CHAPTER - V
ENZYMATIC STUDIES ON VA MYCORRHIZAL
SEEDLINGS OF Exbucklandia populnea
INTRODUCTION

Vesicular-arbuscular mycorrhiza enhances growth and improves phosphorus nutrition of plants (Mosse, 1973a). The improved phosphorus nutrition results from an increased efficiency in phosphorus uptake from the soil (Sanders and Tinker, 1971). It has been demonstrated that the external hyphae of VA mycorrhizal fungi translocate phosphate from the soil to the host root which involves active processes (Pearson and Tinker, 1975; Cooper and Tinker, 1978). Transport of phosphate takes place most probably in the form of polyphosphate granules present in vacuoles by cytoplasmic streaming (Cox et al., 1975; Cox et al., 1980).

In VA mycorrhizal system, little is known of the physiological processes involved in mycorrhizal infection and the mechanisms involved in the fungal transport of phosphorus (Pearson and Tinker, 1975) and its subsequent transfer from the fungal hypha into the host cell (Cox and Tinker, 1976). Alkaline phosphatase enzyme specific to VA mycorrhiza has been reported in onion and tobacco (Gianinazzi-Pearson and Gianinazzi, 1976), which could play a role in the assimilation of phosphorus by mycorrhizal roots (Gianinazzi-Pearson and Gianinazzi, 1978). Ultra-structural studies have indicated that the vacuoles which contain polyphosphate granules are characterised by intense alkaline phosphatase activity (Gianinazzi et al., 1979). Alkaline phosphatase activity may be linked to the phosphate transport
mechanism of VA fungi (Gianinazzi-Pearson and Gianinazzi, 1978). Surface activity of roots may also contribute to the process of phosphorus absorption by the roots (Bieleski, 1973).

In the present investigation, the changes in the activity of phosphatase enzyme were studied in the mycorrhizal and non-mycorrhizal roots of *Exbucklandia* seedlings associated with the formation of VA mycorrhiza which could explain the mechanism of phosphorus uptake by mycorrhizal plants.

**MATERIALS AND METHODS**

The experiment consisted of mycorrhizal and non-mycorrhizal seedlings of *Exbucklandia populnea* grown and supplied with four different levels of single-superphosphate. The activity of phosphatase in the roots of seedlings was assayed. The details of methods are as follows.

**Preparation of pots**: Plastic pots of 2L capacity were used for growing seedlings of *Exbucklandia populnea*. Local garden soil was autoclaved twice and 2kg of the soil was taken to each pot. The soil was treated with four different levels of single superphosphate (SSP) viz: 0, 0.1, 0.5 and 5.0 g per pot. For each level of SSP five replicate pots were maintained. The fertilizer (SSP) was thoroughly hand mixed with the pot soil.

**Inoculation of VA endophyte**: The inoculum consisted of
soil infested with VA endophyte *Glomus* spp. which was pot cultured and maintained on maize plant. For each level of SSP two sets of mycorrhizal treatments were given. One designated as mycorrhizal and the other as non-mycorrhizal. Mycorrhizal set received the inoculum of VA endophyte (*Glomus* spp), whereas, same amount of autoclaved inoculum along with a filtrate of inoculum washing was added to the non-mycorrhizal set. The methods of inoculation were the same as described in Chapter II.

Seedling preparation and plantation: The seedlings of *Exbucklandia populnea* were raised from the seeds in petri plates. The method of seedling preparation and their plantation in the pots were similar to those described in Chapter II.

Growth conditions and harvesting: The experiment was set up in the month of August, 1985 and was carried out in a net house situated at the University campus under natural climatic conditions. The pots were watered weekly with tap water. The seedlings were grown for a period of four months after which they were harvested. The complete seedlings with intact root system were carefully excavated and brought to the laboratory for the assay of phosphatase activity, measurement of growth characteristics and assessment of VA mycorrhizal infection in the roots.

Preparation of cell free extracts: The fresh roots were
detached from the seedlings and washed under running tap water and finally with the distilled water. The roots were chopped into pieces and chilled in refrigerator. 2g of the chilled roots were macerated in a pestle and mortar at 4°C using chilled 0.1M borate buffer at pH 8.8. The macerate was centrifuged at 3000 rpm for 20 minutes. The supernatent was collected and the activities of soluble acid phosphatase and alkaline phosphatase were determined in the supernatent.

