for chitinase was 5.0 and 3.0 to
6.0 respectively. Optimum temperature and stable temperature range for peroxidase was 20°C and 10°C to 30°C respectively. Optimum molarity in sodium citrate buffer (pH, 5.0) was found to be 0.1 M. Optimum incubation time for maximum chitinase was found to be 3 hours. Effect of eight metals ions and reducing agents was checked on chitinase production; out which HgCl₂ was found to be inhibitory; while in presence of β- mercaptoethanol its activity was enhanced. Out of four substrates tested chitinase showed activity only with colloidal chitin.

**Defense enhancement**

Present chapter focused on priming effect of mycorrhization on tomato plants considering production and expression of Peroxidase, polyphenol oxidase, catalase and chitinase. *Fusarium oxysporum f. sp. lycopersici* was chosen as a pathogen for the study on the basis of production and expression of peroxidase enzyme when it was inoculated with tomato plants. Direct inoculation technique was selected out of three techniques tested on the basis of disease index values. *Glomus geosporum* MBAL colonized plants with and without pathogen showed higher production of total protein, total phenol, peroxidase, polyphenol oxidase, catalase and chitinase than uncolonized plants. These results were further supported by statistical analysis (paired t-test and one way ANOVA). Isoenzyme analysis showed that presence mycorrhiza might induce differential isoenzyme pattern in peroxidase, catalase and chitinase. Out of all four enzymes, peroxidase showed highest production while; chitinase showed more prolonged production. In colonized plants with or without pathogen, catalase was the first enzyme to be expressed followed by peroxidase and polyphenol oxidase and then chitinase. In presence of pathogen enzyme expression is more rapid than plants without pathogen.

**Glomus geosporum MBAL and tomato fruit quality**
The beneficial effect of *Glomus geosporum* MBAL colonization on tomato fruit quality was checked. Statistical comparison (t-test, 95% confidence interval) was made of various parameters between tomato fruits grown on colonized and uncolonized plants. Parameters like fruit yield, number of fruits/truss, total phenol, total protein, total lycopene content, total soluble solids, titrable acidity, vitamin C content, antioxidant activity were significantly higher in fruits grown on colonized plants; while days taken for fruit to ripe and fruit shape index didn't show any significant difference.

**Glomus geosporum MBAL mediated remediation**

This chapter deals with mycorrhiza mediated resistance in presence of soil contaminated with heavy metals (Amlakhadi, Ankleshwar). Soil analysis of soil contaminated at 0 day and 100 days divulged that there was considerable decrease in organic carbon, phosphorous content and electric conductivity of soil containing colonized plants as compared to that of uncolonized plants. These results are substantiated by statistical comparison (t-test). Colonized plants responded with better growth (fresh weight and root length) in contaminated soil than uncolonized plants. There was sizeable increase in total protein, total phenol, total chlorophyll and soil glomalin (protein produced by VAM) content in colonized plants after 100 days. Defense related enzymes like peroxidase, polyphenol oxidase, catalase and chitinase were significantly higher in colonized plants than uncolonized plants after 100 days. Soil analysis of Amlakhadi soil showed that all the metals except Ni were above WHO permissible limit. ICP-OES analysis of both colonized and uncolonized showed significant reduction in heavy metal concentration; but VAM colonized soil had less heavy metals than uncolonized plants. Lead was beyond detection limit after 100 days in soil; while Nickel was beyond detection limit in only
colonized plants. Plants after 100 days did not show any significant difference in heavy metals concentration.
Conclusion

Soil microbial community influences important ecosystem services such as plant productivity, carbon storage, nutrient retention and cycling, and water pollution among others. Therefore, soil microbial community directly and/or indirectly has important consequences on food security. Arbuscular mycorrhizal (AM) fungi are soil fungi that develop symbiotic associations with most plant species. These fungi colonize the plant root and the soil around the root and can provide water to the host plant. In addition, AM fungi can uptake nutrients from the soil solution, transport them, and transfer to the plant. Thus, AM fungi help the plant to attenuate water stress effects and enhance plant growth and yield. Advantages of VAM can be exploited for better growth and yield in agricultural countries like India. Following conclusions were drawn from the study:

- Statistical analysis of results of VAM colonization revealed that it has beneficial effects on tomato growth.
- An indigenous VAM present in Vallabh Vidyanagar, Gujarat was identified and a feasible, zero expense and rapid technique to transfer VAM to tomato plants was developed.
- Knowing of dominant morphotype in this area and its beneficial effect on one of the most important plant tomato is a concrete step towards mycorrhizal research in this locality. This knowhow will lead to development of technique to commercially produce VAM starter cultures to farmers and horticulturists for betterment in quality and quantity of tomato plants.
• VAM has a priming effect on tomato plants against fungal pathogen. Analysis of production, expression and sequence of defense related enzymes supported the idea.
• It was also concluded that beneficial effects of VAM continued till fruit stage. VAM colonization also aids in phytoremediation via phytostabilization.