Cell-bound phosphatase activity in cotton (gossypium hirsutum L.) roots

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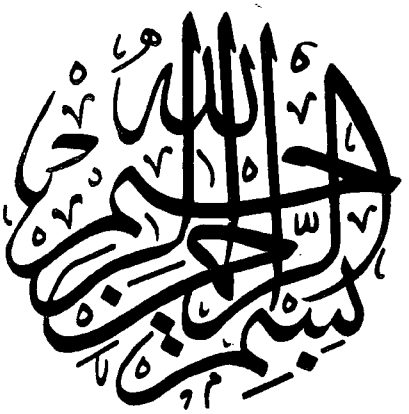

Cell-bound phosphatase activity in cotton (*Gossypium hirsutum* L.) roots

by

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A thesis submitted for the degree of Doctor of Philosophy

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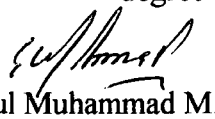
This thesis is entirely my own work. It has not previously been submitted for any other degree or diploma.



Gul Muhammad M. Baloch

DEDICATED TO MY PARENTS
MY WIFE, BROTHERS AND SISTERS

ABSTRACT

A study was made of phosphorus nutrition in cotton cultivars derived from Pakistan with particular reference to phosphatase activity. Cultivars used were Qalandri, Rehmani, Reshami and TH-3/83; Qalandri was used for most of the phosphatase characteristic studies, since this cultivar exhibited the highest germination percentage and seedling establishment.

Investigations of soil parameters from cotton growing region of Pakistan were also carried out. Soil pH was above 8.0 in all sites studied. Soil organic component levels were very variable between sites and within sites at different depths. Soil potassium was found to be at levels regarded as being sufficient for sustaining plant growth at all sites. However, levels of combined nitrogen and inorganic phosphate were in the range of values which could be regarded as being limiting to plant growth. The importance of phosphatase in the mobilization of organic phosphorus was therefore apparent.

Phosphomonoesterase (PMEase) activity, assayed using two substrates p-nitrophenyl phosphate (pNPP) and 4-methylumbelliferyl phosphate (4-MUP), was investigated and was found associated with roots of hydroponically-grown seedlings. Staining studies indicated that the enzymes were present in the cell wall. Phosphodiesterase activity was also detected in roots, using bis-pNPP as the substrate.

PMEase activity was found to have its greatest activity at pH values between 4.0 and 6.0 for all cultivars. In *cv* Qalandri the optimum pH for PMEase activity was 5.5 and the optimum temperature was 55°C when assays were incubated over a 1h period using pNPP or 4-MUP as the substrate. Levels of activity were higher using pNPP than with 4-MUP, but an absolute direct comparison could not be made since the concentrations of the individual substrates used were different.

Plants grown in phosphate-limitation conditions showed enhanced PMEase activity. Enhancement of PMEase activity was associated with the levels of phosphate in the plant growth nutrient solution rather than with those in the plant itself. This activity was highest in 19-day old seedlings, under the conditions of growth employed here.

A comparison between cultivars showed some differences in the level of PMEase activity and in the specific pH optimum for enzyme activity.

Limitation of combined nitrogen to seedling also resulted in enhanced PMEase even when plants were under phosphate-sufficient conditions. A similar effect of potassium-limitation was also found and this was associated with reduced uptake of phosphate into the seedlings. No effect of magnesium-limitation on PMEase activity was found.

A comparison was also made with other plants; cotton, *Primula farinosa*, rice, wheat and algae. All had associated phosphatase activity, with cotton showing significantly higher root acid phosphatase activity than the other higher plant species. Acidic phosphatase activity was found in the alga *Draparnaldia* whilst *Stigeoclonium* had alkaline phosphatase.

ABBREVIATIONS

AMP	adenosine monophosphate
AMeP	2-amino-2-methyl-1-propanol
bis-pNPP	bis-(p-nitrophenyl) phosphate
°C	degrees Celsius
cAMP	cyclic adenosine monophosphate
CAPS	3-(cyclohexylamino)-1-propanesulphonic acid
DMG	3, 3-dimethylglutaric acid
d. wt	dry weight
EDTA	ethylenediaminetetra-acetic acid (disodium salt)
f. wt	fresh weight
g	gramme
h	hour
ha	hectare
HEPES	N-2-hydrxymethyl piperazine-N'-2-ethanesulphonic acid
kg	kilogramme
Km	Michaelis-Menten constant
l	litre
M	molar
m	metre
min	minute
mg	milligramme
ml	millilitre
mM	millimolar
mm	millimetre
NNED	N-1-naphthylethylenediamine dihydrochloride
nm	nanometre
PAR	Photosynthetically Active Radiation
Pi	inorganic phosphorus
PDEase	phosphodiesterase
PMEase	phosphomonoesterase
pNP	p-nitrophenol
pNPP	p-nitrophenyl phosphate
ppm	parts per million
s	second
S.D.	standard deviation
µg	microgramme
µl	microlitre
µM	micromolar
µmol	micromol
4-MUP	4-methylumbelliferyl phosphate
4-MU	4-methylumbellferone
Vmax	maximum rate
v/v	volume per volume

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CHAPTER 1
INTRODUCTION

1.1 General introduction

Cotton (*Gossypium* sp.) is an important fibre crop. Prior to the introduction of man-made artificial fibres, it was the only plant raw material from which human clothing and other necessary textiles were manufactured. Cotton is generally a nutrient-demanding crop and therefore the investment in time, resources and fertilizer to maintain it is high. Phosphate supply is difficult and expensive to maintain with a reliance on importation which accounts for approximately 70 % of the total expenditure on agriculture of Pakistan (Salem *et al.*, 1986). Therefore, it is important to examine the ability of crops to produce high yield with minimum fertilizer input.

This project was designed to look at phosphatase activity in cotton. In order to do so, however, it is necessary to consider other plants eg. algae which are well-characterised for their production of phosphatase in relation to phosphorus content.

1.2 Origin of cotton

One of the most important areas for cotton production in Pakistan is Sindh province. The Indus valley has been well known for its cotton fibre production for hundreds of years. Santhanam and Hutchinson (1974) stated that *herbaceum* cotton was domesticated from the Arabian and Balochistan race *acerifolium*, and that the *Gossypium arboreum* was first brought into cultivation in Gujrat or Sindh. Fryxell (1979) stated that the development of cotton textile appears to have taken place not in Africa but in the Indus Valley in Sindh. Excavations of Moin-jo-Daro have shown that before 2700 BC cotton was grown in the Indus valley, Sindh (Pakistan) (Gulati & Turner, 1928). The first

documented record of cotton production was during 1500 BC in the Hindu Rigveda. Hutchinson *et al.* (1947) stated that it has only been selected by man as a source of raw material for textiles. They concluded that the origin, as well as the distribution of cultivated cotton, is intimately connected with the utilisation of its lint by man. The old world cotton probably originated somewhere in the southern half of Africa and later spread eastwards (Hutchinson, 1954).

1.21 Physiology of cotton

Cotton is a plant located in the hotter regions of the earth. Like other green plants, it needs high temperature, water, light and nutrients for efficient growth. The cotton plant is more complex structurally than any other major field crop. Its yield is limited drastically by the complex of insect pests and also it is highly responsive to environmental factors in its growth, development and production. Cotton produces vegetative and reproductive growth simultaneously over a relatively long period of time (130-180 days). The plant has a vertical stem with two kinds of branches, called monopodial and sympodial branches, in addition to a tap root system with lateral roots. The simple leaves have short stalks and its seed is mostly pear-shaped and varies in size according to variety and cultural conditions.

Thorp, (1960) considered that the optimum soil temperature for germination is 34°C. It has been observed that large seeds give a higher germination percentage as compared to the small seeds (Balls, 1910). However, Rogers (1943) found that the light, medium and heavy seeds did not show consistent differences in terms of the crop yield. The flowering and fruiting behaviour of upland cotton (*Gossypium hirsutum* L.) is as a day-length neutral plant. However, there are short-day cotton plants which develop only vegetative branches during long days. Cotton plants are very sensitive to growing temperatures: the minimum and maximum temperature has a great effect on plant

behaviour. Extreme temperatures also affect growth, with younger plants being more susceptible, but more able to recover quickly. Humidity and age of the plant tissue are important factors determining the cold injury response (McMichael & Powell, 1971).

In terms of cotton plant productivity a considerable proportion of assimilates are utilized in the formation of the fruit and the seeds Gerik *et al.* (1994) found that the accumulation d.wt for the root, stem , leaves, and bolls was 0.4, 12.2, 11.6 and 24.6 g plant^{-1} respectively. Bassett *et al.* (1970) working with cotton plants reported that the distribution of dry matter in cotton plants was stem, 23.1%; leaves 17.4%; burs 16.3%; seed 25.3% and lint 17.9%. However, soil type can have a marked effect on the specific distribution percentages. Mullins and Burmerster (1990) stated that total dry matter productions of 35% shoots, 18.5% leaves, 13.5% burs, 19% seed and 14% lint, was found for plants in Decatur soil, copared with an average of 16% shoot, 14%, leaves, 18% burs, 30% seed and 22% lint for those from Norfolk soil.

1.22 Cotton roots

The cotton plant has primarily a tap root system with many laterals called secondary roots. A study of the root system is very important, because roots not only anchor the plant firmly in the soil, but also absorb the nutrients from the soil for the development of the plant. The tap root grows rapidly downwards at 2.5 cm d^{-1} for several weeks after planting (Bassett *et al.*, 1970). The secondary roots grow close to the surface of the soil and completely fill the soil area around the plant with a network of lateral roots. These lateral roots run more or less horizontally, and constitute the bulk of the feeding roots and are concentrated in the moist soil in order to absorb water or nutrients. The size of the cotton plant root depends on the soil environment, *viz.* physical texture, fertility, soil temperature. Soil moisture is a major factor affecting root growth

rates and distribution (Grimes & El-Zik, 1982). The depth of the root system depends upon soil moisture, aerobic status conditions, soil pH and nutrient supply and availability.

1.3 Phosphorus in the environment

1.31 Introduction

Phosphorus is essential to plant growth and the eleventh most abundant element in nature. The average concentration of phosphorus available in the environment is estimated as 0.1% by weight, and it is thus classed as a macro-element, but the concentration of phosphorus in the plant is usually lower than nitrogen, potassium and calcium. Phosphorus is a necessary nutrient and is defined as a rate-limiting element, even more important to plants than potassium or calcium. It mostly occurs in the oxidised form, either as phosphates or organic phosphorus (P) compounds which can be divided into following groups:

1. Orthophosphates
2. Polyphosphates (chain phosphates)
3. Metaphosphates (ring phosphates)
4. Ultraphosphates (branched ring phosphates)

Orthophosphates are generated from the weathering of rocks or from biological metabolism or degradation. Polyphosphates and metaphosphates are produced by biological activity. Orthophosphates and polyphosphates are frequently introduced into water by man (Broberg & Persson, 1988). Rigler's (1973) classification of analytical phosphorus fractions is given below.

Tot P = Total phosphorus

PP = Particulate phosphorus

SP = Soluble phosphorus

SRP = Soluble reactive phosphorus

SUP = Soluble unreactive phosphorus

Particulate P (colloidal P) is derived from five sources:

1. Cells of plants bacteria and animals;
2. Weathering products such as primary or secondary minerals;
3. Direct precipitation of inorganic P or adsorption into other precipitates;
4. Degradation and fragmentation of cells providing organic detritus;
5. Flocculation of organic macromolecules, resulting in larger sized aggregates;

Particulate organic P in aquatic ecosystems dominates total organic P, and its significance has been comprehensively reviewed by Broberg and Persson, (1988). It is not readily available to plants.

1.32 Phosphorus available in solution

The phosphorus most commonly absorbed by plants is largely as the primary and secondary orthophosphate ions ($H_2PO_4^-$ and HPO_4^{2-}), which are present in the soil solution. The amount of each depends largely on the soil solution pH. Many organisms have two uptake systems for phosphate, one being ``diffusive" and the other`` rapid", the latter occurs when the internal phosphorus concentration is low and is presumably growth-limiting (Ducet *et al.*, 1977). Both these uptake systems need energy, but Whitton, (1967) observed colonies of *Nostoc* which appeared to take up phosphorus passively when the external concentration was very low. Many different substrates can be utilised as phosphorus sources by algae and cyanobacteria (blue-green algae) which are capable of phosphatase activity (Livingstone *et al.*, 1983; Al-Mousawi, 1984; Mahasneh *et al.*, 1990; Whitton *et al.*, 1990; Islam & Whitton, 1992). Broberg (1985) showed that the availability of different phosphorus compounds to algae is dependent on the algal enzyme pool, the phosphorus status of the algae, the orthophosphate uptake rate, the nature of the phosphorus compounds and environmental conditions.

1.33 Kinds of phosphorus in the soil

Phosphorus in soils may be divided into two major categories, organic and inorganic. The amounts of phosphorus in these categories varies widely. The extreme values reported in the surface samples of soils (Ghani & Aleem, 1943) range from 3% organic and 97% inorganic on the one hand, to 75% organic and 25% inorganic on the other hand. The content of organic phosphorus in soils increases as the content of nitrogen increases and as the pH decreases. Manchanda *et al.* (1982) observed that fertilization with phosphate under saline conditions increased salt tolerance of barley and wheat plants.

1.331 Inorganic phosphorus

Most inorganic phosphorus occurs characteristically in the clay fraction, from which it cannot be separated by physical methods. Inorganic phosphorus (Pi) may be classified according to its physical, mineralogical or chemical nature, or a combination of these. Only a fractional percentage of the total phosphorus in the environment is concentrated in deposits consisting of mainly phosphate minerals (Brink, 1978). Phosphate deposits may be classed into three broad apatite groups [Ca_5 (PO_4, CO_3)$_3$ (F, Cl, OH)], apatite deposits of igneous and metamorphic origin, sedimentary phosphorites and guano-related deposits. The inorganic phosphorus in soils is present principally as calcium, iron, and aluminium complex deposits. Most phosphorus, possibly as much as 95%, occurs as fluoroapatite [$Ca_{10}Fe_2(PO_4)6$] which is a common, stable, accessory mineral in most rock types. Fluoroapatite is normally present in concentrations of less than 1% (McKelvey, 1973). The direct evidence regarding the nature of inorganic compounds of phosphorus in soil has been obtained by microscopic examination of individual grains from the sand and silt fractions. Apatite accounts for 95% or more of the phosphorus in igneous rock. The ferrous phosphate, vivianite [Fe_3(PO)4 $8H_2O$] has

been found on occasion under conditions of poor drainage. Various indirect means have been employed in investigating the nature of inorganic phosphorus in soils. Phosphorus is eventually liberated from its so-called resistant minerals and through uptake by plants enters the biosphere (Jansson *et al.*, 1988). Work on the mineralogical aspects of this subject was reviewed by Mason and Berggren (1941) who cited work on P-bearing zircon [$ZrSiO_4$] in which PO_4 groups substituted for as much as 25% of the normal complement SiO_4 groups. Such phosphate groups are probably incorporated into the mineral during crystallisation and occur scattered throughout the structure.

1.332 Organic phosphorus

In the soil 15-85% of the total phosphorus is organic. With increasing soil depth the organic phosphorus component declines. At the same time, the proportion of total phosphorus that is in the organic form is greater at the surface than in subsurface horizons. Organic phosphorus, which cannot be utilised directly by plants, is reliant on the role of micro-organisms for its conversion to inorganic forms which can then be absorbed. By their action, bacteria, fungi, and actinomycetes make the bound element in the remains of the vegetation and in soil organic matter available to succeeding generations of plants. The organic phosphorus entering a soil is largely derived from plant litter, though animal remains and faeces may be important under heavy grazing conditions. The known groups of organic phosphorus compounds in this material are inositol phosphates, phospholipids, nucleic acids, nucleotides and sugar phosphates, but only inositol phosphate has been detected, in quantity, in soil and even this may be of secondary micro-biological origin. Nucleic acids are the major organic compounds which contain phosphorus in plants and micro-organisms; Adam *et al.* (1954) reported the presence of these substances in the soil. Cosgrove (1967) observed that organisms occur in the soil and rhizophere which are

capable of dephosphorylating all known organic phosphorus compounds of plant origin and that plant roots may also have similar phosphatase activity at their surfaces.

Dissolved organic phosphorus (DOP) can be regarded as the more important fraction, as it is readily available to the biota and is rapidly turned-over. Some pools of DOP do not undergo rapid hydrolysis by phosphatases, and these compounds may constitute a major part of the DOP pool. One such pool of DOP compounds are nucleotides or polynucleotides. Phillips (1964) reported six DOP fractions from sea water, three of which were identified as nucleotides or polynucleotides. Broberg and Persson (1988) stated that up to 4.2% of the total P in bogs could be attributed to nucleic acids. Minear (1972) noted that up to 50% of high molecular weight DOP excreted from organisms was DNA or its fragments. Hino (1989) reported that 65% of DOP in lake waters was composed of compounds between 300-1000 daltons molecular weight. Addition of phosphodiesterases alone did not show phosphorus release from high molecular weight DOP compounds, but a combination of phosphodiesterases and phosphomonoesterases increased the amount of Pi released by 30% when compared to the release of Pi by phosphomonoesterases alone.

1.4 Function of phosphorus in plants

The concentration of inorganic phosphorus in plants is, of course, influenced by the supply in the soil, and measurements of inorganic phosphorus in plant tissues are sometimes made to provide an index of the supply of phosphorus in the soil. Phosphorus plays a vital role in plants, it is absorbed from soil and remains in the oxidised state, and occurs in both organic and inorganic forms as the central element of the phosphate group. Inorganic phosphorus is usually determined in extracts obtained by treating plant tissue with an acid such as trichloracetic acid that removes the orthophosphate present in the solution or in a water-soluble form as well as the orthophosphate present in sparingly

soluble forms. Phosphorus is stored in seeds as phytin, the calcium or magnesium salt of inositol hexaphosphoric acid. This compound is hydrolysed enzymatically during germination, and the phosphate is thereby changed to the inorganic form, from which it is used to develop the seedling. Phosphorus has a major function in plant metabolism as a carrier of energy. The most important carrier of high energy phosphate is adenosine triphosphate. Phosphorus is also an important component in photosynthesis, the initial reaction of which is light energy trapping for the splitting of water, in the presence of inorganic phosphate, adenosine diphosphate and the coenzyme nicotinamide adenine dinucleotide phosphate, with the production of the reduced form of nicotinamide adenine dinucleotide phosphate, adenosine triphosphate, and molecular oxygen.

1.41 Phosphorus content of cotton

The phosphorus content of a plant not only varies with species, but changes with variety and locality. Plant analysis is used as a diagnostic tool and as a guide in planning crop production programmes. Plant analysis has been used to evaluate fertiliser practices and to investigate problems of poor growth, it has a promising role in the assessment of the adequacy of plant nutrient supply. It is therefore essential to understand the growth and mineral uptake patterns of plant.

Environmental conditions have a definite influence on the rate of nutrient absorption, hence different nutrient concentrations are found in plants with varying environmental regimes. Olson and Bledsoe (1942) reported, for non-irrigated cotton, that dry matter production rates within a given growing season was dependent on the location of the plant. The cotton plant when growing upon a fertile soil develops its reproductive tissue largely on the basis of a continued uptake of phosphorus with only minor exhaustion of the phosphorus of the leaves and stem (Fiske & Subbarow, 1925). Mullins and Burmester (1990) observed the phosphorus distribution in cotton plant averaged 11.7% in

shoots, 19.5% in leaves, 16.0% in burs and 52.8% in seed. Cotton seed has the highest concentration of P at maturity (Nelson, 1980). Bhatt and Appukuttan (1971) investigated nutrient uptake by bushy and short branch cotton varieties. The two varieties gave the same cotton yield, but the short branch variety took up less N P K. The response of plants to different external ion levels are, however, also due to the inherent growth rate potential at the seedling stage and to the reserve pool of the element in the seeds (Chapin & Bieleski, 1982). Schjorring and Pauljensen (1984) reported that the ratio between the phosphorus concentration in shoots and roots increased with the P status of the seedlings grown at low to intermediate external phosphorus levels, but decreased at higher phosphorus levels.

Russell and Martin (1953) showed that the transfer of phosphorus to plant shoots could be retarded either by a low initial phosphorus status in the plants or by low external concentration increments in either the phosphorus status of the plants or in the external concentration could increase upward movement.

Nayakekorala and Taylor (1990) reported that at all growth stages of cotton plants, total phosphorus uptake from the upper soil layer was greater than that from the lower soil layer. They further observed that phosphorus flux from either layer was greater during early growth stages than during later stages. A significant decrease in phosphorus flux occurred when plant reached the flowering stage.

Cotton requires higher culture-solution concentrations of phosphorus than other plants. The minimum inorganic phosphate concentration for optimum plant growth in nutrient solution was found to be between 3 and 13 μM (Asher & Edwards, 1983).

Interaction also occurs between phosphate and other ions within the soil. Award *et al.* (1990) stated that increasing the inorganic phosphate content of the growth medium from 1 to 10 μM increased the growth of tomato plants grown in 100 mM NaCl. Martinez and Lauchi (1991) studying the short term effects of salinity (150 mM NaCl) on phosphorus translocation in cotton plants raised in the nutrient solution, reported that

salinity inhibited phosphorus translocation from root to shoot. This inhibition was more evident at higher Pi concentrations in the root medium. Increasing Pi by 33-fold in the solution resulted in a 4.3-fold increase in phosphorus in the root under saline conditions, but only in a 1.8-fold increase in the shoot. In older shoot tissue total phosphorus was elevated in the salinized plants. Salinity decreased phosphorus recirculation from the cotyledons to the young leaf of cotton seedlings (Martinz & Lauchi, 1991). Salinity is a threat to oilseed crops in areas with poor quality underground water, as the only source of irrigation. Seedling emergence in saline conditions has been reviewed by Creda *et al.* (1977). Tolerance of salt at emergence and early growth stages are considered desirable traits in crop production programmes on problem soils (Norlyn & Epstein, 1984).