Quantitative assay for soluble acid phosphatase activity:
The substrate used was p-nitrophenyl phosphate. The substrate solution was prepared by dissolving 50mg of p-nitrophenyl phosphate in 10ml distilled water. 0.5ml of the substrate solution along with 0.5ml of 0.1M acetate buffer pH 4.0 was taken in test tubes and to it was added 0.1ml of enzyme extract and the mixture was inoculated in water bath at 35°C for a period of 30 minutes. The reaction was terminated by adding 3.4ml of 0.1 N NaOH and the OD of the mixture was recorded at 410nm with spectrophotometer.

Quantitative assay for alkaline phosphatase activity: 0.5ml of substrate solution prepared above was taken in test tubes along with 0.5 ml of tris-citric acid buffer pH 8.5. To the mixture was added 0.1ml of enzyme extract and inoculated at 35°C for 30 minutes. The enzyme reaction was stopped by adding 3.4ml of 0.1N NaOH and OD of the mixture solution recorded at 410nm.
The O.D. recorded for both the enzymes was converted to UM of p-nitrophenol by referring to standard p-nitrophenol curve. Standard p-nitrophenol curve was prepared by taking known concentrations of p-nitrophenol solution and adding 3.4ml 0.1N NaOH solution and reading OD at 410nm. The enzyme activity was expressed as UM PNP released/min./g fresh weight of roots.

Growth measurement and assessment of mycorrhizal infection:

The fresh weight of shoot of the harvested seedlings was recorded. The roots were fixed in FAA. 25 root segments (1cm) were randomly selected from the sample, cleared and stained by the method of Phillips and Hayman (1970). The percent VA infection was assessed by the microscopic method of Allen et al. (1982).

RESULTS

Plant growth and mycorrhizal infection: Average fresh weight is presented in Table 5.1. It was observed that shoot fresh weight of mycorrhizal seedlings was significantly (p < .05) higher than those of non-mycorrhizal ones at 0.1 and 0.5g SSP/pot. The shoot fresh weight of both mycorrhizal and non-mycorrhizal seedlings increased with the increase in the level of single superphosphate (SSP), however, at 5.0g SSP/pot level there was little difference between the two. The level of VA mycorrhizal infection was maximum (60%) at
Table 5.1 Fresh weight of mycorrhizal and non-mycorrhizal *Exbucklandia populnea* seedlings grown in a range of superphosphate level on per plant basis.

<table>
<thead>
<tr>
<th>Treatment (g.SSP/pot)</th>
<th>Fresh weight of shoot/plant ( \text{g} )</th>
<th>Mycorrhizal</th>
<th>Non-mycorrhizal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>( 0.203 \pm 0.038 )</td>
<td>0.203 \pm 0.038</td>
<td>0.139 \pm 0.033</td>
</tr>
<tr>
<td>0.2</td>
<td>( 0.324 \pm 0.025^* )</td>
<td>0.324 \pm 0.025</td>
<td>0.153 \pm 0.027</td>
</tr>
<tr>
<td>0.5</td>
<td>( 0.462 \pm 0.036^* )</td>
<td>0.462 \pm 0.036</td>
<td>0.175 \pm 0.023</td>
</tr>
<tr>
<td>5.0</td>
<td>( 0.403 \pm 0.044 )</td>
<td>0.403 \pm 0.044</td>
<td>0.390 \pm 0.049</td>
</tr>
</tbody>
</table>

\( ^* \): Values are mean of 10 replicates with standard error.

\( ^* \): Significantly different \( (p < 0.05) \) from non-mycorrhizal.
Table 5.2  Infection level of mycorrhizal seedlings of *Exbucklandia populnea* grown for 3 months in autoclaved soil in a range of single superphosphate levels.

<table>
<thead>
<tr>
<th>Treatment (g.SSP/pot)</th>
<th>Number of infected root segments (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>0.2</td>
<td>60</td>
</tr>
<tr>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>5.0</td>
<td>0</td>
</tr>
</tbody>
</table>
Og and 0.1g SSP/pot and it decreased greatly at 0.5g SSP/pot and at higher level of 5.0g SSP/pot VA-mycorrhizal infection was absent (Table 5.2).