Marschner and Ismail (1986) reported that with Zn-deficiency the pH of the nutrient solution decreased from 6.0 to 5.0 and Zn-sufficient cotton plants raised the pH of the nutrient solution to about 7.0. Phosphorus uptake rate in Zn-deficient plants was increased by a factor of 2 to 3, whereas the uptake rates of potassium, calcium, and particularly NO_3 decreased (Christensen & Jackson, 1981). Longeragan *et al.* (1982) noted that the enhanced phosphorus uptake can induce phosphorus toxicity in older leaves when phosphorus supplies are relatively high. Christensen and Jackson (1981) stated that in Zn-deficient plants the uptake and translocation of phosphorus from roots to shoots is enhanced considerably.

1.5 Phosphatases

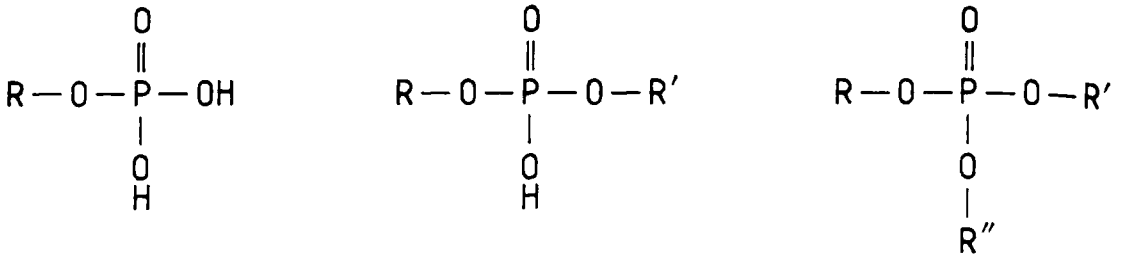
Phosphatases are enzymes which promote the degradation of a wide variety of complex phosphorus compounds into orthophosphate (Pi) and an organic moiety (Jansson *et al.*, 1988). Phosphatases have an essential role and function in the nutrient dynamics of the environment. The term phosphatase is often used for the enzyme which catalyses the

hydrolysis of both esters and hydrates of phosphoric acid and is used synonymously with phosphomonoesterases, abbreviated to PMEase (Flynn *et al.*, 1986). Similar, but functionally different, enzymes are the phosphodiesterases, abbreviated PDEase which hydrolyse a range of compounds including the nucleases.

1.51 Formula of phosphate esters

The general formula for different phosphate esters are given below,

Fig. 1.1



PHOSPHOMONOESTER PHOSPHODIESTER PHOSPHOTRIESTER

R denotes the organic part of the phosphate esters. Based on McComb *et al.* (1979) reported that phosphatases may be involved in metabolic processes other than hydrolysis, for example in transport of substances across membranes and synthesis of new organic phosphates. The most common catalytic breakdown is by phosphomonoesterase (PMEase). The reaction mechanism is divided into four steps (McComb *et al.*, 1979).

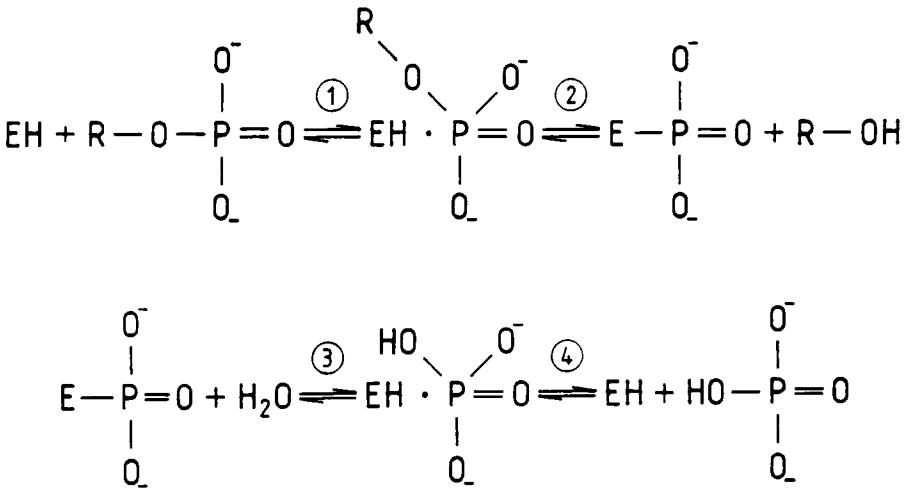
1. Non-covalent binding of the substrate to the enzyme.
2. Alcohol release from the complex and Pi becomes covalently bound to the enzyme forming a phosphoryl-enzyme compound.
3. Conversion of the phosphoryl enzyme compound through uptake of water, to a

non-covalent complex.

4. Release of Pi and regeneration of free enzyme.

Any of the steps 2-3 can be rate-limiting for all of the reaction (McComb *et al.*, 1979). Reaction schemes for the enzyme catalysis of phosphate esters as described by McComb *et al.* (1979).

Fig. 1.2 Reaction scheme for the enzyme described by McComb *et al.* (1979)



1.52 Sources of phosphatases

1.521 Introduction

Phosphatases have been observed in bacteria, algae, bryophytes and higher plants (Siuda, 1984; Press & Lee, 1983).

Acid phosphatases are enzymes of wide specificity which cleave phosphate ester bonds and, thus, play an important role in the mineralization of organic P in the environment, where their activity may be correlated with a low level of free inorganic ions (Spiers & McGill, 1979; Appiah & Thomas, 1982). Acid phosphatases generally have their optimum between pH 4.0-6.0.

1.522 Bacterial phosphatase activity

Bacteria are one of the most important contributors to environmental phosphatase activity. A large number of workers have concentrated on PMEase in hetrotrophic bacteria, with most research on PMEase structure and function in *E. coli* (McComb *et al.*, 1979). PMEase activity in freshwater hetrotrophic bacteria has been demonstrated in lake waters (Jones, 1972; Chrost *et al.*, 1984).

1.523 Algal and cyanobacterial phosphatase activity

Phosphatase activity has been observed in many species of cyanobacteria (blue-green algae) and algae, though not universally (Healey, 1982). Frequently, the phosphatase activity has been observed in cultured cyanobacteria and algae (Healey, 1973; Doonan & Jensen, 1980; Flynn *et al.*, 1986; Grainger *et al.*, 1989; Whitton *et al.*, 1990, 1991; Islam & Whitton, 1992). Phosphatase activity has been located on the cell surface and in cell membranes (Moller *et al.*, 1975; Flynn *et al.*, 1986). It has been reported that the extracellular enzyme is released into the algal culture (Wynne, 1981; Siuda, 1984; Grainger *et al.*, 1989; Whitton *et al.*, 1990, 1991; Islam & Whitton, 1992).

Algae have been used as assay organisms in a number of studies and, whatever species are used, the assay techniques are similar in theory. It has been reported that algal species could be used as indicators of clean or polluted water and lists of indicator

assemblages have been produced (Palmer, 1959). Whitton (1984) reported that chemical analyses of algal material could give an integrated picture of pollution and could also give a better indication of the chemical fraction likely to affect the ecosystem than chemical water analyses. The presence of *Stigeoclonium* has often been taken as indicative of organic pollution (De Vries and Kamphof, 1984). It has been also reported that *Stigeoclonium* occurs in many unpolluted and heavy metal polluted waters (McLean, 1974).

Ihsan *et al.* (1990) reported that a strain of *Calothrix viguieri* isolated originally from the surface of a mangrove root, showed marked morphological and physiological responses to the salinity of the medium when grown to phosphorus limitation in a fresh water medium, each trichome ended in a long hair and the culture showed marked cell-bound phosphomonoestrase and phosphodiestrase activities when grown with various phosphorus sources (inorganic & organic) to phosphorus limitation in medium including 5.6 g l^{-1} NaCl, hairs were absent, phosphomonoestrase activity was much lower and phosphodiestrase activity absent. With five of the seven organic phosphorus sources tested the yield was also lower in saline medium. Reduction of NaCl concentration led to development of hairs, whereas increase in NaCl led to the hairs being shed.

1.524 Phosphatase activity in higher plants

Enzymatic activity in root soil interfaces maintains soil fertility through complex bio-chemical processes. In the majority of the soils, organic phosphorus to the extent of 30-80 % of the total phosphorus has to be hydrolysed by phosphatase enzymes to convert it into available forms. Production and distribution of the enzyme by different crops in mineral and organic soils (Chhonkar & Tarafdar, 1984) and also in arid soils has been assessed (Tarafdar *et al.*, 1988). Increased microbial and root activity may have accounted for higher enzyme activity, including phosphatase (Tarafdar & Chhonnkar,

1978). Kumar and Tarafdar (1989) reported that acid and alkaline phosphatase activity in the rhizospheres of sunflower was many time higher on saline than on control soils.

It has been reported that most plants are rich in non-specific and specific acid phosphatases, but do not contain any appreciable alkaline phosphatase activities (McComb *et al.*, 1979; Lee, 1988). It has been observed that many plant tissues contain acid phosphatase activity, including plant roots. The activity is localised in apical meristems and outer surface cells (Esterman & McLaren, 1961; McLaren & Gahan, 1970; Shaykh & Roberts, 1974). Phosphatase activity appears in media in which cells of higher plants have been grown (Yamaoka *et al.*, 1969).

McLean and Gahan (1970) reported that the hydrolase activities present in the root cap may help in the breakdown of intracellular material, thereby loosening the cells. In wheat, comparison of root and leaf phosphatase activity in plants grown in nutrient solution showed that leaf phosphatase was not necessarily a better measure of phosphorus status of the growing plants than root phosphatase (McLachlan & DeMarco, 1982). Besford (1979a) observed acid phosphatase activity in seven plant species, including wheat in sand culture. Besford (1979b) further suggested that it may be possible to use this enzyme as an indicator of P-limitation in all the crops tested, except dwarf rice. It has been reported that acid phosphatase which was secreted by tomato root and suspension cultured cells, increased under P-limitation (Goldstein *et al.*, 1988). Plant root phosphatase may be involved in the mineralization of organic phosphorus present in the soil or in added organic material (Chang & Bundurski, 1964). The amount of organic phosphorus hydrolysed by clover root phosphatases surpassed the amount of phosphorus taken up by the plant by a factor of 20 (Tarafdar & Claassen, 1988).

1.525 Phosphatase activity of plants grown under conditions of phosphate limitation

When a plant is grown in a P-deficient substrate the intracellular and cell wall phosphatase activities increase as the tissue phosphorus concentration decreases (Bieleski, 1973). Surface phosphatase activity increases in phosphorus-deficient plants (Boutin *et al.*, 1981; McLachlan & DeMarco, 1982; Dracup *et al.*, 1984). Besford (1979) interpreted a strong relationship between the intracellular phosphatase activity and the phosphorus status of the tomato leaf and suggested that the enzyme activity could be used for an early detection of P-deficiency in tomato plants. P-deficiency causes a 4-12 fold increase in phosphatase activity in at least four lower organisms; *Escherichia coli* (Torriani, 1960), *Saccharomyces* (Suomalainan *et al.*, 1960), *Euglena gracilis* (Blum, 1965), and *Neurospora crassa* (Nye, 1967). Reid and Bieleski (1970) reported that P-deficiency caused a 10-50 fold increase in phosphatase activity and the appearance of a new isoenzyme in the higher plant *Spirodela oligorrhiza*. The phosphatase activity of cell walls from *Agrostis tenuis* was two-fold higher when plants were grown with 1.0 μM phosphate than when plants were grown with 100 μM phosphate (Wool house, 1969). Strother (1980) observed that the increased phosphatase activity in P-deficiency is possibly a response favouring phosphate homeostasis. Acid phosphatase activity of the intact roots of the young plants is influenced by the phosphorus status of the growth medium (Bieleski, 1973). Kummerova (1986) stated that starvation induced an increase in the activity of phosphatases on the root surface of *Zea mays* and that this enhancement was especially pronounced in new roots. McLachlan (1976) noted that increases in inorganic phosphorus in the resulting medium reduced the phosphatase activity of the plant roots. Most of the acid phosphatase activity was noted at the surface of wheat roots (Ridge & Rovira, 1971). Besford (1979b) observed that soluble phosphatase activities also increased with P-deficiency in the leaves of wheat. P-deficiency in higher plants has been shown to increase the activity of acid phosphatase in homogenised leaves (Besford,

1978a). Phosphatase activities were influenced by water deficit and leaf age as well as by P-deficiency (Barrett-Lennard *et al.*, 1982). Dracup *et al.* (1984) suggested that increased phosphatase activity was localised in P-deficient clover root cell walls.

1.526 Influence of pH on phosphatase activity

Jansson *et al.* (1988) stated that phosphatases typically have maximum hydrolysing capacity at different pH values. The enzymes can be catagorised as alkaline and acid phosphatase. It has been reported that the optimum pH measured for *Eriophorum vaginatum* at pH 3.5-4.0 is lower than that reported for other plants *e.g.* between 5.0 and 6.0 for wheat root surface phosphatases (McLachlan, 1980) and between 4.5 and 5.0 for *Aegilops* roots from soil of pH 7.4-7.6 (Silberbush *et al.*, 1981), and clover (Dracup *et al.*, 1984). The pH optimum for alkaline phosphatase activity has been shown to vary with the substrate concentration, the type of substrate (Walker & King, 1950), and the buffer employed (Zittle & Della, 1950). Since it has been shown that the pH optimum can be reduced from a non-physiological to physiological pH, it is logical to assume that there is optimum alkaline phosphatase activity *in vivo*. It may be that the pH optima of other enzymes change with variation in substrate concentration *e.g.* urease (Howell & Summer, 1934).

High pH values have been found necessary for optimum activity of alkaline phosphatase *in vivo*. The pH values in the living cell structures on the other hand are near neutral. McComb *et al.* (1979) reported that factors affecting phosphatase activity are temperature, ionic strength, pH and metal ions. Jansson *et al.* (1988) observed that external lake water phosphatases mostly have pH optima in the alkaline region.

Acid phosphatases generally seem to be active in the internal cell metabolism.

Ross *et al.* (1951) noted that as the concentrations of the substrates were lowered the pH optima for alkaline phosphatase activity were found to be nearer neutral pH values.

1.527 Effect of ions and phosphatase

Metal ions inhibit enzyme reactions by complexing the substrate, combining with the active groups of enzymes or by acting with the enzyme substrate complex. Inhibition also depends on the nature and size of the ions (Alverez, 1962). Juma and Tabatabai (1988) reported that acid phosphatase activity in higher plant roots of maize and soybean is inhibited by metal ions. The inhibition of the acid phosphatase in plants by metal ions revealed that Zn (II) and W (VI) have little effect on acid phosphatase activity in maize roots, but these ions inhibited activity in soybean roots by 25% and 62%, respectively (Juma & Tabatabai, 1988). Hasegawa *et al.* (1976) reported that Hg (II), Cu (II), Fe (III), Zn (II) and Co (II) inhibited acid phosphatase activity of wheat roots by 100, 82, 82, 46, and 31% respectively. Willett and Batey (1977) noted that in serpentine-tolerant strains of *Festuca rubra* the activity of these enzymes *in vivo* was affected differently by external Ca^{2+} concentration , but not by Mg^{2+} or Ni^{2+}, which are considered among the factors responsible for serpentine toxicity.

Johnston and Proctor (1984) observed that when root surface phosphatases of a serpentine and non-serpentine clones of *F. rubra* were assayed under a range of ionic concentrations, based on those in the soil solutions, they were found to be altered. Meikle Kilrannoch serpentine clones showed greatest activity in culture solutions which had high concentrations of magnesium and nickel, similar to that in the soil. The phosphatase from the non-serpentine clone was partially inhibited in the same culture solution.

1.6 Staining methods for phosphatase

Siuda (1984) observed that the enzyme activity was extracellular and cell-bound in some organisms. Moller *et al.* (1975) noted acid phosphatase activity in the cytoplasmic fraction of marine diatoms. Wynne (1977) suggested that acid phosphatase activity was located within cells of *Peridinium cinctum.* Jansson *et al.* (1981) found four different acid phosphatases on the basis of their molecular weights. Siuda (1984) has suggested that acid phosphatases are located within the cells (cellular) rather than in contact with the surrounding medium. The acid phosphatase synthesis generally is not inhibited by Pi and acid phosphatases are probably constitutive enzymes produced for internal P metabolism (Jansson *et al.*, 1988).

Histochemical studies of root apices have been concerned primarily with the distribution of enzymes in the tissues of different roots (Benes & Opatrna, 1964; McClurkin & McClurkin, 1967; Hall 1969; McLean & Gahan, 1970; Ashford & McCully, 1970; Benes, 1971; Hall & Davie, 1971 & Onofeghara, 1972). It has been found that phosphatase activity in some microorganisms increases as a result of phosphorus deficiency (Torriani, 1960; Price, 1962; Blume, 1965; Nye, 1967). This enhanced phosphatase activity appears to be located in the cell wall whereas most of the activity in cells adequate in phosphorus is located only at particular sites in the cytoplasm (Weimberg & Orton, 1964; Sommer & Blum, 1965). Reid (1968) stated that when sections were stained to locate phosphatase much of the activity in P-deficient tissue appeared to be localised in the epidermis. An unspecified amount of acid phosphatase activity is associated with epidermal cells of many plant roots (Shaykh & Roberts, 1974). DeJong (1965) found that the bulk of the phosphatase activity in onion roots was located in the intracellular spaces between the epidermis and the hypodermis.

Bieleski and Johnson (1972) reported that specific staining showed phosphatase in control plants of *Spirodela oligorrhiza* was located primarily in and around the vascular strands; in P-deficient plants it was located in the epidermis of the root and under surface of the frond. Localisation of PMEase activity was carried out using-5-bromo-4-chloro-3-indolyl phosphate (BCIP) as an organic phosphorus substrate (Coston & Holt, 1958; Holt & Withers, 1958). All plants showed high activity in the meristematic zone. This is associated with the root cap cells (Jensen, 1956; Gahan & Maple, 1966; Benes & Opatrna, 1964).

1.7 Nitrogen

1.71 Nitrogen in cotton plants.

Nitrogen is one of the essential macroelements which is of high importance for the growth of plants and crop production, and deficiency can occur in crops unless nitrogen is applied as a fertilizer.

In cotton Gerik *et al.*, (1994) reported that nitrogen content of the root, leaf, stem, and boll were 0.008, 0.023, 0.141, 0.213 g N plant^{-1}, respectively. Mullis and Burmester (1990) observed that the distribution of nitrogen as 15.9% stem, 27.6% leaves, 14% burs and 42.4% seed. Bassett *et al.* (1970) noted that crops of mature cotton plants contained 142 kg N, 19 kg P, and 127 kg K ha^{-1}, they further reported that about 70 kg N, 11 kg P, and 21 kg K ha^{-1} were contained in the seed.

1.711 Inorganic combination of nitrogen

In inorganic combined form, nitrogen occurs in soils as nitrous oxide (N_2O), nitric oxide (NO), nitrogen dioxide (NO_2), ammonia (NH_3), ammonium (NH^+_4), and nitrate (NO^-_3). The first four of these forms are gases and the last two are ionic forms found in

the soil solution. Nitrite and nitrate occur exclusively, or almost exclusively, as freely diffusible ions in the soil solution. Most of the ammonium occurs in exchangeable and nonexchangeable forms. Only a little nitrogen occurs in an ionic form in the soil solution. Ordinarily, the ammonium is exchangeable with soil-solution forms. The nitrite and nitrate collectively constitute less than 2% of the total nitrogen in soil, however, these forms are of great qualitative importance, because they are the forms used by plants.

1.712 Organic combination of nitrogen

The organic nitrogen added to soil in plant and animal residues is largely proteinaceous in nature. The microbial attack to which these materials are subjected in soils probably results in nearly complete disappearance of the original protein and its partial replacement by microbial protein, with the remainder of the nitrogen being changed to inorganic or elemental forms. Protein certainly exist in soil, but the only isolation of protein that appears to have been accomplished is the separation and crystalization of a mixture of subtances having urease activity (Briggs & Segal, 1963).

1.72 Function of nitrogen in plants

As well as its fundamental role as a component of protein, nitrogen and its metabolism within a plant are also related very closly with phosphate metabolism and it is likely that factors having an effect on one can alter the other.

1.8 Effect of salinity on higher plants

One of the major problems in the cotton growing regions of Pakistan is the increased levels of salts which can affect yield. The effects of salinity on cotton are similar

to those reported from studies on other species of higher plants. Javed *et al.* (1993) reported that the number of nodules per plant, nodule weight and nodule size of *Cicer arietinum* plants decreased with increasing salinity levels. Javed *et al.* (1993) compared growth, seed yield and nodulation of *Cicer arietinum* at 0, 0.1, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 % levels of salinity in sandy clay loam. Fresh weight of shoots and roots after 8, 12, and 15 weeks salinity treatment generally decreased with increasing salinity levels. Compared to vegetative growth, reproductive growth was more affected since pods were formed up to 0.4 % NaCl levels, whereas, seeds were formed at NaCl level of up to 0.1 % only. Saline soils are common in the regions of arid and semi-arid climate where transport of soluble salts to the ocean does not occur because of low rainfall (Eaglesham & Ayanaba, 1984). Gobar *et al.* (1975) observed that the saline decline in seed cotton yield due to salinity was generally minimised through the applications of nitrogen fertiliser at the relatively moderate salinization conditions (compensation effect), but a reverse trend was noticed at the high salinity.

Microbial activity in saline soils plays an important role in the amelioration of salt affected soils (Malik, 1978). Mirza and Tariq (1992) compared growth and nodulation of *Sesbania sesban* at eight levels (0-2.0 %) of NaCl salinity in sandy clay loam soil. Dry weight of shoots and roots and numbers of nodules per plant decreased with increasing salinity levels. Nodule dry weight, however, increased at low levels of salinity (0.1 % & 0.2 % NaCl) and decreased at higher levels. Yousef and Sprent (1983) grew *Vicia faba* under salt stress and found that the number of nodules per plant decreased that was partially compensated by producing larger nodules. Kurth *et al.* (1986) observed in hydroponically grown cotton seedlings, both length and weight of the primary root were enhanced by moderate salinity (25-100 millimolar NaCl) in the presence of 10 millimolar calcium, but the roots became thinner.

1.9 Aims

The main aims of the project were

1. To investigate the potential of cotton roots to hydrolyse and use organic phosphates in their environment through phosphatase activity.
2. To characterise those conditions of phosphate nutrition which influence the production of phosphatase.
3. To localise phosphatase activity within the root system in relation to altered phosphate nutrition.
4. To investigate different cotton cultivars for their capacity to produce phosphatase, in order to assess their suitability for growing in inorganic phosphate-poor soils.
5. To investigate some aspects of the influence of selected soil environmental factors on phosphatase activity and to relate these to conditions in cotton growing areas of Pakistan.

CHAPTER 2
MATERIAL AND METHODS

2.1 Plant material

Cotton cultivars used were Qalandri, Rehmani, Reshami, TH 3/83. Whilst these are all modern cultivars the chronological order of origin and derivations are as follows:

1. Qalandri = (*G. hirsutum* var M4 x *G. anomalum*) x *G. barbadense* var Karnak.
2. Rehmani = *G. hirsutum* 21 x McNair TH-14920.
3. Reshami = Coker 100 A x Latifi (H-61-29).
4. TH-3/83 = H-59-1 (Qalandri) x Acala-1517.
5 Wheat (*Triticum aestivum) cv* Jauhar = Nayab NF 600 RAD
6 Rice (*Oriza sativa) cv* Chota Bawalia

2.2 Seed origin and treatment

The seeds of cotton were obtained from Cotton Section Agriculture Research Institute Sindh and prior to use were stored dry in the dark at 20 °C. Healthy, viable seeds of cotton were surface sterilised by treatment with 5% sodium hypochlorite solution for 10 minutes. The seeds were then washed 6 times in distilled water to remove traces of the chemical. The seeds were planted on vermiculite in small plastic trays and incubated at 25°C, with 100% relative humidity in continuous darkness under automatic water sprinklers until germination took place, usually after 5 days.

It was observed that the germination ability of the cultivars were different. The most efficient was Qalandri followed by Reshami, TH-3/83 and Rehmani. This may reflect a real difference in the physiology of the different cultivars or may mainly have been due to a differential effect of storage conditions.

2.3 Plant growth conditions

After germination, uniform cotton seedlings were selected and were grown on in honey jars. Seedlings were inserted through holes in the plastic lids as shown in Fig 2.1 The jars, containing water initially, were kept at the 25°C growth room temperature with a 16-h photoperiod, light flux was 100 μmol photon m^{-2} s^{-1} PAR, supplied by banks of Phillips Warm White fluorescent tubes. Dark period temperatures were ambient, usually around 15°C. After two days the seedlings had adjusted to the culture conditions and were transferred to aerated Hoagland solution culture (Table 2.1). For various treatments plants were grown in modified media, Figs. 2.2, 2.3 and 2.4.

Table 2.1 Composition of mineral salts in Hoagland growth medium used to raise cotton plants.

Salt	Concentration
KCl	1.02 g l^{-1}
$Ca(NO_3)_2$	0.492 "
$NH_4H_2PO_4$	0.23 "
$MgSO_4.7H_2O$	0.49 "
H_3BO_3	2.86 mg l^{-1}
$MnCl_2.4H_2O$	1.81 "
$CuSO_4.5H_2O$	0.08 "
$ZnSO_4.7H_2O$	0.22 "
$H_2MoO_4.H_2O$	0.09 "

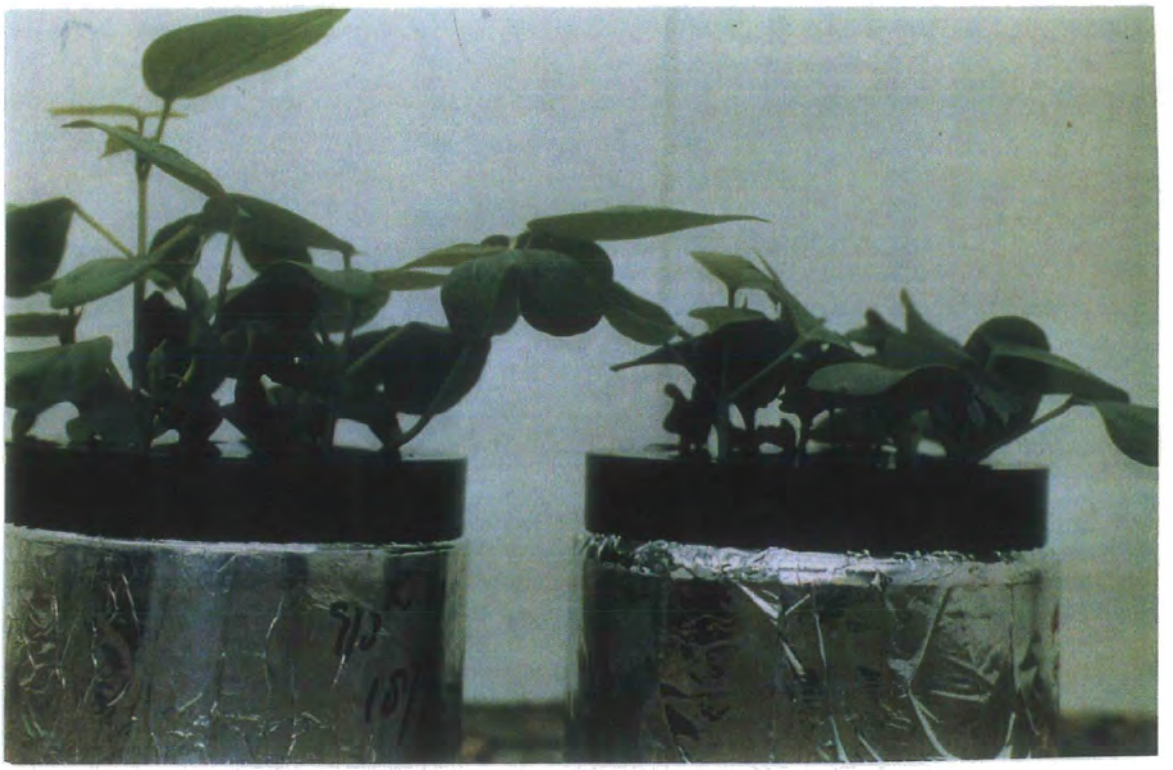


Fig. 2.1 Cotton seedlings grown with and without phosphorus added to the growth medium (left +P, right -P)

Fig. 2.2 Cotton seedlings grown without and with nitrogen added to the growth medium (left -N, right +N)

Fig. 2.3 Cotton seedlings grown without and with potassium added to the growth medium (left -K, right +K)

Fig. 2.4 Cotton seedlings grown without and with magnesium added to the growth medium (left -Mg, right +Mg)

Eight seedlings were placed in each honey jar, containing 500 ml nutrient solution The pH of the solution was adjusted to pH 6.0 using sodium hydroxide. The glass honey jars were wrapped with aluminium foil to exclude the light from the roots of the plants and to prevent growth of algae. The nutrient solution was changed weekly. Plants were harvested for experimental purposes as required. In most of the experiments plants grown for 19 days in the honey jar culture system were used. For experimental treatment purposes the nutrient solution was replaced with one of specific composition. For P-limitation, Hoagland solution was modified by omitting $NH_4H_2PO_4$ and replaced it with NH_4Cl, Fig 2.1.

Light measurements were taken with a Macam Q 101 light meter (Macam Photometrics Ltd., Scotland). All incident light was measured as photosynthetically active radiation (PAR). The readings were recorded as photon flux density (μmol photon m^{-2} s^{-1}).

2.31 Split root experiments

For the split root experiments seedlings were maintained in honey jar cultivation, using water only, for two days after germination. The water was then replaced with culture solution without added phosphorus. After six days secondary roots developed and at this stage the seedlings were used for further treatments.

Seedlings were taken and arranged so that each half of the complete root system was immersed in culture solution in individual glass tubes supported side by side. In this way each half of the root system could be treated as a separate unit by manipulation of the culture solutions. Seedlings were grown on with one set of roots in solution with added phosphorus and the other without added phosphorus. After fourteen days of growth the two sets of roots were harvested individually and used for phosphatase activity as described later.

2.4 Common laboratory procedures adopted

2.41 pH

All pH measurements were carried out using an Ingold Combination WTW E50 Electrode and EIL Meter (model 7050). The probe was calibrated with BDH standard buffer solutions, prepared in MilliQ water. Immediately before a measurement was taken the pH meter was calibrated using buffer solutions, one higher and another lower than the pH of the solution required. The standard buffer solutions were used at room temperature.

2.42 Weighing

Measurements were carried out on Electronic ER-182 A Balance (A & D Company Limited Japan).

2.43 Root dry weight

After recording the phosphatase data roots were washed with distilled water, blotted dry and transferred to (5 x 2 cm) glass vials. The vials were put in the oven at 105 °C overnight and then cooled in the desiccator for at least 30 min before recording the dry weight. Dry weights were used to convert the calorimetric readings to a weight basis (μmol mg-1 d. wt) and depending on the incubation time used, the above value is converted into rate of enzyme activity per hour (μmol mg-1 d. wt h^{-1}).

2.44 Cleaning of apparatus

All the glassware was washed in tap water containing 2% Decon phosphate-free detergent (Decon Laboratories Ltd., England) for 12 h and then rinsed six times in distilled

water. All the volumetric glassware was dried at room temperature, plastics were dried at 40°C. Universal and snap-top bottles were dried in an oven at 105°C. The plastic cuvettes were washed three time in tap water and rinsed six times in distilled water.

2.5 Phosphatase assays

2.51 Preparation of plant materials

The secondary healthy, clean and white roots were excised 0.5 cm below the hypocotyl from the seedlings for the assay. The roots from different seedlings were bulked and washed four times thoroughly in assay medium to remove contaminants. The roots were then separated into bunches of equivalent size for addition to assay bottles and were kept in dishes of assay medium until required.

In the preliminary experiments roots of Primulas were treated and prepared in the same way.

2.511 Algae

Two species of algae (1) *Stigeoclonium* and (2) *Draparnaldia* were chosen for preliminary study. Each species required individual treatment. The material was harvested using a silicon rubber tube on a glass rod to remove the culture from the walls of the conical flask. Specimens were centrifuged at 8000 x g for 10 min and washed with assay medium. They were then harvested again by centrifugation. The material was suspended in a small amount of assay medium and homogenized with a Virtis 45 homogeniser. For the homogenisation there was no standard fixed time, for the different cultures, therefore, separate timings were noted for each culture until a uniform fine suspension was obtained. After homogenisation a simple assay was carried out to check how much material was needed. Assay medium was then added to the homogenised

material up to the required quantity. The assays were terminated by removing the organism by filtration and then the terminator was added to the filtrate. The 30-40 ml of diluted organism was taken for dry weight determination.

2.52 Stock solutions

All salts were from BDH Analar grade stock and were prepared in MilliQ water and then kept in the refrigerator at 4.0°C until required. The stock solutions were renewed every 3 months.

2.53 Assay medium

The assay media were made up from stock solutions which were prepared and stored in a refrigerator, the dilution of the stock solution was made using Milli-Q water. The media were used for up to one week before being discarded.

Table 2.2 Concentrations of mineral salts used in assay medium of modified CHU 10-D (1942)

Stocks	g l^{-1}	Volume ml^{-1}	Salt mg l^{-1}	µmoles l^{-1}
$NaHCO_3$	15.85	1.0	15.85	188.60
$CaCl_2.2H2O$	35.83	1.0	35.83	243.70
$MgSO_4.7H_2O$	25.0	1.0	25.0	101.43
KCl	4.28	0.5	2.14	28.70
FeEDTA stock		0.125		
$FeCl_3.6H_2O$	9.70		1.21	4.28
Na_2EDTA	13.35		1.67	4.78
Microelement stock		0.25		
$MnCl_2.2H_2O$	0.181		0.045	0.23
$ZnSO_4.7H_2O$	0.222		0.056	0.19
$CuSO_4.5H_2O$	0.079		0.020	0.08
$CoSO_4.7H_2O$	0.042		0.011	0.039
H_3BO_3	2.86		0.715	11.56
$NaMoO_4$	0.027		0.0068	0.028

Table 2.3 Elemental concentrations in assay medium

Element	mg l^{-1}	µM
Ca	9.78	244.01
Na	4.45	193.59
Mg	2.47	101.63
S	3.26	101.67
K	1.12	28.64
EDTA	1.56	4.78
Fe	0.25	4.48
B	0.125	11.56
Zn	0.013	0.199
Mn	0.012	0.218
Cu	0.005	0.079
Mo	0.003	0.031
Co	0.002	0.034

For the preparation of one litre of assay medium 1 ml $NaHCO_3$, 1 ml $MgSO_4.7H_2O$, 1 ml $CaCl_2.2H_2O$, 0.5 ml KCl, 0.25 ml Fe EDTA and 0.125 ml of micro nutrient low Mn+N were added to a volumetric flask, then the final volume was made up to one litre with Milli-Q water. Trace elements were used as chelating agents in order to prevent the precipitation of iron in the medium.

2.531 Assay buffers

All the chemicals used in the buffer solution were obtained from British Drug House Ltd (BDH), except HEPES and CAPS which were supplied by Sigma Co, USA. Buffers were prepared in the Chu 10-D assay medium to a final concentration of 50 mM and were stored in the refrigerator at 4°C until required, the buffer solutions were made up fresh every three months.

Table 2.4 Buffers used to determine the effect of pH on phosphatase activity

pH	Buffer	Buffering capacity	pka at. 20°C
3.0	DMG-NaOH	3.0-7.6	3.66 and 6.20
3.5	DMG-NaOH	3.0-7.6	3.66 and 6.20
4.0	DMG-NaOH	3.0-7.6	3.66 and 6.20
4.5	DMG-NaOH	3.0-7.6	3.66 and 6.20
5.0	DMG-NaOH	3.0-7.6	3.66 and 6.20
5.5	DMG-NaOH	3.0-7.6	3.66 and 6.20
6.0	DMG-NaOH	3.0-7.6	3.66 and 6.20
6.5	DMG-NaOH	3.0-7.6	3.66 and 6.20
7.0	HEPES-NaOH	6.8-8.2	7.50
8.0	HEPES-NaOH	6.8-8.2	7.50
9.0	Glycine-NaOH	8.6-10.6	9.60
10.0	Glycine-NaOH	8.6-10.6	9.60
11.0	CAPS-NaOH	9.8-11.1	10.40

2.54 Substrates

Phosphatase activity was assayed using three different substrates in order to investigate a wide range of enzyme activity (Table 2.4).

Table 2.5 Substrates used for assaying phosphatase activity.

Reagent	Supplier
p-nitrophenyl phosphate disodium (pNPP)	Sigma Chemical Co., U.S.A
bis p-nitrophenyl phosphate sodium salt (bis pNPP)	Sigma Chemical Co., U.S.A
4-Methylumbelliferyl phosphate (MUP)	Sigma Chemical Co., U.S.A

It was intended that a direct comparison should be made between pNPP and 4-MUP as suitable substrates for phosphatase assays. However, the calculated concentration of pNPP was based on the molecular weight of the chemical where no account was made for water of crystalization. Consequenctly the pNPP and 4-MUP concentrations are not directly comparible; the standard concentrations used were 71 μM as a corrected value of pNPP, and 100 μM for MUP.

2.55 Assay conditions

All experimental containers required for the experiment were oven dried at 105°C before initiating the experiment. The snap-caps vials were properly labelled as required. Modified assay medium as formulated by CHU-10D was used (Table 2.2). The roots harvested from cotton plants were washed with assay medium four times and transferred to sterilised Petri dishes in assay medium. The previously-prepared assay medium, buffer and substrates, placed under 4°C in a fridge, were taken out before starting the experiment and

left for 10 min to equilibrate to room temperature. Into every labelled snap-cap bottle 1.4 ml of assay medium was pipetted and roots were added. The relevant buffers, 1.5 ml from each, were then added to each snap-cap bottle. Assays were initiated by the addition of 0.1 ml of required substrate, including to the control bottles, (without root material). The bottles then were immediately transferred to a shaking water bath and were incubated at 25° C for 30 min. The experiment was terminated by the addition of 0.3 ml of the appropriate terminator. After termination of the assay the organism was taken out from the snap-cap vials with forceps and were kept in pre-weighed vials for dry weight determination as described. The assays solutions for pNPP and 4-MUP were poured into the cuvettes and the relevant absorbances were then recorded in the spectrophotometer or fluorimeter.

2.56 Terminator

The terminator was made up in assay medium and kept at room temperature. When pNPP was used as the substrate the terminator was 5M NaOH. For bis-pNPP substrate 1 M NaOH terminator was used.

For 4-MUP substrate the terminator consisted of different concentrations of K_2HPO_4 + NaOH + EDTA used according to the pH of the assay buffer. For pH`s from 3.0 to 5.5 the concentration of terminator used was 100 mM NaOH plus 100 mM K_2HPO_4 and 2.5 mM EDTA. Above pH 6.0 a mixture of 50 mM NaOH, 50 mM K_2HPO_4 and 2.5 mM EDTA was used.

2.57 End product determination

2.571 Colorimetry

All colorimetric analyses on phosphatase activity were carried out using a Shimadzu Digital-Double-beam spectrophotometer (model-150-2). Quartz cuvettes with a path length of 1.0 cm were used to read absorbance of the assay solutions at 405 nm. The yellow colouration was the result of the hydrolysis of pNPP to pNP.

2.572 Fluorimetry

A Baird-Atomic Fluoripoint spectrofluorimeter was used for fluorescence measurements of phosphatase activity when using 4-MUP as a substrate. Plastic cuvettes, with a path length 1.0 cm, were used for all fluorescence readings at wavelengths of 444 nm for emission, 356 nm for excitation.

2.6 Histological localization of phosphatase activity

For the cellular location of phosphatase activities three staining techniques were used, with different organic phosphate substrates.

2.61 5-bromo-4-chloro-3-indolyl phosphate (BCIP)

The localisation of PMEase activity by staining was carried out by using 5-bromo-4-chloro-3-indolyl phosphate (BCIP) as an organic substrate (Coston & Holt, 1958; Holt & Withers, 1958). The white clean roots of *cv* Qalandri, with and without phosphorus, were washed four times in assay medium. Assay medium, 1.4 ml, was pipetted into snap-cap vials and roots were added. 1.5 ml of pH 5.5 assay buffer (DMG-NaOH) was then added to each snap-cap vial. The assay was started with addition of 0.1 ml of BCIP. The vials were transferred immediately to a water bath at 32°C and shaken for 15 min.

Following incubation the roots were then washed three times in assay medium prior to examination of segments under the high power [x 400 or x 1000] of a microscope.

2.62 Naphthol AS-MX phosphate

Localization of PMEase activity was also investigated by microscopy using 3-hydroxy-2-naphtholic acid 2, 4-dimethylanilide phosphate sodium salt (naphthol AS-MX phosphate) as the organic P source and diazotized 4-benzylamino-2,5-dimethoxyanilide zinc chloride (Fast Blue RR diazonium salt) as the coupling agent; the product is a violet insoluble dye. The staining medium consisted of 12 ml of assay medium plus 0.5 ml of 1 mM naphthol AS-MX phosphate alkaline solution (Sigma Chemical co, 1984) and 7.5 mg of Fast Blue RR diazonium salt. The mixture was stirred continuously using a magnetic stirrer during the preparation. The staining medium was adjusted to pH 5.5 using 1 M NaOH and then filtered through a GF/C glass microfibre filter and was used immediately after preparation. Plant roots were washed three times in assay medium, resuspended in the staining medium, left for 15 min at 32°C, washed three times in assay medium and were examined under a high powered microscope (x 400 or x 1000) to locate staining.

2.63 β-naphthyl phenylphosphonate.

PDEase activity was tested using β-naphthyl phenylphosphonate as the organic P source and diazotized 2-methyl-4-[(2-methyphenyl) azo] benzendiazonium sulphate salt (Fast Garnet GBC sulphate salt) as the coupling agent; the product is deep red organic insoluble dye. The staining medium consisted of assay medium at pH 5.5, 1 mM naphthyl phenylphosphonate and 0.1% Fast Garnet GBC sulphate salt. The staining medium was filtered through 0.22 μM nitro-cellulose filter and used immediately after preparation. Material was washed three times in assay medium and resuspended in the staining medium,

left for 15 min at 32°C, washed three times and examined under a high powered microscope (x 400 or x1000). Microtouch medical gloves were used throughout as Fast Garnet GBC sulphate salt is a possible carcinogen.

2.7 Plant analysis

Plant analysis is the determination of the concentration of an element or extractable fraction of an element in a sample from a particular part or portion of crop sampled at a certain time or stage of morphological development (Munson & Nelson 1973). According to Fageria (1984) plant analysis, in simplest terms, is a study of the relationship of the nutrient content of the plant to its growth. Plant analysis is a valuable tool in the diagnosis of nutrient-related yield limitations. Interpretation of plant analysis requires a knowledge of the ideal composition or critical nutrient levels for a given crop when its growth or yield is at a maximum; however, critical plant compositon criteria must be obtained for specific sampling period during the plants development, because the concentrations of most elements vary with the maturity of the crop (Evanylo et al. 1988). The critical concentration is defined as the minimal concentration in a specific plant part from plants producing 90 to 95 % of maximum dry matter (Morghan 1985).

Phosphorus concentration in cotton plants may be increased by P fertilization (Cope 1984; Khasawned & Copeland 1973; Sharpley & Reed 1982; Stelley & Morris 1953). In greenhouse sand culture trials, an increase in P in the solution had a direct effect on the level of P in the plants (Ergle & Eaton 1957; Joham 1951). From the information provided by Bassett et al. (1970) and Kapp et al. (1953) cotton tissue can be listed in order of increasing P concentration: roots, stems, leaves, immature leaves, fruiting parts, and finally seed. Root growth and P uptake were found to be closely related (Khasawneh & Copeland 1973). The amount of P taken up per unit length of root was the same regardless of P concentration in solution.