Soluble acid phosphatase activity: The observation of soluble acid phosphatase activity in the roots of *Exbucklandia populnea* seedlings is presented in Table 5.3. It was observed that the activity of acid phosphatase in the roots of mycorrhizal and non-mycorrhizal seedlings did not differ significantly. However, in mycorrhizal plants it was slightly higher in comparison to non-mycorrhizal ones at 0, 0.1 and 0.5g SSP/pot. Soluble acid phosphatase activity decreased slightly with the increase in the concentration of single superphosphate (SSP).

Soluble alkaline phosphatase activity: The activity of soluble alkaline phosphatase was low (Table 5.3). There was no significant difference in the activity of alkaline phosphatase between mycorrhizal and non-mycorrhizal plants. However, with the increasing concentration of single superphosphate the activity of soluble alkaline phosphatase in both mycorrhizal and non-mycorrhizal seedlings greatly decreased in comparison to the activity at 0g. SSP/pot.

**DISCUSSION**

In higher plants activity of acid phosphatase normally varied between 0.3 to 1.5 e.u/g fresh weight of
Table 5.3 Soluble acid and alkaline phosphatase enzymes activities (JIM PNP released/min/g. fresh weight of roots of 3 months old seedlings of *E*bucklandia populnea.

<table>
<thead>
<tr>
<th>Treatment (g.SSP/pot)</th>
<th>Soluble acid phosphatase enzyme activity</th>
<th>Soluble alkaline phosphatase enzyme activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+M</td>
<td>-M</td>
</tr>
<tr>
<td>0.0</td>
<td>0.209±.021</td>
<td>0.282±.037</td>
</tr>
<tr>
<td>0.2</td>
<td>0.337±.043</td>
<td>0.278±.025</td>
</tr>
<tr>
<td>0.5</td>
<td>0.306±.048</td>
<td>0.232±.031</td>
</tr>
<tr>
<td>5.0</td>
<td>0.198±.033</td>
<td>0.202±.038</td>
</tr>
</tbody>
</table>

*: Significantly different from that of 0g. SSP/pot at 5% level.
tissues and deficiency of phosphorus may cause an increase in the activity of acid phosphatase (Bieleski, 1973). The results of the present experiment show that the activity of acid phosphatase in the roots of seedlings of *Exbucklandia populnea* was almost close to the lowest range of acid phosphatase reported by Bieleski (1973) in higher plants. It was observed that inoculation of VAM and or addition of superphosphate to the soil did not affect the activity of acid phosphatase as no significant differences could be observed in the activity of this enzyme with respect to mycorrhizal treatment or phosphate treatment of soil. However, Woolhouse (1969) reported a significant stimulation of acid phosphatase activity in the roots of *Agrostis tenuis* grown in solution culture under phosphorus deficient conditions. The differences in the results of Woolhouse (1969) and that of present investigation could be due to the differences in the species of plants and also due to the conditions of plant culture.

The results of quantitative assay of alkaline phosphatase activity showed that there was no significant difference between soluble alkaline phosphatase activity of mycorrhizal and non-mycorrhizal plants supplied with no phosphorus in the soil. However, activity of alkaline phosphatase significantly decreased in the presence of 5.0g SSP/pot. Gianinazzi-Pearson and Gianinazzi (1978) had also reported large differences in the soluble alkaline phosphatase activity of roots between the non-mycorrhizal plants.
which were supplied with high level of exogenous soluble phosphate and the mycorrhizal plants growing under the phosphorus deficient conditions of the soil. The non-mycorrhizal plants got phosphorus directly from the soil whereas, in phosphorus deficient conditions phosphorus was made available to the plants by mycorrhiza. The results, therefore, support the hypothesis that metabolism of the phosphorus between the mycorrhizal and non-mycorrhizal plants may be different (Gianinazzi-Pearson and Gianinazzi, 1978).

Activity of phosphatase enzymes in the root could also be involved in the establishment of VA mycorrhizal infection (Woolhouse, 1975). Mycorrhiza specific phosphatase enzyme activity specific to mycorrhizal infection in the roots of onion have been demonstrated by Gianinazzi, Gianinazzi-Pearson and Dexheimer (1979) in ultrastructural studies.
Plate 5.1

Effect of different levels of single super phosphate on the growth of seedlings of *Exbucklandia populnea.*
PLATE-5.1

NON-INOCULATED

INOCULATED 0 0.2 0.5 5.0

S-G-P (g. Pot⁻¹)