2.71 Phosphorus analysis

Four stock solutions were prepared for phosphorus analysis. From these solutions, three reagents were prepared daily for use. The following stock solutions and reagents were used for phosphorus analysis.

Table 2.7 Stock solutions and reagents used for phosphorus analysis

Stock solution	Reagent
1. Phosphate standard 1 in Digestion Reagent	(K2O8S2,D)
2. H_2SO_4-Antimony Mixed Reagent	(C6H8O6,B,C)
3. Molybdate	
4. Digestion acid (conc. H_2SO_4)	

Stock solutions were stored in 1000 ml bottles and wrapped with aluminium foil. Additionally, a stock solution of 16M HNO_3, Spectrosol grade, was used for the digestion of the cotton material. Stock solutions were made as communal stocks approximately every two months and were stored at 4°C, with the exception of HNO_3.

Cotton seeds, roots and leaves were prepared for dry weight determinations, before analysis. After the dry weights were recorded approximately 0.25 g of each replicate was digested in 5 ml 4M HNO_3 in snap-cap vials in a test tube boiling rack in a fume cupboard for 1 h at 100 °C. Bubble flasks were placed on the snap-cap vials after 10 minutes digestion. The roots and leaves were ground with a glass rod prior to digestion because of the fibrous components. After digestion, distilled water was used to rinse the condensed liquid which had settled on the bubble flasks, into the snap-caps vials. Each sample was then transferred to an Erlenmeyer flask, diluted to 35 ml with distilled water, and the pHs adjusted to pH 7.0 using 1M NaOH. Phosphate standard solutions did

not have their pH adjusted. The samples were transferred to a measuring cylinder, diluted to 50 ml with distilled water, and then 25 ml returned to the Erlenmeyer flask and 25 ml was discarded. To each sample, including the standards, 5 ml of digestion reagent was added and then 5 ml of mixed reagent value of each sample was measured on a Shimadzu double beam Spectrophotometer UV 150-02 against a MilliQ water blank, at 882 nm. A 4-cm cell was used for each sample. The detection limit for each reading was 0.009 OD units.

2.72 Nitrogen analysis

For the nitrogen analysis three solutions were prepared as communal stocks and oxidising reagent solution was prepared daily, Smart *et al.* (1983). The following stock solutions and reagent were used for nitrogen analysis (Table 2.6).

Table 2.6 Stock solution and reagent used for nitrogen analysis

Stock solution	Reagent
1. Nitrogen standard	(Nitrate)
2. Phosphate standard in digestion	($K_2S_2O_8$ and NaOH)
3. Ammonium chloride (buffer)	(NH_4Cl)
4. NNED	
5. Sulphanilamide reagent	

Stock solutions were made up and kept at 20 °C room temperature. The NNED was put in brown bottles. Bottles containing sulphanilimide were wrapped with aluminium foil to exclude light.

Cotton seeds, roots and leaves were dried in 105°C for 16 h in pre-weighed snap-cap vials. All the samples were then put in a desiccator and after 30 min dry weight was recorded. The samples were ground with a glass rod and to each 150 ml of oxidising solution was added following transfer to conical flasks. Each flask was sealed with aluminium foil prior to being autoclaved for 1.5 h at 120°C before being cooled to room temperature. After autoclaving samples were filter through Whatman No1. filter paper. All the filtered samples were diluted by removing 1 ml from each and making up to 50 ml by adding MilliQ water, 5 ml of buffer was then added to each sample. The total mixture was then passed through a cadmium column at a rate of < 25 ml in 4 min, 25 ml samples were collected. Sulphanilamide reagent (0.5 ml) was added to each 25 ml sample from the column. After 5 min 0.5 ml NNED was added each and after a further 30 min the absorbance of the solution was measured at 543 nm (1 cm cells).

2.8 Soil sampling methods

Soil samples were collected from six cotton growing districts of Sindh viz Sanghar, Mirpurkhas, Nawabshah, Khairpur, Sukkur, Hyderabad. From each district areas 21 samples were drawn at three depth levels, taking 7 samples from each depth as follows, 0-15 cm D1, 15-30 cm D2 and 30-45 cm D3. After collection the samples were analysed with the cooperation of Soil Fertility Section, Agriculture Research Institute Tandojam Sindh, Pakistan. The data were recorded and statistically analysed.

2.81 Field Sites

Soil sampling is the most important step for soil testing. From soil testing one can determine the nature of the soil profile and the physical and chemical properties. Such information is essential to modern management systems which aim to predict the

productivity of soil. The success or failure of soil analysis depends on collecting the representative soil samples. For this study the following steps were taken:

(1) The field was divided into different areas. The samples were collected from the area which represented uniformity in topography, texture, colour of the soil, depth, drainage and crop management.

(2) Soil samples were taken from different positions to take into account soil variability.

(3) Particulars of the area sampled, location, crop grown, manuring history were recorded on an information sheet.

(4) All the samples were labelled with sample number, Village, Taluka and District.

The following characters were determined:

(a) Soil pH

(b) Electrical conductivity

(c) Organic matter exchangeable potassium

(d) Available phosphorus

(e) Total nitrogen

2.82 Core sampling

For the soil sampling a soil auger, with a cavity of 12" long and 4" diameter, was used. Each soil depth sample consisted of a mixture of seven soil cores.

2.83 Determination of soil pH

Samples of 30 g air-dried soil were taken in 250-ml shaking bottles and 150 ml distilled water was added. The suspensions were shaken on a mechanical shaker for 30 min and then filtered through Whatman filter paper No. 40. The filtrate were transferred to a 100-ml beaker for pH measurements. The pH meter was calibrated with standard

buffer solution tablets pH 4.0, 7.0 and 9.0. The temperature of the pH meter was also maintained at 20°C. The glass electrode was rinsed with distilled water, and readings were recorded.

2.84 Determination of soil conductivity

The soil and water were mixed together at a ratio 1:5, shaken on the mechanical shaker for 30 min, and a clear extract was obtained by filtration. All the samples were measured by using a conductivity meter. The conductivity was expressed as $\mu S\ cm^{-1}$ at 30°C.

2.85 Determination of soil organic matter

Reagents used for standard of Soil Fertility Dept., Sindh, Pakistan:

1. 1 N $K_2Cr_2O_7$: 49.04 g in one l, of distilled water.
2. Diphenylamine indicator: 0.5 g of reagent grade indicator was dissolved in 20 ml distilled water and 100 ml of concentrated H_2SO_4
3. Ferrous solution: A. 0.05 N ferrous solution was made up by dissolving 196.1 g Fe $(NH_4)_2\ (SO_4)_2 .\ 6H_2O$ in 800 ml distilled water to which was added 20 ml concentrated H_2SO_4 and the mixture was diluted to one litre.
4. 85% H_3PO_4.
5. NaF salt.
6. Concentrated H_2SO_4 not less than 96% purity.

A 2 g soil sample was taken and 10 ml of 1 N K_2Cr_2O7 solution was pipetted onto it and both were mixed by swirling and shaking the flask. 20 ml of concentrated H_2SO_4 was then added and mixed by gentle rotation for 1 min to ensure the complete contact of the

reagents with the soil. The mixture was allowed to stand for 20-30 min. The standard blank (without soil) was run in the same way.

Back titration: Ferrous solution (3) diluted to 200 ml with distilled water and 10 ml of H_3PO_3, 0.2 g NaF salt and 30 drops of diphenylamine indicator were added. The solution was back titrated with ferrous solution. In the beginning, the colour was dull green with chromous salts then this colour changed to turbid blue as the titration proceeded. At the end point this colour sharply changed to green.

2.86 Determination of soil exchangeable potassium

5 g soil samples were taken in 150-ml shaking bottles, and 100 ml of 1N ammonium acetate solution were added to each. The bottles were stoppered and shaken for 30 min. The suspension was filtered/ centrifuged. The potassium content of filtrates was determined by flame photometer at a wavelength of 770 mμ.

2.87 Determination of available phosphorus in soil

Reagents

1. Sodium bicarbonate 0.5 N, pH 8.5: Dissolved 42.0 g of $NaHCO_3$ in one l of distilled water and adjusted the pH to 8.5 with 1.0 N NaOH.
2. Activated charcoal: Phosphorus-free activated charcoal was used.
3. Sulphuric acid 0.5 N: 14 ml of concentrated sulphuric acid diluted to one l with distilled water.
4. Solution A:

(i) Potassium antimony tartarate 0.124 g, added with 400 ml of distilled water and 70 ml of concentrated H_2SO_4.

(ii) 6 g ammonium molybdate dissolved in 300 ml of distilled water.

(iii) When the antimony-acid solution was cold, then one l ammonium molybdate solution was added to it.

5. Solution B:1.056 g Ascorbic acid was added to every 200 ml solution A. (fresh solution was made up each time).

6. Standard phosphorus solution: Potassium dihydrogen phosphate (KH_2PO_4) 2. 195 g was dissolved in a l of distilled water, this was the 500 ppm stock solution. 5 drops of toluene were added to prevent microbial activity. The standard was made up by diluting this stock as required.

5 g of soil sample was taken in a 250 ml flasks to which was added 100 ml of reagent 1 then mixed with one teaspoon of reagent 2. The suspensions were shaken for 30 min on a mechanical shaker and were then filtered through Whatman filter paper No. 40. 10 ml aliquots of the each filtrate were transferred to 25 ml volumetric flasks to which were added 10 ml of reagent 3 to neutralise the $NaHCO_3$. The flasks were left overnight for removal of CO_2. 4 ml of reagent 5 and 1 ml of distilled water were added and contents of the flasks were immediately shaken. A blue colour appeared in 1-2 min which reached its maximum intensity in about 10-15 min. Finally readings of the standard and samples were recorded on a spectrophotometer at 680 nm wavelength.

2.88 Determination of total nitrogen

Reagents

1. N/10 sulphuric acid
2. N/10 NaOH
3. Methyl red indicator solution
4. Salt mixture containing K_2SO_4 (10 parts) $FeSO_4$ (1 part) $CuSO_4$ (0.5 parts)

10 g of soil samples were transferred to clean dry Kjeldhal flasks, moisten with 10 ml of distilled water, shaken well and allowed to settle for 30 min. Samples were supplemented

with 35 ml concentrated sulphuric acid and 10 g of a mixture of K_2SO_4, $FeSO_4$ and $CuSO_4$, and 1 g of salicylic acid and 5 g $Na_2S_2O_2$. The samples were heated on a low flame which was then gradually raised to high. The samples were continuously digested by boiling until the mixture was colourless. After cooling 50 ml of distilled water was added in each flask; these were swirled well and the samples were cooled under a tap. The solution was transferred to a 800-ml flask by decantation. Contents of the digestion flask were washed with distilled water. The volume was made to 400 ml and a few pieces of porcelain were added to prevent bumping. The distillation was made in presence of a known quantity of ammonia N/10 sulphuric acid (20 ml). Excess acid was titrated with N/10 NaOH using methyl red indicator. The same procedure was carried out with a blank, absorbance was recorded on a spectrophotometer at a wavelength of 680 nm.

2. 9 Computing

Four computer programs were used for this study. All the routine calculations were performed on Microsoft Excel. The text was written up on the Word for Windows version 2.0. The graphic output was carried out under the Sigma Plot (4.1) suite of software.

The statistical analyses were carried out on Minitab using 2-way ANOVA. In the analyses comparison was made between single variables and the interaction between the variables. eg. Chapter 6, section (6.1) condition = ± , concentration = different concentrations of NaCl, condition x concentration =i nteraction between the two variables.

CHAPTER 3
AGRO-ECOLOGICAL COTTON CONDITIONS IN SINDH (PAKISTAN)

3.1 Introduction

Cotton is one of the most important commercial crops worldwide and Pakistan usually ranks as the fifth biggest producer of cotton in the world. Pakistan is situated between 24° and 27° N and 62° E and stretches over 1600 km north to south and 885 km east to west with a total area of 796,095 km^2. Out of 20 million hectares of cultivated area in Pakistan, 14 % is devoted to cotton production. Cotton, therefore, plays a vital role in the economy of Pakistan, and the country earns substantial foreign exchange from the export of both cotton fibre and its products. In 1992, Pakistan produced 1.85 $x10^6$ t cotton, from which 0.51 $x10^6$ t were exported (Cotton World Statistics, 1992)

The country comprises four provinces Punjab, Sindh, Balochistan and North west frontier province, of which Punjab and Sindh are well known for their crop productions. Sindh province is situated in the south-west of Pakistan. The Indus river flows through Sindh making the province the main source of the agricultural production in Pakistan. The total land area of Sindh is 14.09 million hectares, the cultivated area is 5.45 million hectares of which the total area under cotton crop (1991-92) was 548 thousand hectares (Cotistics, 1992).

The main cotton growing areas of Sindh are shown in Fig. 3.1. Many different cultivars are grown locally. The soil conditions vary in pH, nutrient, matrix and water availibility. A study was made to obtain information on the soil environment from which the specific cotton seeds were obtained.

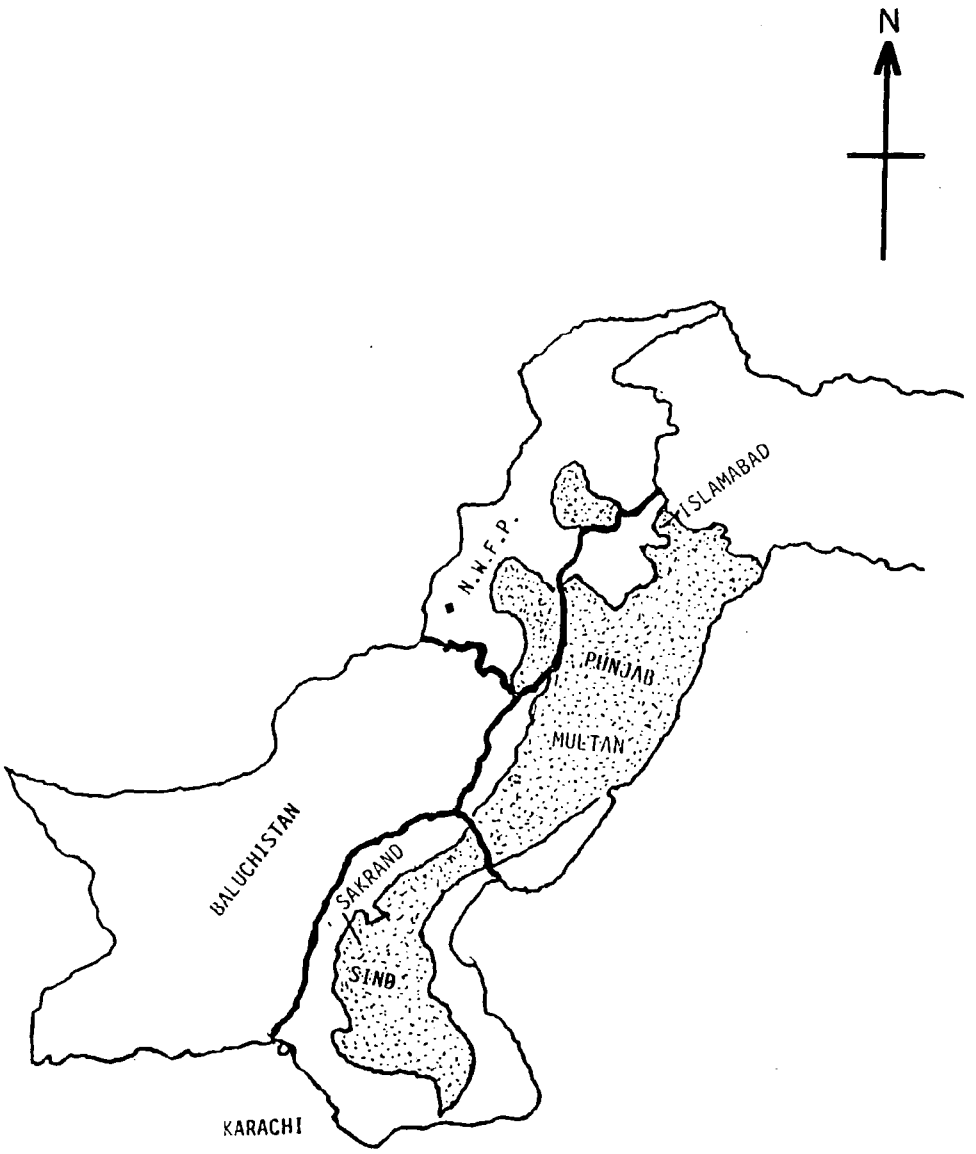


Fig. 3.1 Map of Pakistan showing cotton growing areas.

▦ Cotton growing areas.

3.2 Climate

Cotton is grown under the temperature range of 40-45°C in the northern area, and 38-43°C in the southern area of Sindh. The mean annual rainfall varies from 18 to 50 mm with mean humidity of 60 and 85 % in the south and north respectively.

Sindh has been divided in to three cotton growing zones according to agro-climatic condition and soil types. It includes northern, middle areas and southern zones. In the northern zone cotton is grown in two distrticts called Sukkur and Khairpur. In the middle zone cotton is grown in Nawabshah and Sanghar districts, while in south cotton is cultivated in the districts of Hyderabad and Mirpurkhas.

3.3 Soil analysis

Soil analysis is one of the important factors in the diagnoses of weak plant growth and the suitability of a site for crop production. The main purpose of soil analysis is to determine the quantity of fertilizer required to prevent economic loss of crop value because of nutrient deficiency. Soil tests provide an index of the available nutrients in the soil. The second purpose of soil testing is to monitor the quantity of available nutrients present over time. This information is useful for evaluation of fertilization, crop zoning and in making decisions about waste.

The uptake of nutrients by plants is known to depend on soil pH. Generally, plants grown in solution have highest nutrient uptake at about pH 5 to 6. At higher and lower pH values the nutrient uptake decreases dramatically. Dunlop and Bowling (1978) found that the external pH affected the electrical potential difference between the roots and the solution, and that this factor was closely related to phosphate uptake. The soil pH is also believed to increase the microbiological activity (Halstead *et al.* 1963).

Nitrogen (N) availability indices are a measure of the potential of a soil to supply nitrogen to plants when conditions are ideal for mineralization. Surface soil usually

contains 0.08 to 0.4 % total nitrogen, mostly in the organic form (Bremner 1965a). The amount of nitrogen that is mineralized each year is commonly in the range of 1 to 3 % (Broadbent, 1984). Most of the nitrogen being mineralized in soil obviously comes from a fraction of the soil organic matter that is easily decomposed. Therefore, it is likely that a mild acid or alkaline extractant could make a suitable chemical isolation medium (Keeney & Bremner, 1966).

The purpose of testing soil for phosphorus is to examine the quantity of supplemental phosphorus required to prevent economic loss of crop value because of phosphorus deficiency. A soil test indicates an index of the available phosphorus in the soil which in turn can be used to predict the amount of supplemental phosphorus needed. Generally 1 % of the soil volume is occupied by the plant roots (Barber, 1984), therefore, influencing the root system, size or morphology will likely effect the quantity of the soil phosphorus that is available to a plant. Simulated sensitivity analysis has demonstrated the large impact that the rate of root elongation has on phosphorus uptake (Silberbush & Barber, 1983).

The exchangeable potassium is also one of the most important elements in crop development, commonly, its insufficient supply in the soil limits crop yield. Potassium plays a specific role in the mechanism of opening and closing of stomata. When nitrogen and potassium are in short supply, the plants are stunted, their leaves are small and rather ashy grey in colour, they die prematurely, and the fruit and seed are small in quantity and size (Drew, 1975; Hackett, 1978). Nitrogen and potassium deficiency effects are general and are seen in crops on all soils,however, deficiency is most common on sandy soil. The soil texture also affects the amount of water retained at field capacity (McGowan, 1984). The effect of soil structure is the most important factor in determining the soil water retention.

3.4 Results

3.41 Soil pH

The results were obtained using the methods (2.71). Significant results were obtained for soil pH (Table 3.1) among the samples drawn from D1 ($P \leq 0.05$), D2 ($P \leq 0.001$), and D3 ($P \leq 0.05$). The alkaline nature of the soil was common in every location of study. The highest pH was recorded for district Khairpur (D1 = 8.66, D2 = 8.82, D3 = 8.86) while the lowest pH levels (D1= 8.29, D2= 8.34) were noted at district Mirpurkhas. The increasing trend in pH levels was found as samples were taken from increasing depths. The incremental trend in pH was constant in every district from D1 to D3 except at D1 in district of Sukker and Khairpur its order was disturbed. This may be because of some unforeseen errors in the sampling.

Table 3.1 pH at different soil depths (cm below surface).

District	D 1 (0-15 cm)	D 2 (15-30 cm)	D 3 (30-45 cm)
Sanghar	8.42	8.47	8.51
Mirpurkhas	8.29	8.34	8.40
Nawabshah	8.37	8.39	8.42
Khairpur	8.66	8.82	8.86
Sukkur	8.52	8.65	8.70
Hyderabad	8.57	8.58	8.61
F (df)	3.02 (5)	5.17 (5)	4.94 (5)
P≤	0.308	0.605	0.642

3.42 Conductivity

Highly significant results for conductivity (Table 3.2) among districts were noted ($P \leq 0.001$). The depth levels were also highly significant ($P \leq 0.001$). Conductivity varied from district to district and at different soil depths within and between their districts. The highest conductivity was observed in samples of Sanghar (451), and lowest conductivity was noted for Khairpur (224).

Table 3.2 Conductivity ($\mu S\ cm^{-1}$) at different soil depths (cm below surface).

District	D 1 (0-15 cm)	D 2 (15-30 cm)	D 3 (30-45 cm)
Sanghar	451	360	393
Mirpurkhas	429	385	346
Nawabshah	347	351	310
Khairpur	274	224	244
Sukkur	450	341	305
Hyderabad	263	326	281
F (df)	1250 (5)	73 (5)	68 (5)
$P \leq$	308	605	642

3.43 Organic Matter

Significant differences were recorded among districts for organic matter (O.M.) (Table 3,3) content present at different sampling depths, D1 ($P \leq 0.05$), D2 ($P \leq 0.01$), and D3 ($P \leq 0.05$). Highest O.M. was found at district Khairpur (1.47) while the least was at Hyderabad (0.29). The O.M. declined for the increasing depth in every district except for

Sukkur where it increased in D2 with respect to, D1. This unpredictable result may again have been the result of disparity in collecting the soil samples.

Table 3.3 Organic matter (ppm) at different soil depths (cm below surface).

District	D 1 (0-15 cm)	D 2 (15-30 cm)	D 3 (30-45 cm)
Sanghar	1.32	1.16	1.03
Mirpurkhas	1.17	1.06	0.89
Nawabshah	1.07	0.89	0.59
Khairpur	1.47	1.20	0.92
Sukkur	0.86	0.92	0.80
Hyderabad	0.88	0.42	0.29
F (df)	2.45 (5)	4.07 (5)	2.97 (5)
P ≤	0.052	0.005	0.024

3.44 Total Nitrogen

For the nitrogen content (Table 3.4) non-significant results obtained for all districts. Significant results were noted for the depth levels, D3 ($P \leq 0.05$) and significant results also obtained for D2 ($P \leq 0.01$). The data showed highest nitrogen levels in Sanghar (0.066) while the lowest were in Hyderabad (0.015).

Table 3.4 Total nitrogen (ppm) at different soil depths (cm below surface).

District	D 1 (0-15 cm)	D 2 (15-30 cm)	D 3 (30-45 cm)
Sanghar	0.066	0.058	0.052
Mirpurkhas	0.059	0.054	0.041
Nawabshah	0.053	0.048	0.033
Khairpur	0.061	0.062	0.047
Sukkur	0.044	0.042	0.040
Hyderabad	0.046	0.024	0.015
F (df)	2.34 (5)	3.93 (5)	2.66 (5)
P ≤	0.061	0.006	0.038

3.45 Available Phosphorus

Analysis of soil samples showed non-significant variation for available phosphorus (Table 3.5) among districts. Significant differences ($P \leq 0.05$) were observed for depth levels. The highest available phosphorus was detected in Sanghar districts (1.93) at D1 and the lowest available phosphorus level was found in Hyderabad (0.48).

Table 3.5 Available phosphorus (ppm) at different soil depths (cm below surface).

District	D 1 (0-15 cm)	D 2 (15-30 cm)	D 3 (30-45 cm)
Sanghar	1.93	1.28	0.56
Mirpurkhas	1.31	1.11	0.53
Nawabshah	1.81	0.88	1.66
Khairpur	1.93	1.08	1.00
Sukkur	1.58	1.18	0.53
Hyderabad	0.63	0.67	0.48
F (df)	0.94 (5)	0.57 (5)	0.56 (5)
P ≤	0.469	0.724	0.729

3.46 Exchangeable Potassium

Highly significant results among districts for exchangeable K (Table 3.6) were recorded ($P \leq 0.001$). Also highly significant differences ($P \leq 0.001$) were noted for depth levels. The highest exchangeable K of 184 ppm and lowest 42 ppm for Sukker district were recorded. The exchangeable K also declined with increasing depth levels.

Table 3.6 Exchangeable potassium (ppm) at different soil depths (cm below surface).

District	D 1 (0-15 cm)	D 2 (15-30 cm)	D 3 (30-45 cm)
Sanghar	153	127	116
Mirpurkhas	145	125	101
Nawabshah	162	126	95
Khairpur	154	127	104
Sukkur	110	92	74
Hyderabad	184	156	129
F (df)	4.77 (5)	6.80 (5)	4.38 (5)
$P \leq$	0.002	0.0	0.003

CHAPTER 4

4.1 Initial investigations of PMEase activity in cotton

Initial experiments were carried out in order to establish the existence of PMEase activity in cotton roots and to characterise some of the properties of the system. In order to achieve this plants of select cultivars were grown as described in the Materials and Methods and PMEase activity was assayed using systems which had already been successful with other plant species. In order to maximise PMEase activity and regulate its production cotton seedlings were grown with and without phosphorus added to the growth medium.

For these initial experiments only pNPP was used as the substrate and the *cv* Rehmani was used as the experimental plant. A narrow range of pH values for the assay medium was also employed. It should be noted, however, that in subsequent experiments two substrates were used for the assay of PMEase activity, pNPP and 4-MUP. As stated in Materials and Methods (Chapter 2) the actual absolute concentrations of these two substrates used were different therefore a direct comparison between rates can not be made when a full concentration range of substrate has not been used. This anomaly will be discussed further in Chapter 8.

Table 4.1 shows that PMEase activity was present in the roots of *cv* Rehmani. The data indicate that the plants grown without phosphorus added to the growth medium gave more PMEase activity at all the pH values, with the lowest rate at pH 7.0 and the highest at pH 6.0. In contrast it was noted that plants grown with phosphorus added to the growth medium showed lower activities but the least activity was at pH 7.0 and the highest at pH 6.0. The ratio of PMEase activity was recorded as 1.34 at optimum pH. Highly significant differences ($P = \leq 0.001$) were obtained between the plant growing conditions. Highly significant differences ($P = \leq 0.001$) were found between pH values.

Table 4.1 indicates that significant differences ($P = \leq 0.01$) were recorded for the interaction between pH and growth conditions.

Table 4.1 PMEase activity (μmol mg d. wt^{-1} h^{-1}) in cotton seedling roots (*cv* Rehmani) grown without and with phosphorus added to the growth medium; pNPP substrate was used (n = 4, ± SD).

pH	PMEase activity (μmol mg d. wt^{-1} h^{-1})	
	- P	+ P
3.0	0.089 ± 0.001	0.056 ± 0.001
4.0	0.109 ± 0.004	0.080 ± 0.003
5.0	0.135 ± 0.005	0.103 ± 0.002
6.0	0.172 ± 0.001	0.128 ± 0.003
7.0	0.078 ± 0.001	0.043 ± 0.001
ANOVA	F (df)	Prob.
pH	2469 (4)	** 0.001
Condition	2822 (1)	** 0.001
pH x condition	16 (4)	* 0.01

4.2 Influence of cutting roots on their PMEase activity

In order to assess the effect of cutting injury on root PMEase activity and its variability, an investigation was performed using different-sized pieces of root in the assays. Seedlings were grown in medium with or without added phosphorus; equal quantities of fresh weight of whole roots were harvested for each treatment. Each treatment sample was divided into two equal amounts and the roots were then cut into

batches of either 1-cm or 4-cm segments. The 1-cm and 4-cm segment samples were then assayed for PMEase activity at pH 6.0 which was indication as giving the highest rate.

Table 4.2 shows that 1-cm length of roots gave higher PMEase activity than the-4 cm length in both treatments. Plants grown without added phosphorus displayed higher activity in all cases. The results indicate that some PMEase activity was being detected in the cut cells as well as on the surface of the roots. To minimise this effect 4-cm segments, including root tips, were used routinely in subsequent experiments. Also the variability of activity between replicates was less in the larger segments.

Table 4.2 Influence of root segment size from *cv* Rehmani seedlings, grown without and with phosphorus added to the growth medium, on PMEase activity, pNPP was used as substrate. Activity is expressed as µmol mg d. wt^{-1} h^{-1} (n = 4, ± SD).

Segment size and f. wt (mg)	Root weight	d. wt (mg)	PMEase activity (µmol mg d. wt^{-1} h^{-1})	
	- P	+ P	- P	+ P
1 cm, 80	2.1 ± 0.14	2.05 ± 0.13	0.172 ± 0.013	0.116 ± 0.005
4 cm, 80	2.35 ± 0.06	2.35 ± 0.06	0.142 ± 0.003	0.089 ±0.003
ANOVA			F (df)	Prob.
Root types			53 (1)	* 0.01
Condition			187 (1)	** 0.001
R.typ x condition			N.S	-

4.3 Influence of pH on PMEase activity of *cv* Qalandri

Once PMEase activity had been shown to be associated with cotton roots, further characterisation was undertaken. Because *cv* Qalandri gave better and more consistent

germination, and because of a greater availability of seeds, this was used as the standard test plant.

A more precise range of pH activity was investigated in order to establish the relative amounts of acid and alkaline PMEase present. In addition pNPP and 4-MUP were utilized in order to assess the range of substrate specificity and sensitivity of the activity (Fig. 4.1)

With both substrates acid PMEase activity was observed. The activity increases from pH 4.0 reaches at its maximum 5.5 and declined after pH 6.5 onward. The maximum rate of activity was noted at pH 5.5 with both the substrates. pNPP showed higher PMEase activity than 4-MUP at all pH levels.

The plants grown without phosphorus added to the growth medium also showed higher PMEase activity than the plants grown with phosphorus added to the growth medium. However, the peak pH for activity with both plant growth conditions was similar with both substrates.

The data in Table 4.3 show highly significant differences ($P = \leq 0.001$) between pH values. Highly significant differences ($P = \leq 0.001$) were observed for the plants grown with and without phosphorus added to the growth medium. Highly significant differences ($P = \leq 0.001$) were obtained between substrates. The interaction of pH, substrates and conditions was also highly significant ($P = \leq 0.001$).

PMEase activity appears to be primarily acidic; however, some activity was detected above pH 7.0.

Table 4.3 Summary of statistical analyses of different conditions of the assays.

ANOVA	F (df)	Probability
pH values	1454 (11)	** 0.001
Conditions	5199 (1)	** 0.001
Substrates	626 (1)	** 0.001
pH x conditions	102 (11)	** 0.001
pH x substrates	28 (11)	** 0.001
Conditions x substrates	217 (1)	** 0.001
pH x subs x conditions	9 (11)	** 0.001

4.4 Relative activities using different substrates

PMEase activity in *cv* Qalandri roots was assayed at the optimum of pH 5.5 using the two substrates in order to assess their relative utilisation. Initially both substrates were used at 71 µM (pNPP) and 100 µM ml-1 (4-MUP). Table 4.4 indicates that in both the conditions of plant growth acid PMEase activity was observed, with both substrates. The highest PMEase activity, (0.150 µmol mg d. wt^{-1} h^{-1}) was noted with pNPP in the plants grown without phosphorus added to the growth medium, and the lowest acid PMEase activity, 0.058 µmol mg d. wt^{-1} h^{-1} was obtained with 4-MUP substrate in the plants grown without phosphorus in the growth medium. However, as indicated earlier no direct comparison can be made between rates with the two substrates because of differences in concentration.

Highly significant differences ($P = \leq 0.001$) were found between the substrates. Also highly significant differences ($P = \leq 0.001$) were noted between the plant growth conditions.

Fig. 4.1 Influence of pH on PMEase activity of roots of cotton *cv* Qalandri grown without and with phosphorus added to the growth medium pNPP and 4-MUP used as substrates

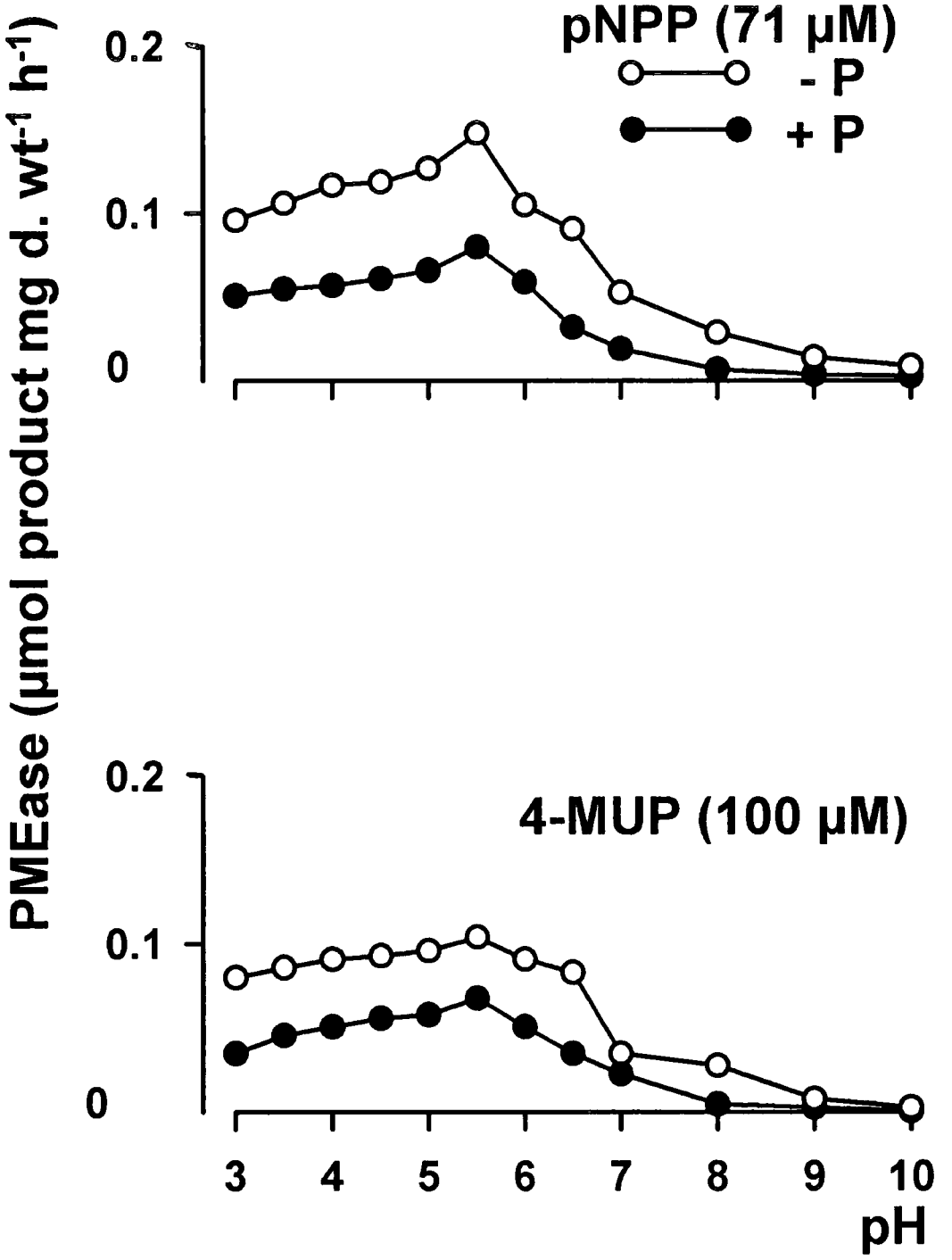


Fig.4.2 Effect of phosphate on PMEase activity of cv Qalandri grown with different phosphorus concentrations added to the growth medium pNPP and 4-MUP used as substrates

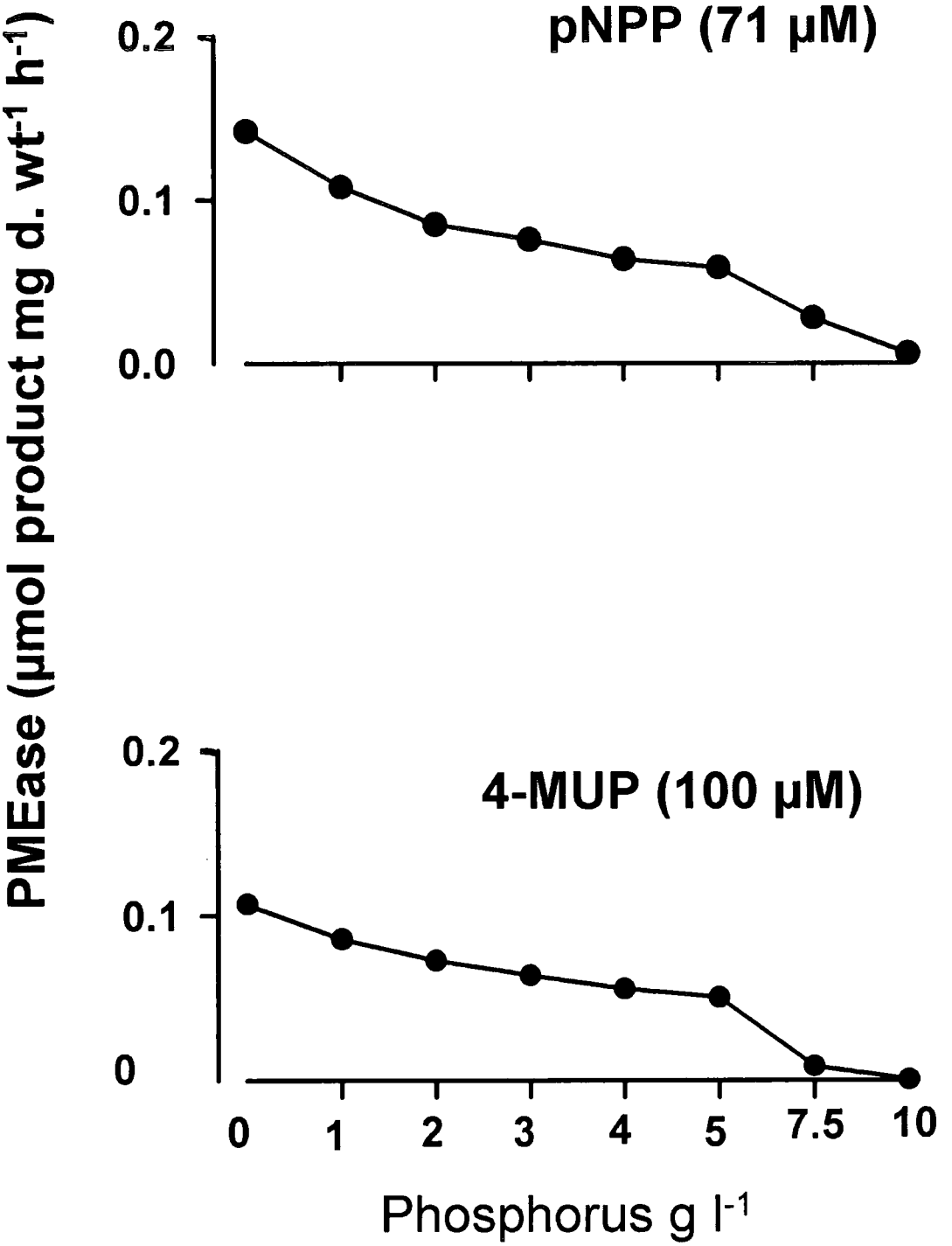


Table 4.4 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown without and with phosphorus added to the growth medium using pNPP and 4-MUP as the substrates (n = 4, ± SD).

Substrates	PMEase activity (μmol mg d. wt^{-1} h^{-1})	
-	- P	+ P
pNPP	0.150 ± 0.001	0.089 ± 0.004
4-MUP	0.105 ± 0.005	0.058 ± 0.003
ANOVA	F (df)	Prob.
Substrates	694 (1)	** 0.001
Conditions	1412 (1)	** 0.001
Substrate x condition.	25 (1)	* 0.05

These results indicate that the phosphatase activity is greater with pNPP as the substrate than with 4-MUP.

4.5 Effect of different phosphate concentrations on PMEase activity in cotton (*cv* Qalandri) roots

In order to assess the most suitable level of phosphate to be added to the plant growth medium in order to suppress PMEase activity the effects of different phosphate concentrations were investigated. For this experiment eight concentrations of phosphate and two substrates (pNPP, 4-MUP) were used.

The effect of different concentrations of phosphate measured using 71 μM pNPP and 100 μM 4-MUP are shown in Fig.4.2. When pNPP was used as a substrate the maximum activity (0.142 μmol mg d. wt^{-1} h^{-1}) was recorded in P-deficient plants. A similar result was obtained when 4-MUP was used as substrate, the highest rate of activity was 0.108 μmol mg d. wt^{-1} h^{-1}. When the concentration of P was increased in the growth medium,

the rate of PMEase activity decreased. When concentrations of phosphate in excess of 0.8 g l^{-1} were used PMEase activity was suppressed for both substrates. The differences between substrates (Table 4.5) were highly significant ($P = \leq 0.001$). The highly significant ($P = \leq 0.001$) differences were recorded among the concentrations of P

Table 4.5 Summary of statistical analyses of results of the comparison of PMEase activity when different concentrations of phosphate were added to the growth medium.

Source	F (df)	Prob.
Concentrations	49 (7)	0.001
Substrates	17 (1)	0.001
Concentration x substrates	N.S	

4.6 Influence of substrate concentration (pNPP or 4-MUP) on PMEase activity of *cv* Qalandri

The objective of this experiment was to standardise the particular concentration of substrate for further study. Also it was important to investigate the capability of the enzyme to hydrolyse the organic phosphate. For this experiment two substrates pNPP & 4-MUP, at optimum pH of 5.5, and one cv were used.

Fig. 4.3 indicates that when pNPP was used as the substrate the highest PMEase activity (0.490, 0.249 µmol mg d. wt^{-1} h^{-1}) was found with the 354 µM concentration, for both growing conditions (without and with phosphorus added to the growth medium) respectively. It was observed the activity increased from a substrate concentration of 18 µM and reach its highest level at 354 µM, afterward the PMEase activity was the same up to 710 µM.

Fig. 4.3 shows that the similar trend of PMEase activity was noted with 4-MUP substrate, while the rate of activity was lower than for the pNPP substrate at all the concentration levels.

4.7 Time course of PMEase activity in *cv* Qalandri

For these experiments three concentrations of pNPP (71, 177 , 710 μM) and 4-MUP (100, 250, 1000 μM), one pH and one cv were used.

The PMEase activity was measured using pNPP and 4-MUP substrates over a 50 min period with readings being taken every 10 min. The time course was started when the substrate was added to the assays which were carried out at pH 5.5. The rate of activity was calculated at half time of the intervals and showed a linear increase over the incubation period for pNPP, Fig.4.4. The rate of activity was still linear up to 50 min in both the conditions P-limited and P-sufficient at the highest concentration of substrate. However, the activity entered a stationary phase for the lower phosphate concentration treatment.

In the case of 100 μM 4-MUP substrate the PMEase activity showed a linear increase over the incubation time (Fig.4.5). At lower concentrations the maximum rate of activity was noted at 50 min in both the conditions, P-limited and P-sufficient.

4.8 Localization

The previous experiments established that cotton roots had PMEase activity associated with them and it was observed that the activity present was acidic. The purpose of this investigation was to assess the location of the PMEase activity in the tissues of the roots of *cv* Qalandri seedlings grown with and without phosphorus added to the growth medium. In order to do this experiments were carried out using different

organic phosphate substrates as described in Materials and Methods which would allow localization by staining.

Fig. 4.3 Effect of concentrations of pNPP & 4-MUP substrates on PMEase activity of *cv* Qalandri seedlings grown without and with phosphorus added to the growth medium

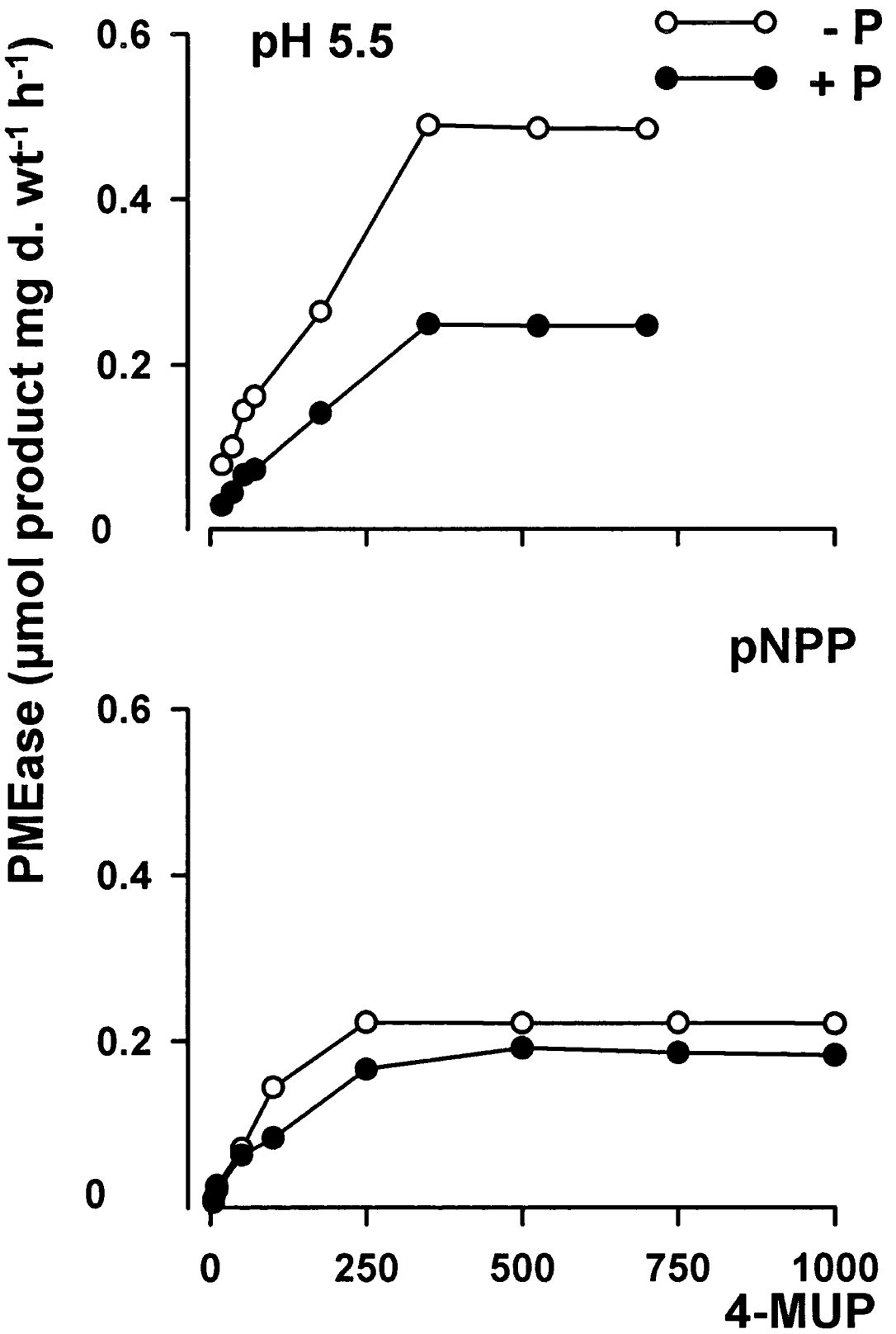


Fig.4.4 Influence of incubation on PMEase activity of roots of *cv* Qalandri grown without and with phosphorus added to the growth medium pNPP used as substrate

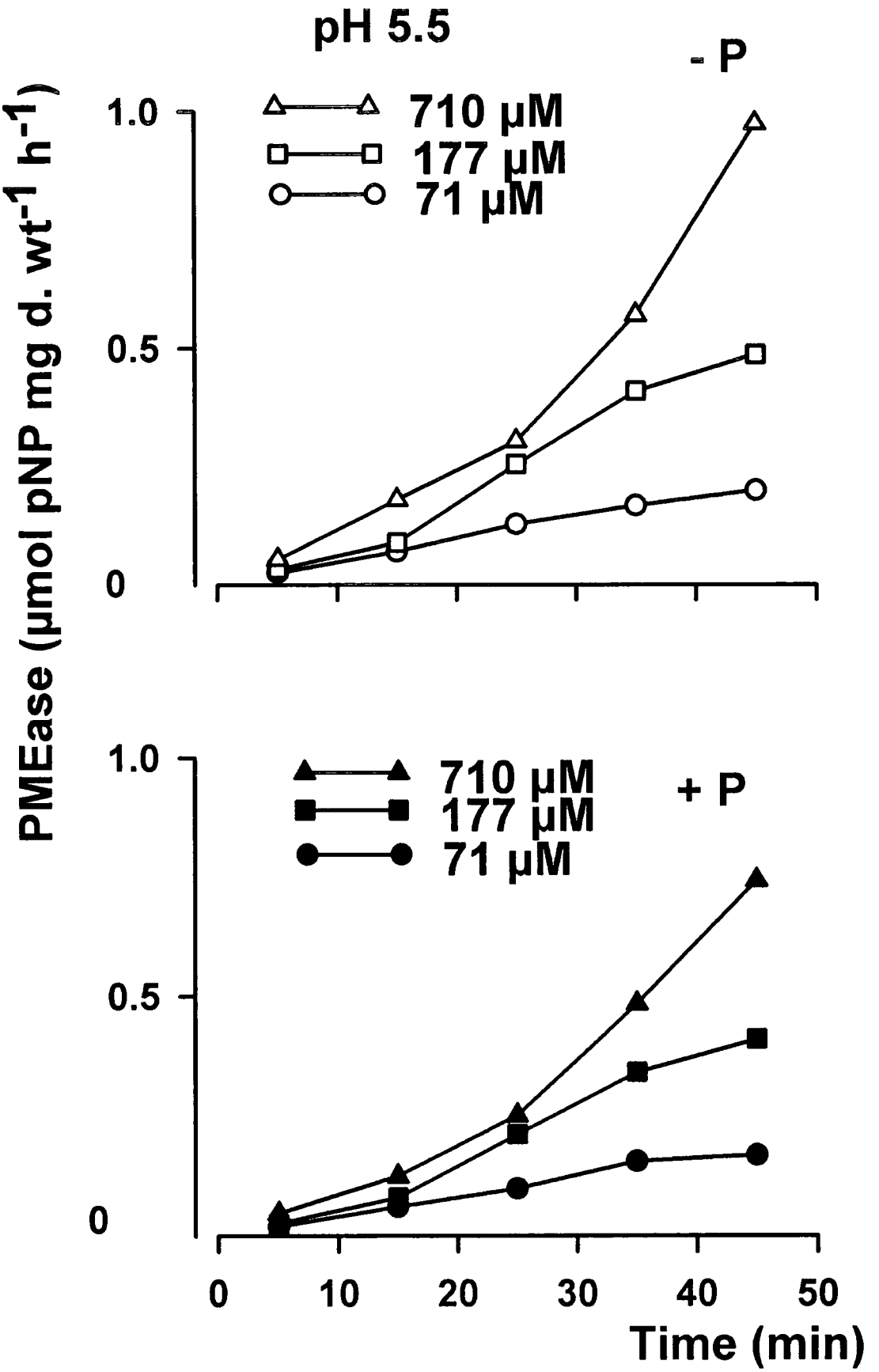
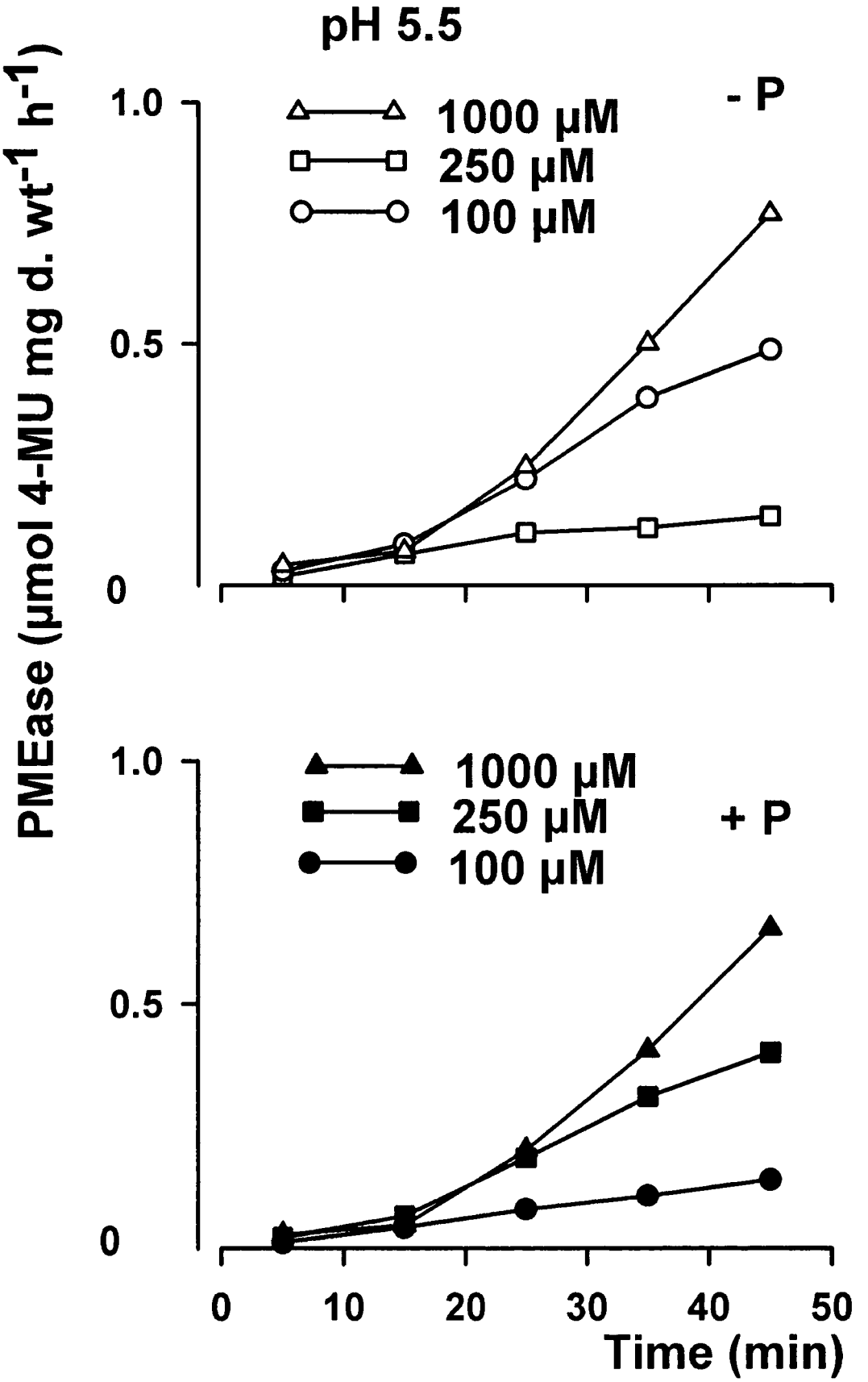


Fig.4.5 Influence of incubation on PMEase activity of roots of *cv* Qalandri grown without and with phosphorus added to the growth medium 4-MUP used as substrate



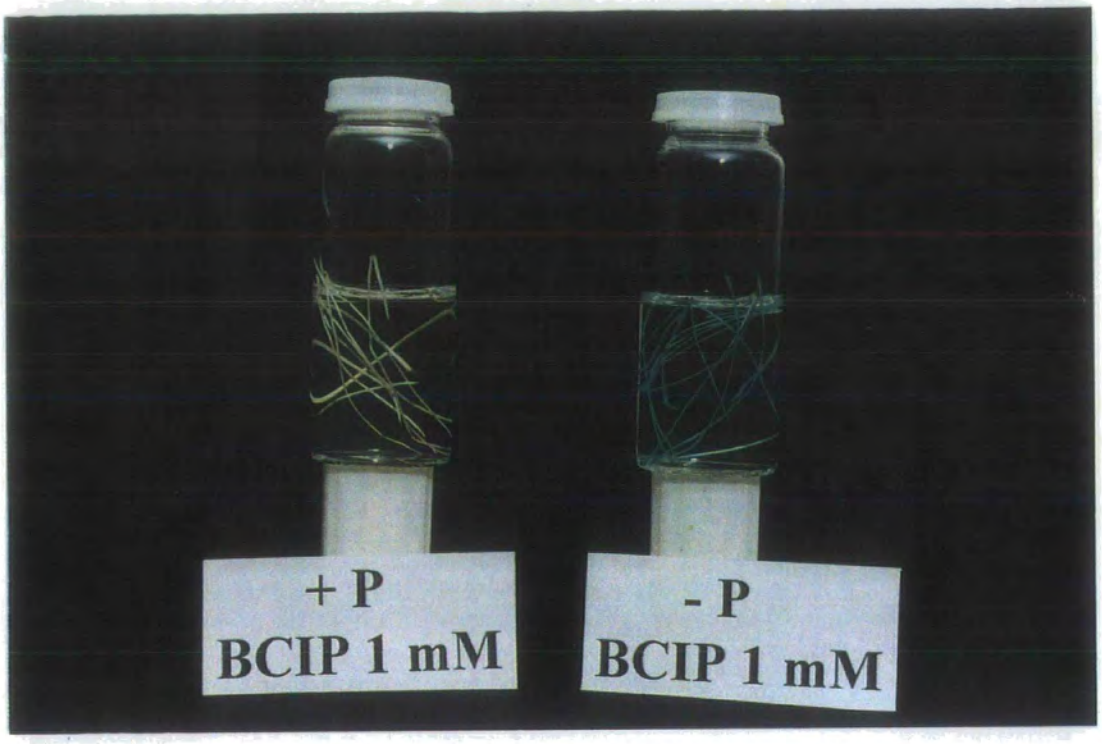


Fig. 4.6 Roots of cotton plants from growth conditions where phosphate were added or omitted stained with BCIP for 30 min.

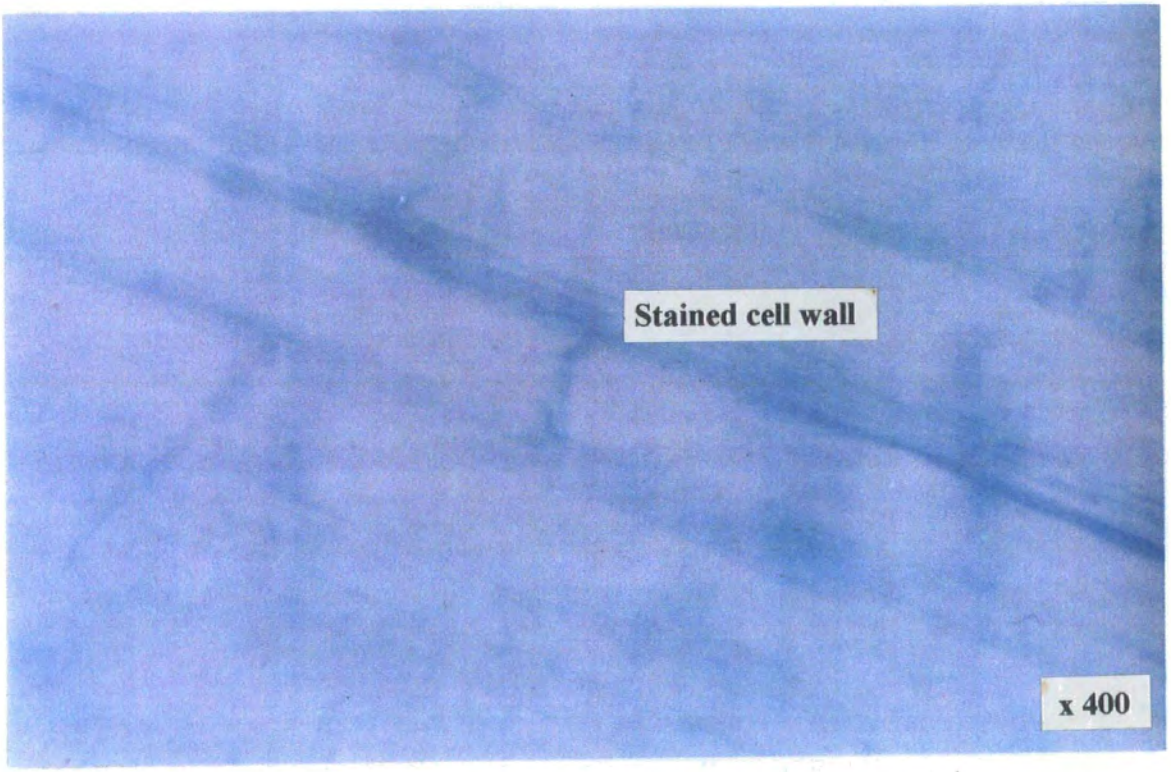


Fig. 4.7 Localization of PMEase activity in cell walls of cotton roots of plants grown without phosphorus added to the growth medium stained with BCIP.

Fig.4.6 shows that by staining with BCIP it was possible to observe PMEase activity at the root surface, especially in the cell walls. After 30 min of incubation at 25 °C temperature in BCIP the P-limited plant roots exhibited strongly stained tissues. In these plants the roots contained high PMEase activity. When the lateral roots were examined at 400 x magnification, the stained cells were found to contain a small amount of stain. In the case of P-sufficient plants roots were very lightly stained.

When Fast Garnet GBC salt β-naphthyl phenyl phosphate was used the PDEase activity was clearly visible as staining in the whole tissue Fig. 4.7.

No enzyme activity was observed with naphthol AS-MX phosphate and Fast blue RR salt. No staining was found in either P-limited or P-sufficient growth conditions.

4.9 Effect of temperature on PMEase activity in roots of *cv* Qalandri

An experiment was carried out to determine the influence of temperature on PMEase activity of *cv* Qalandri seedlings grown with and without phosphorus added to the growth medium in order to establish optimum conditions and range of activity.

For this experiment six different temperatures, two substrates (pNPP, 4-MUP), and two phosphorus conditions were used.

The influence of temperature on PMEase activity of *cv* Qalandri was quantified by using pNPP and 4-MUP (Fig. 4.8). Temperature optima of 55°C for PMEase activity with both the substrates were recorded. The maximum rate of activity (0.177 µmol mg d. wt $^{-1}$ h $^{-1}$) was observed in roots of plant grown under P-limited condition while using pNPP as substrate. With the same substrate less activity (0.144 µmol mg d. wt $^{-1}$ h $^{-1}$) was noted in the roots of plants grown under P-sufficient conditions. While a lower rate of activity (0.126 µmol mg d. wt^{-1} h^{-1}) was observed with 4-MUP substrate in P-sufficient plant roots.

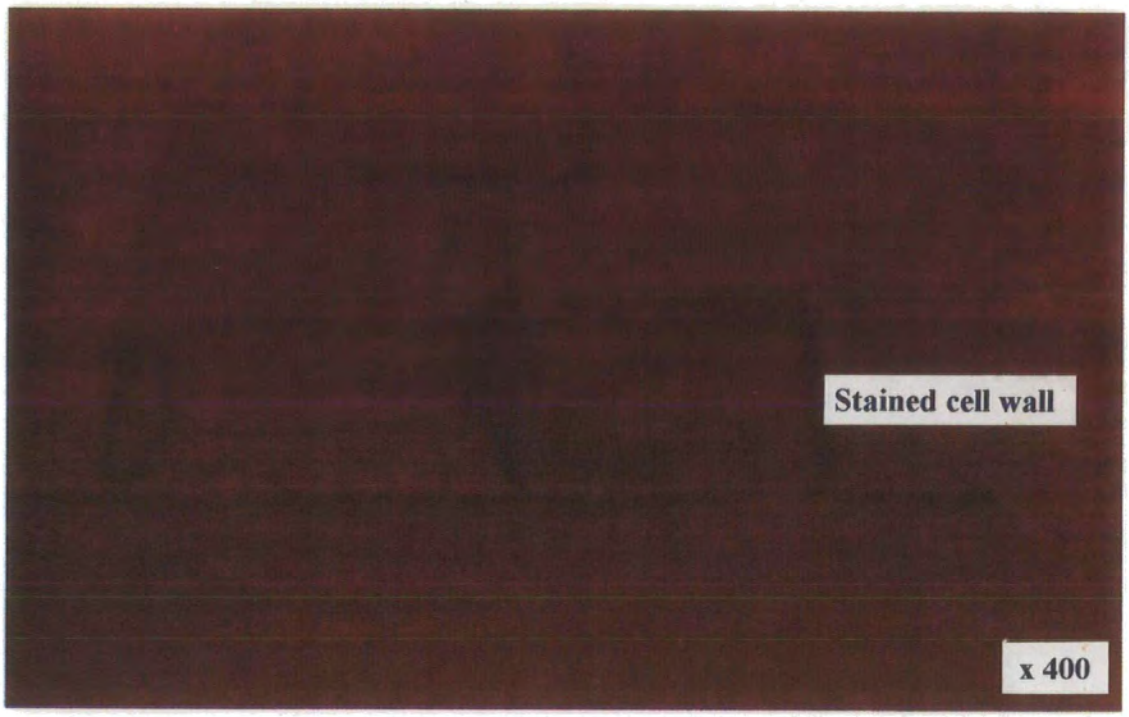


Fig.4.8 Localization of PDEase activity in whole tissues of cotton roots of plants grown without phosphorus added to the growth medium stained with β-naphthyl phenyl phosphate

Fig.4.9 Effect of different temperatures on PMEase activity of roots of *cv* Qalandri grown without and with phosphorus added to the growth pNPP and 4-MUP used as substrates

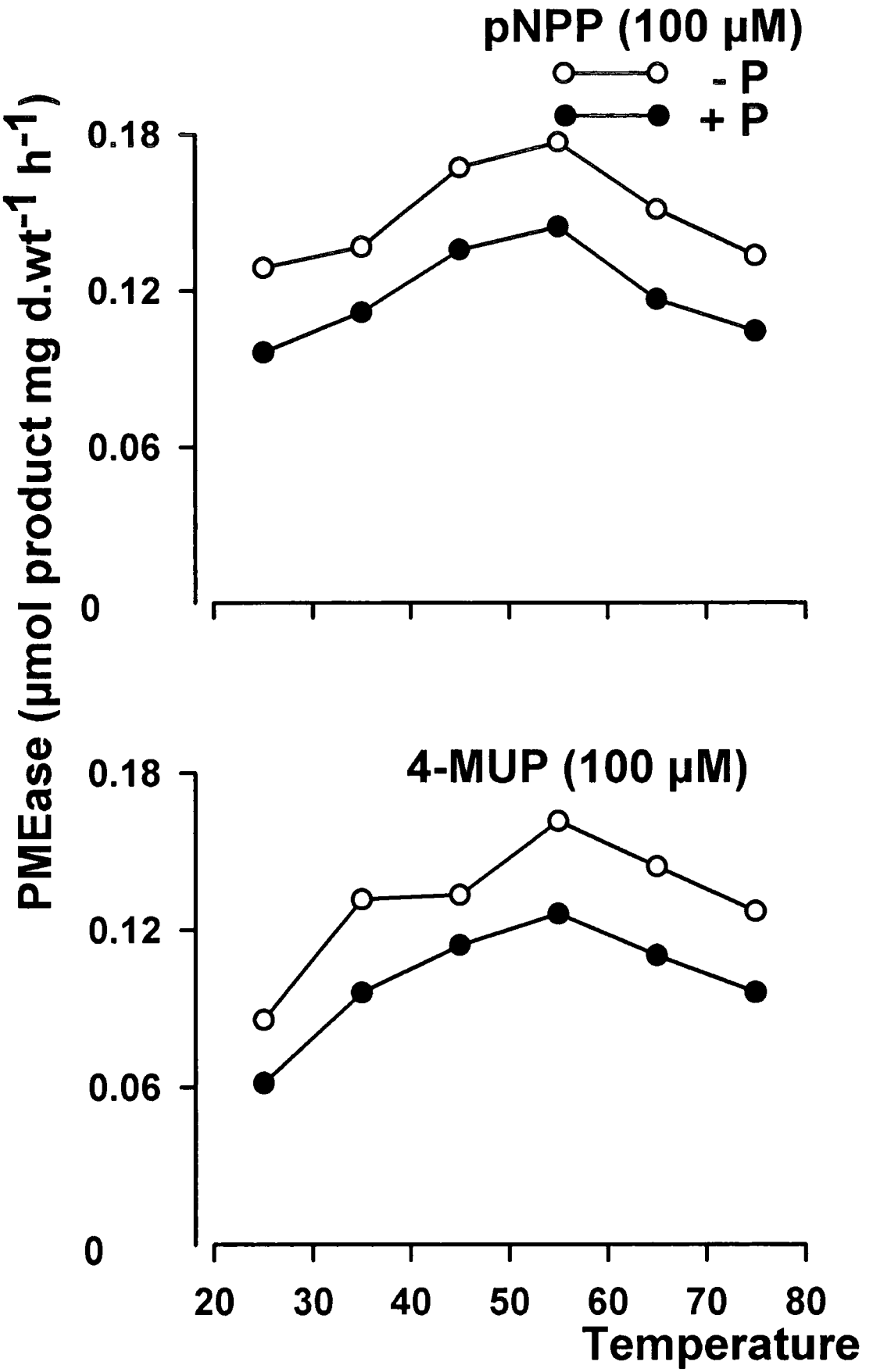


Table 4.6 indicates that there were highly significant differences ($P = \leq 0.001$) between activities at different temperatures. The interaction between temperature and growth conditions were statistically significant differences ($P = \leq 0.05$). The data also indicate that interactions among temperature, growth conditions and substrates were significant ($P = \leq 0.05$).

Table 4.6 Summary of statistical analyses of effects of different temperatures on PMEase activity of *cv* Qalandri roots.

ANOVA	F (df)	Probability	
Temperature	542 (5)	**	0.001
Conditions	1797 (1)	**	0.001
Substrates	625 (1)	**	0.001
Temperature x condition	4 (5)	*	0.05
Temperature x substrate	55 (5)	**	0.001
Condition x substrate	N.S		
Temp. x condition x substrate	5 (5)	*	0.01

4.10 Effect of ions on PMEase assay

It has been shown that PMEase activity in a number of organisms is influenced by the levels and types of ions included in the assay medium.

In order to study this an investigation of the effect of ions on the PMEase assay was made. In order to do so Na, Ca, Mg or K was omitted individually from the assay medium. The effects of individual ions on PMEase activity of *cv* Qalandri roots was

assayed on plants grown with and without phosphorus added to the growth medium. For this experiment one substrate and an assay pH of 5.5 was used.

The activity was measured using 4-MUP as the substrate (Fig.4.9). The highest activity (0.209 μmol mg d. wt^{-1} h^{-1}) was recorded in control P-deficient plants where all ions were present in the assay. The differences between conditions (Table 4.7) were highly significant ($P = \leq 0.001$). Non-significant differences were noted among the ions. The interaction between conditions x ions also were non-significant

Table 4.7 Effect of different ions on the PMEase assay using 4-MUP as substrate.

Ions	PMEase activity (μmol mg d. wt^{-1} h^{-1})	
	- P	+ P
Na	0.146 ± 0.01	0.085 ± 0.01
Ca	0.143 ± 0.01	0.073 ± 0.01
Mg	0.147 ± 0.02	0.091 ± 0.00
K	0.165 ± 0.01	0.078 ± 0.01
Control	0.209 ± 0.02	0.082 ± 0.00
ANOVA	F (df)	Probability
Treatment	N.S	** 0.001
Condition	60 (1)	-
Treatment x condition	N.S	-

4.11 Retention of product in, and leaching of activity, from roots of (*cv* Qalandri)

In order to assess the retention of PMEase activity in the roots, assays were carried out using a range of pH values, and two substrates. During normal assay procedures

surface PMEase in particular was measured, however, the probability exists that the substrate entered the cells and then was hydrolysed by cellular PMEase. Also the secretion of cellular phosphatase activity onto the cell surfaces could cause erroneous and variable surface PMEase activities. In order to clarify these probabilities the following assay was performed.

Seedlings were grown without phosphate added to the growth medium to maximise PMEase activity, and were assayed in the normal way over range of pH values with pNPP and 4-MUP substrates. At the end of 30-min incubation the tissue was removed from the assay tube and was washed in assay medium, and the PMEase activity was assayed again as before for one h.

Table 4.7 shows that less then 1 % of original activity was found during the second assay. However with 4-MUP at pH 7.0 and above there was increase up to 11 % at pH 10.0. This could reflect leaching of enzyme or reaction product and may be related to the higher pH values altering root permeability problems. No such responses was seen for pNPP.

Since assays were performed routinely at pH 5.5 it is unlikely that product retention or enzyme leaching would be of any significance since only 0.13 % of the original assay activity was detected at this pH.

Table 4.8 PMEase activity and its retained percentage in the roots of cotton which had been assay for 30 min and then for a further hour following washing. pNPP (71 µM) and 4-MUP (100 µM) were used as the substrates for *cv* Qalandri.

pH	PMEase activity (µmol mg d. wt^{-1} h^{-1})					
	pNPP			4-MUP		
	Normal activity	Retained activity	% Retained	Normal activity	Retained activity	% Retained
3.0	0.116 ± 0.003	0.00030 ± 0.00019	0.25	0.057 ± 0.001	0.00012 ± 0.00004	0.22
3.5	0.129 ± 0.003	0.00027 ± 0.00018	0.20	0.063 ± 0.001	0.00026 ± 0.00005	0.41
4.0	0.139 ± 0.001	0.00028 ±0.00019	0.21	0.092 ± 0.002	0.00055 ± 0.00001	0.60
4.5	0.145 ± 0.001	0.00036 ± 0.00014	0.49	0.096 ± 0.002	0.00058 ± 0.00002	0.61
5.0	0.157 ± 0.003	0.00036 ± 0.00018	0.23	0.105 ± 0.002	0.00064 ± 0.00003	0.43
5.5	0.196 ± 0.006	0.00044 ± 0.00012	0.13	0.111 ± 0.001	0.00069 ± 0.00001	0.66
6.0	0.171 ± 0.002	0.00045 ± 0.00012	0.26	0.102 ± 0.001	0.00080 ± 0.00002	0.79
6.5	0.121 ± 0.002	0.00054 ± 0.00008	0.10	0.089 ± 0.003	0.00066 ± 0.00002	0.25
7.0	0.093 ± 0.002	0.00057 ± 0.00020	0.87	0.025 ± 0.002	0.00055 ± 0.00003	2.24
8.0	0.056 ± 0.001	0.00067 ± 0.00004	0.38	0.019 ± 0.000	0.00046 ± 0.00002	2.40
9.0	0.040 ± 0.001	0.00073 ± 0.00021	0.14	0.001 ± 0.000	0.00012 ± 0.00001	7.08
10.0	0.028 ± 0.001	0.00079 ± 0.00001	0.78	0.001 ±0.000	0.00009 ± 0.00001	10.98

4.12 Influence of plant age on PMEase activity of *cv* Qalandri

As plants grow their nutritional status changes and with this so could the requirement for PMEase activity. In order to assess the magnitude of this effect PMEase activity was assayed in plants of different ages. Also it was important to standardise plant age; plants which were grown for three different times were utilized here for further experiments. Seedlings were grown with and without phosphorus added to the growth medium. A range of 12 pH values (3-10) and two substrates were used for this study.

4.121 13-day old seedlings

Seeds were germinated and after five days seedlings were transferred to water culture (see Materials and Methods). After a further 2 days the water was replaced with growth medium, with or without added phosphorus. Assay for PMEase was carried out after a further period of six days. The PMEase activity was measured using the two substrates pNPP and 4-MUP as shown in Fig. 4.10. The maximum rate of activity was observed at pH 3.0 with both substrates being 0.099, 0.086 μmol mg d. wt^{-1} h^{-1} was noted in the plants grown without phosphorus added to the medium with pNPP and 4-MUP substrates respectively. However, another peak of activity was also noted at pH 6.0, (0.092 μmol mg d. wt^{-1} h^{-1}) with pNPP substrate while with 4-MUP substrate the second peak was shifted to pH 5.5 (0.082 μmol mg d. wt^{-1} h^{-1}) after which the activity gradually declined to pH 7.0. Above that pH limit the PMEase activity was negligible. pNPP showed a slightly higher activity compared with 4-MUP at pH 3.0. The overall activity was higher at all pH levels for pNPP compared with 4-MUP. The PMEase activity of roots of P-sufficient plants was less than that of P-limited plants for both the substrates. However, the optimum pH for activity peaks of P-sufficient and P-limited seedlings was similar with both substrates. The statistical differences between (Table 4.9) the plants grown under P-limited and P-sufficient conditions were highly significant ($P = \leq 0.001$). Highly significant differences ($P = \leq 0.001$) were recorded for the age of plants.

4.122 19-day old seedlings

PMEase activity was detected between pH 3-7 (Fig. 4.10) with optimal activity at pH 5.5. These seedlings were grown for 12 days following transfer to nutrient solution. The maximum rate of activity (0.194 µmol mg d. wt $^{-1}$ h $^{-1}$) was noted in plants grown under P-limited condition at pH 5.5, with pNPP as substrate. However, when the activity was tested using 4-MUP as substrate the same results were observed in that the maximum rate of activity (0.157 µmol mg d. wt^{-1} h^{-1}) occurred at pH 5.5. Highly significant differences ($P = \leq 0.001$) were found between substrate (Table 4.9). The ratio of PMEase activity of pNPP to 4-MUP was recorded as 1.23 at their peak pH. Highly significant differences ($P = \leq 0.001$) were noted among pH. levels. It was noted that PMEase activity in these plants was higher for both substrates when compared with the 13-day old seedlings. No peak of activity was detected at pH 3.0 as for the 13-day old seedlings.

4.123 25-day old seedlings

These seedlings were grown for 18 days following transfer to nutrient solution. Over the range of pH values tested, the maximum rate of activity was observed at pH 5.5 with both P-limited and P-sufficient conditions (Fig 4.10). A rate of 0.09 µmol mg d. wt^{-1} h^{-1} was observed in roots of P-limited plants and 0.07 µmol mg d. wt^{-1} h^{-1} was noted in roots of P-sufficient plants while using pNPP substrate.

In the case of 4-MUP different results were noted. Again the peak of activity was recorded at pH 5.5 in both P-limited and P-sufficient conditions. The rates of activity observed were 0.08, 0.07 µmol mg d. wt^{-1} h^{-1} P-limited and P-sufficient conditions respectively.

Table 4.9 shows that the interactions of substrate and phosphorus conditions were highly significant ($P = \leq 0.001$). Highly significant differences ($P = \leq 0.001$) were recorded for interactions among pH and the age of plants. The data also indicate highly

Fig. 4.10 Effect of plant age on PMEase activity of roots of *cv* Qalandri grown without and with phosphorus added to the growth medium pNPP used as substrate

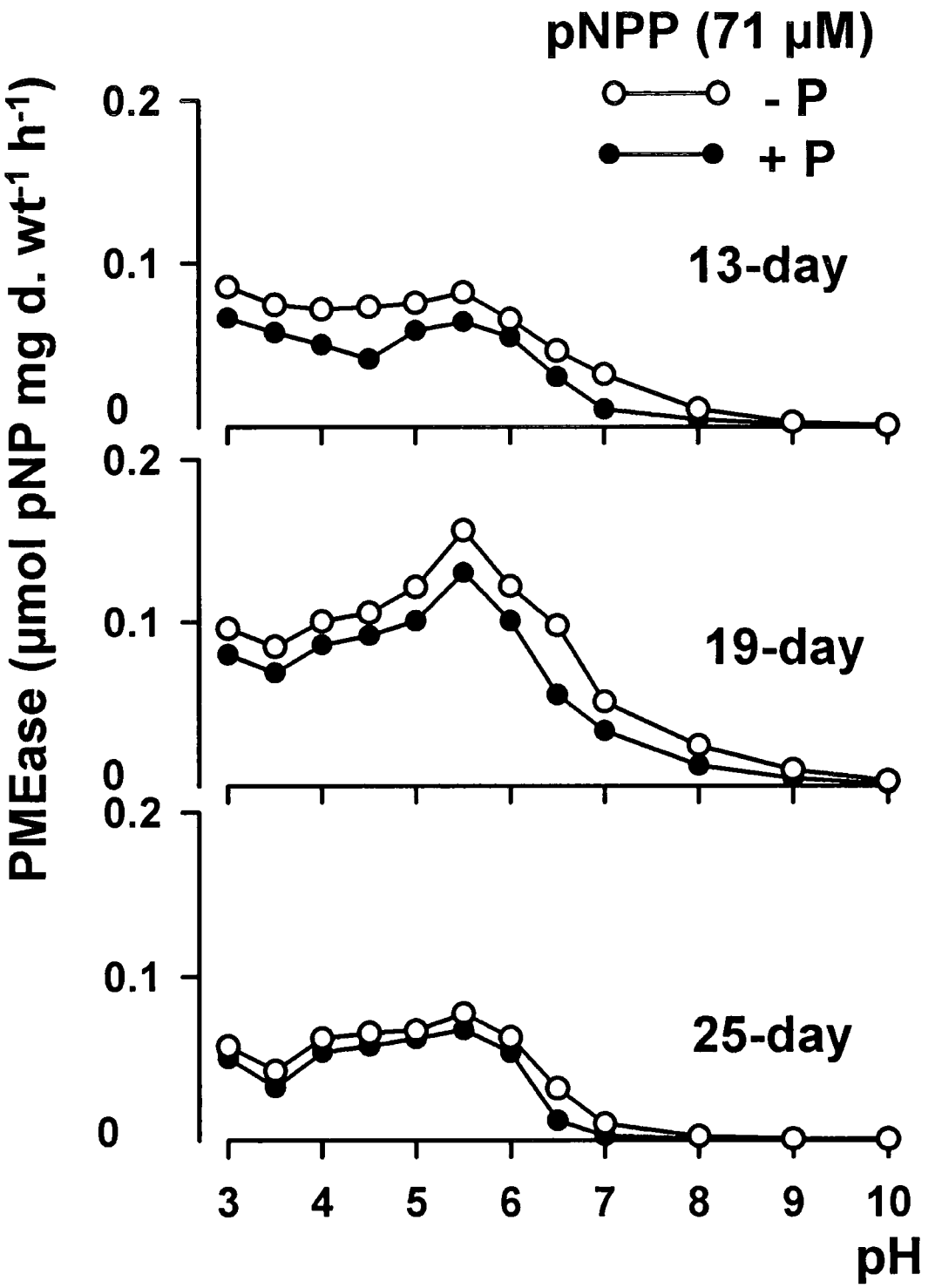
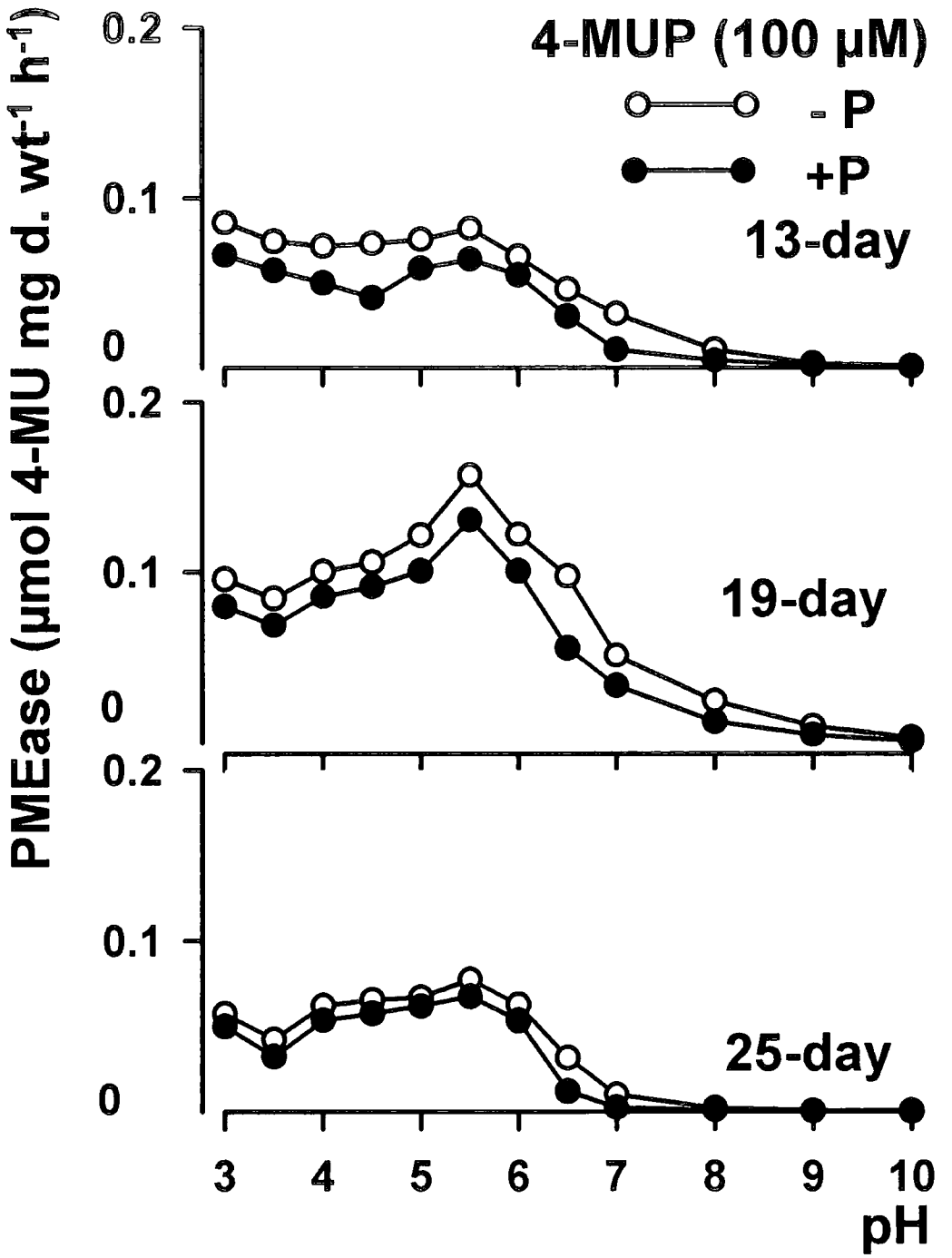


Fig. 4.11 Effect of plant age on PMEase activity of roots of *cv* Qalandri grown without and with phosphorus added to the growth medium 4-MUP used as substrate



significant differences ($P = \leq 0.001$) were observed between phosphorus conditions and the age of plants. Interactions of substrate, pH and age of the plants were highly significant ($P = \leq 0.001$).

These data show that PMEase activity increased from 13-day old seedlings to the 19-day old seedling and then decreased in the 25-day old seedlings. This was observed for both substrates and both growing conditions.

Table 4.9 Summary of statistical analyses of effects of different ages of *cv* Qalandri seedlings on PMEase activity.

ANOVA	F (df)	Probability
Age	1288 (2)	** 0.001
pH	775 (11)	** 0.001
Substrate	197 (1)	** 0.001
Condition	522 (1)	** 0.001
Age x pH	35 (22)	** 0.001
Age x substrate	17 (2)	** 0.001
Age x condition	32 (2)	** 0.001
Age x pH x substrate	4 (22)	** 0.001
Age x substrate x condition	3 (2)	* 0.05

4.13 Effect of growth medium conditions on PMEase activity and phosphorus content in *cv* Qalandri seedling roots

The increase in PMEase activity between the 13-and 19-day old seedlings could result from a build up of phosphate deficiency in the plant as a whole. This would be

particularly the case for the plants grown with phosphate added where phosphorus supply could become limiting.

In order to test this possibility were experiments set up where the level of phosphate in the growth medium was renewed or replaced with medium without added phosphate at times during the 19-day growth period. To do this seedlings were grown for the 12-day treatment period with or without added phosphorus but for some plants the medium was changed at 6 days of treatment. Here the plants were transferred to fresh medium which contained no added phosphorus or phosphorus added to the normal level.

Table 4.10 indicates that when pNPP was used as a substrate the highest PMEase activity 0.15 μmol mg d. wt^{-1} h^{-1} was noted in the plants grown 6 days without phosphorus added to the growth medium followed by 6 days in fresh medium (-P). Fig.4.1 shows clearly that the longer treatment period for seedlings resulted in an increase in PMEase activity both in the media with and without added phosphate (Treatments 1 & 2 and 5 & 6). In both cases highest activity was found in the plants grown without added phosphorus.

Plants which were grown without added phosphate for 6 days followed by a further 6 days in fresh medium without phosphate (7) had higher activity than those grown similarly with media containing phosphorus (8). However, both of these sets of plants had higher activity than those grown in the same type of medium but without it being changed (5 & 6).

When plants were grown in medium without added phosphorus for 6 days followed by a further 6 days in medium with phosphorus (3) there was a supression of PMEase activity. The transfer of plants from conditions of phosphorus addition to those of no phosphorus addition related in a increased PMEase activity (4).

The important determination was that the PMEase activity built up in plants here when the level of phosphate in the plant medium was maintained (8). This indicates that PMEase level is related to the age of the plant rather than phosphate limitation in the growth medium

Root internal phosphate levels are not closely converted with surface PMEase activity. It therefore, is probable that phosphatase activity is modulated by phosphate conditions at the surface of the roots. This is shown in Table 4.10 where under treatment 2 there is a high phosphate level but low PMEase activity compared with 3 where there is a similar PMEase activity but a low phosphate level. Similar effects were seen in a comparison of 4 and 8. The assays using 4-MUP, however, did not show these effect as clearly as in those where pNPP was used.

Table 4.10 Effect of growth medium on PMEase activity (μmol mg d. wt^{-1} h^{-1}) at pH 5.5, and phosphorus content in *cv* Qalandri seedling roots (n = 4, ± SD).

Treatments	Phosphorus content		PMEase activity (μmol mg d. wt^{-1} h^{-1})	
	mg g-1 d. wt	d. wt	pNPP	4-MUP
1	19.17 ± 1.26	0.02 ± 0.00	0.06 ± 0.00	0.04 ± 0.00
2	56.44 ± 1.13	0.03 ± 0.00	0.05 ± 0.00	0.03 ± 0.00
3	14.48 ± 0.75	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
4	14.64 ± 1.18	0.05 ± 0.00	0.10 ± 0.00	0.05 ± 0.00
5	15.62 ± 1.52	0.03 ± 0.00	0.13 ± 0.00	0.08 ± 0.00
6	52.18 ± 2.37	0.06 ± 0.00	0.08 ± 0.00	0.05 ± 0.00
7	13.57 ± 0.46	0.03 ± 0.00	0.15 ± 0.00	0.09 ± 0.00
8	53.75 ± 0.85	0.07 ± 0.01	0.10 ± 0.00	0.05 ± 0.00
ANOVA	F (df) F.Prob.		F (df) F.Prob.	
Treatment	920 (7) **		928 (7) **	
Substrates	-		3771 (1) **	
Treat x subs	-		78 (7) **	

1 = 6 days without phosphorus added to the growth medium

2 = 6 days with phosphorus added to the growth medium

3 = 6 days without phosphorus added to the growth medium, followed by 6 days with phosphorus added to the growth medium

4 = 6 days with phosphorus added to the growth medium, followed by 6 days without phosphorus added to the growth medium

5 = 12 days without phosphorus added to the growth medium

6 = 12 days with phosphorus added to the growth medium

7 = 6 days without phosphorus added to the growth medium, followed by 6 days in fresh medium (-P)

8 = 6 days with phosphorus added to the growth medium, followed by 6 days in fresh medium (+P)

4.14 Split root system

In order to investigate further the influence of phosphate in the root in contrast to that in the medium split root experiments were set up. Here one half of the root system was incubated in a medium to which phosphate was added while the other half was maintained in medium without added phosphorus. PMEase activity was then assayed and compared for the two sets of root tissues.

For this experiment the optimum pH of 5.5, two substrates pNPP & 4-MUP, and *cv* Qalandri were used.

The results in Table 4.11 indicate that the half set of roots without phosphorus added to the medium gave higher PMEase activity with both the substrates pNPP, 4-MUP respectively. The other set of roots grown with phosphorus added to the growth medium gave less PMEase activity also with both the substrates pNPP, 4-MUP. The phosphorus content in the roots which were grown up with phosphorus added to medium showed a higher phosphorus concentration (13.92 mg g^{-1} d. wt) in comparison to the roots grown without phosphorus added to the medium (8.02 mg g^{-1} d. wt).

Table 4.11 Split root system of *cv* Qalandri seedlings, PMEase activity (μmol mg d. wt^{-1} h^{-1}) and phosphorus content (mg g^{-1} d. wt) (n = 4, ± SD).

Substrate	PMEase activity (μmol mg d. wt^{-1} h^{-1})		Phosphorus content			
			Leaf	Stem	Root	
	- P	+ P		-	- P	+ P
pNPP	0.195	0.081	19.44	6.99	8.02	13.92
4-MUP	0.080	0.034	-	-	-	-

4.15 Comparison of PMEase activity of three cotton cultivars

4.151 Overview

The PMEase activity of three different cotton cultivars, from different (origin) parents grown in different ecological zones of Sindh Pakistan were used as detailed in Chapter 2. Each *cv* was assessed for its PMEase activity in terms of pH range, peak activity and substrate utilization. These assessments were carried out on plants which had been grown both without and with phosphate added to the growth medium.

A comparison was made to estimate PMEase activity of the different cultivars in order to assess their potential for utilising of soil organic phosphate with particular reference to soil conditions in Pakistan.

4.152 *cv* Qalandri

The results in Fig. 4.12, where pNPP was used as the substrate, show the maximum PMEase activity at pH 5.5. The activity increased after pH 4.0 to the maximum at pH 5.5 and started to decrease from pH 6.5 to 10.0. Table 4.12 shows that the plants raised without and with phosphorus added to the growth medium showed a highly significant difference in activity ($P = \leq 0.001$). The roots of plants grown without

phosphorus in the medium showed more PMEase activity at all pH levels, the range being 0.009 (pH 10.0) to 0.215 µmol mg d. wt^{-1} h^{-1} (pH 5.5) in comparison with the plant roots grown with phosphorus added to the medium where the range was 0.002 (pH 10.0) to 0.151 µmol mg d. wt^{-1} h^{-1} (pH 5.5). In terms of percentage, the roots of plants grown without phosphorus added to the growth medium yielded 29 % more activity than those with phosphorus, at their peak pH level. Highly significant differences ($P = \leq 0.001$) (Table 4.12) were found among pH levels. It is clear (Fig. 4.12) that the peaks of PMEase activity for without and with phosphorus added plant roots were observed at pH 5.5. Statistically highly significant results were found for the interaction of P-condition and pH levels (Table 4.12).

Fig. 4.12 shows that when the substrate 4-MUP was used acid PMEase activity was recorded with the peak activity at pH 5.5. The activity increased from pH 4.0 and reached its climax at pH 5.5. Activity declined from pH 6.5 onwards. The data (Table 4.12) for the plants grown with and without phosphorus added to the growth medium conditions were highly significant ($P = \leq 0.001$).

The plants grown without phosphorus added to the growth medium showed higher PMEase activity at all pH levels ranging from 0.004 (pH 10.0) to 0.184 µmol mg d. wt^{-1} h^{-1} (pH 5.5) against plants grown with phosphorus added to the growth medium having 0.002 (pH 10.0) to 0.138 µmol mg d. wt^{-1} h^{-1} (pH 5.5). The ratio of PMEase activity of the plants grown without phosphorus added to the growth medium and the plants grown with phosphorus to the growth medium was recorded as 1.33 at peak pH. Highly significant differences ($P = \leq 0.001$) were noted between pH's.

Highly significant differences ($P = \leq 0.001$) were recorded in interaction between pH and phosphorus status condition. For both conditions of the phosphorus a straight line slope (Fig. 4.13) was obtained.

Activity declined from pH 6.5 onwards. The data (Table 4.12) for the plants grown without or with phosphorus added to the growth medium were both highly significant ($P = \leq 0.001$).

4.153 *cv* Rehmani

When pNPP was used as a substrate the maximum PMEase activity was recorded at pH 6.0 (Fig. 4.12). The activity increased after pH 4.0 with a maximum at pH 6.0 and then declined from pH 6.5 to 10.0. Plants in low P conditions gave values of 0.206 µmol mg d. wt^{-1} h^{-1} at pH 6.0 which declined to 0.063 µmol mg d. wt^{-1} h^{-1} at pH 10.0. Plants with P gave PMEase activities of 0.079 µmol mg d. wt^{-1} h^{-1} at pH 4.0 which decreased to 0.012 µmol mg d. wt^{-1} h^{-1} at pH 10.0. The PMEase activity ratio was 2.59 calculated for plants with P compared to those without P. The plants grown without phosphorus added to the growth medium gave 61 % more PMEase activity than plants grown with phosphorus added to the growth medium at their peak pH. The activities at all pH levels (Table 4.12) were highly significantly different for the two sets of plants ($P = \leq 0.001$).

When 4-MUP was used as the substrate it was noted that the PMEase activity increased after pH 4.0 to its peak at pH 5.5. It started to decline from pH 6.5 up to the higher pH of 10.0. The data (Table 4.12) indicated that plants raised without phosphorus added to the growth medium and the plants grown with phosphorus added to the growth medium were highly significantly different from each other in PMEase activity ($P = \leq 0.001$). The plants grown with phosphorus gave low PMEase activity ranging from 0.001 (pH 10.0) to 0.098 µmol mg d. wt^{-1} h^{-1} (pH 4.0) in comparison with the plants grown without phosphorus which showed activities ranging from 0.001 (pH 10.0) to 0.157 µmol mg d. wt^{-1} h^{-1} (pH 5.5). The increased percentage of PMEase activity in plants grown without phosphorus added to the growth medium over the plant grown with phosphorus added to the growth medium was 37 % at peak pH. The ratio of activity at peak pH was 1.59 (without v/s with phosphorus added to the growth medium).

It is evident (Fig. 4.13) that in the roots of the plants grown without phosphorus, higher PMEase activity was detected than in roots of the plants grown with phosphorus at all pH levels. Different pH peaks of activity were noted for without phosphorus added

Fig. 4.12 Influence of pH on PMEase activity of roots of three cotton cultivars grown without and with phosphorus added to the growth medium pNPP used as substrate

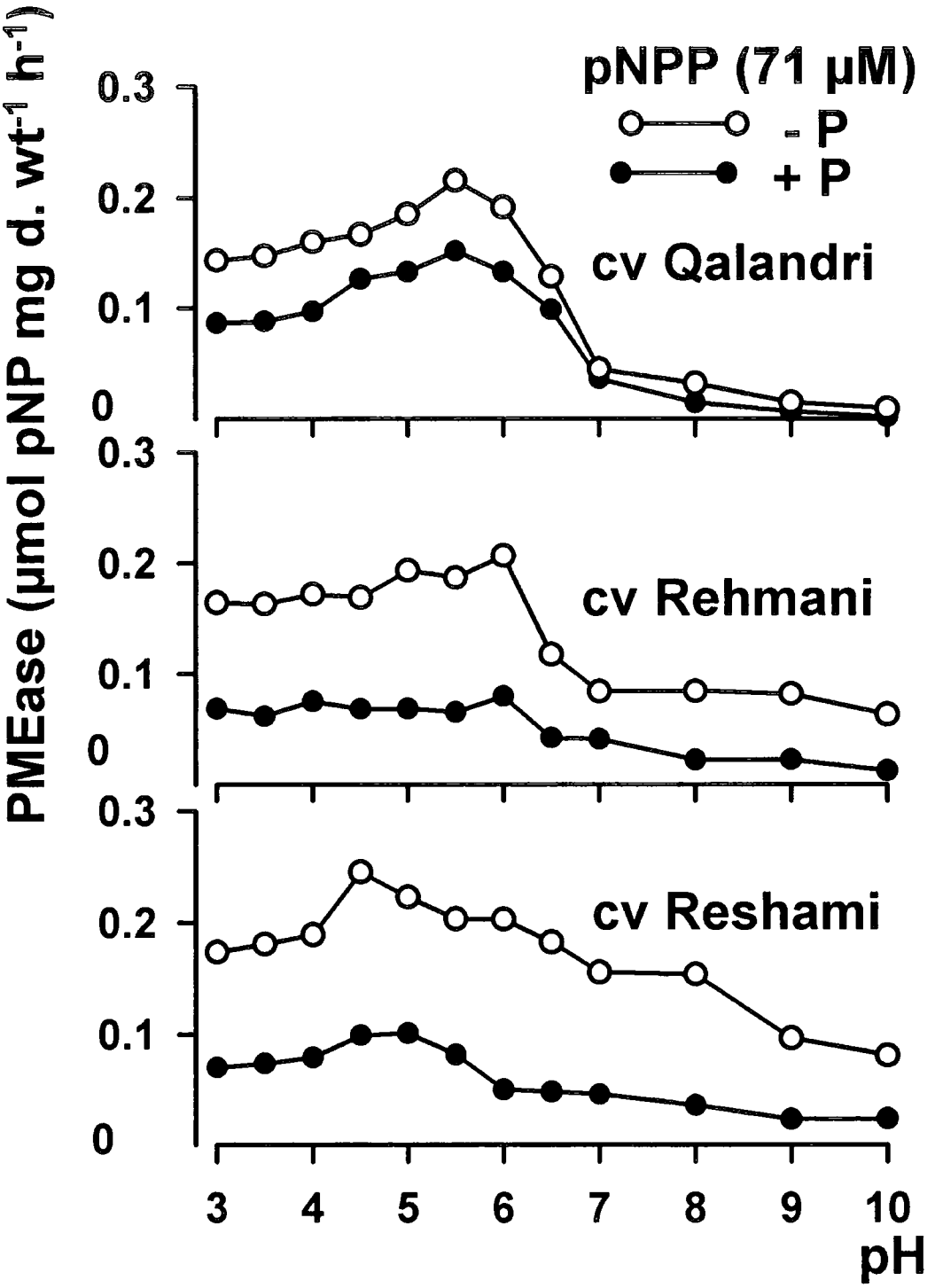
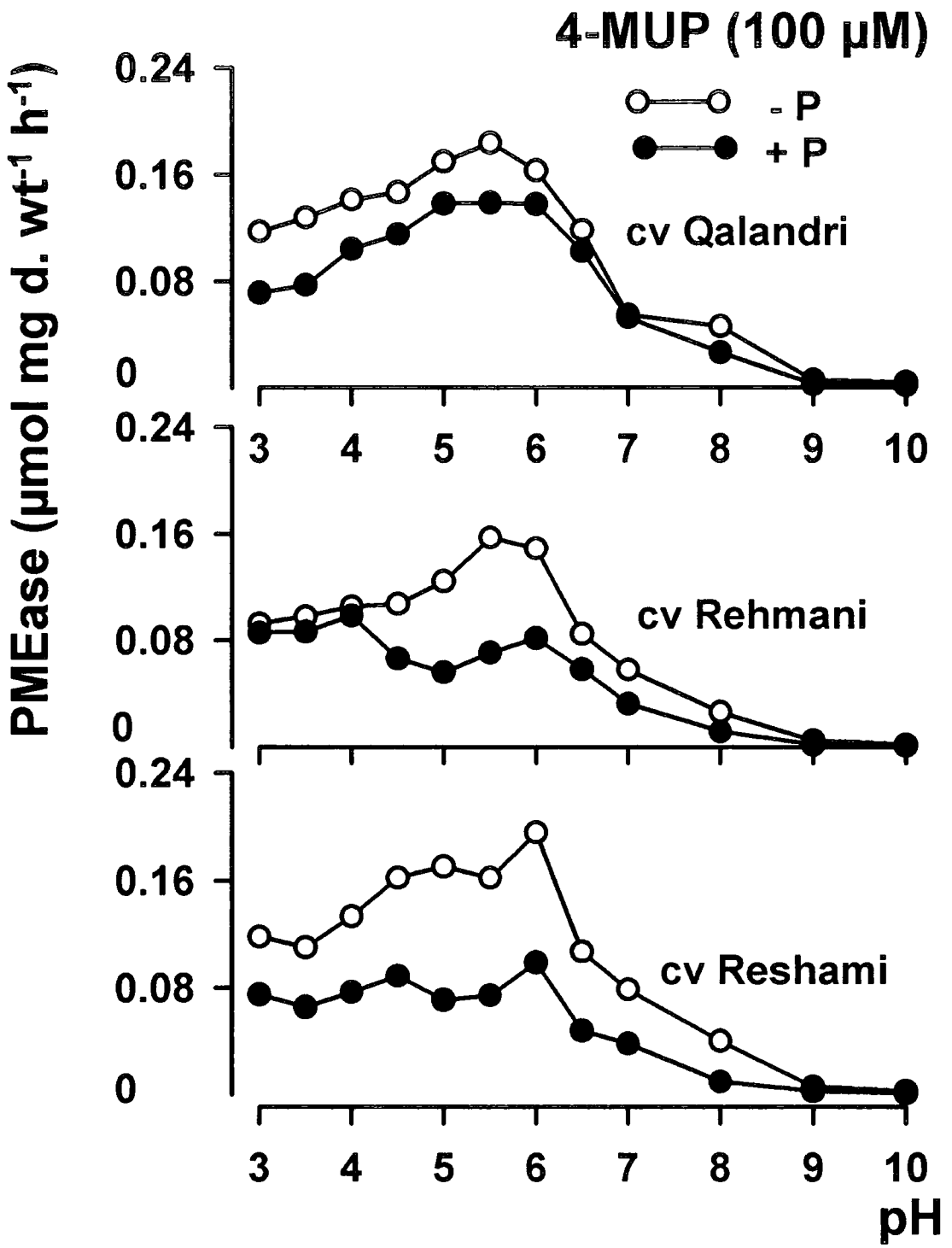


Fig. 4.13 Influence of pH on PMEase activity of roots of three cotton cultivars grown without and with phosphorus added to the growth medium using pNP as a substrate



plants (pH 5.5) and with phosphorus added plants (pH 4.0). For pH the differences were significant ($P = \leq 0.01$) Table 4.12.

4.154 *cv* Reshami

The peak of PMEase activity with pNPP substrate was noted at pH 4.5 (Fig. 4.12). It started to decline slightly after pH 5.0 up to pH 10.0. The data (Table 4.12) show that the plants raised in without phosphorus and with phosphorus were highly significantly different from each other with regard to PMEase activity ($P = \leq 0.001$).

The plants grown without phosphorus added to the growth medium showed higher PMEase activity at all pH levels. Plants grown without the addition of phosphate to the culture media showed PMEase activities of 0.080 µmol mg d. wt^{-1} h^{-1} and 0.245 µmol mg d. wt^{-1} h^{-1} at pH 10.0 and 4.5 respectively. In contrast the plants grown with phosphorus added to the medium culture had PMEase activities of 0.023 µmol mg d. wt^{-1} h^{-1} and 0.100 µmol mg d. wt^{-1} h^{-1} at pH 10.0 and pH 5.0 respectively. The increased percentage of activity of plants without phosphorus over the plants with phosphorus was 58 % at the peak pH. Interaction between pH levels and phosphorus conditions were highly significant ($P = \leq 0.001$).

When 4-MUP was used as the substrates to detect the PMEase activity in cotton cv. Reshami (Fig. 4.13) the peak PMEase activity was recorded at pH 6.0. The activity increased after pH 4.0 and reached its climax at pH 6.0. activity and then started to decrease again from pH 6.5. The data (Table 4.12) indicate that plants grown without phosphorus added to the growth medium and the plants grown with phosphorus added to the growth medium were highly significantly different from each other ($P = \leq 0.001$).

The plants grown without phosphorus added to the growth medium produced higher PMEase activity at all pH levels ranging from 0.1957 (pH 6.0) to 0.0028 µmol mg d. wt^{-1} h^{-1} (pH 10.0). The plants grown with phosphorus added to the growth medium showed 0.0988 (pH 6.0) to 0.0009 µmol mg d. wt^{-1} h^{-1} (pH 10.0). The plants grown

without phosphorus added to the growth medium gave 49 % more PMEase activity than the plants grown with phosphorus added to the medium. The pH values shown (Table 4.12) were highly significant (P = ≤ 0.001). The interaction of between pH and phosphorus conditions were also highly significant (P = ≤ 0.001).

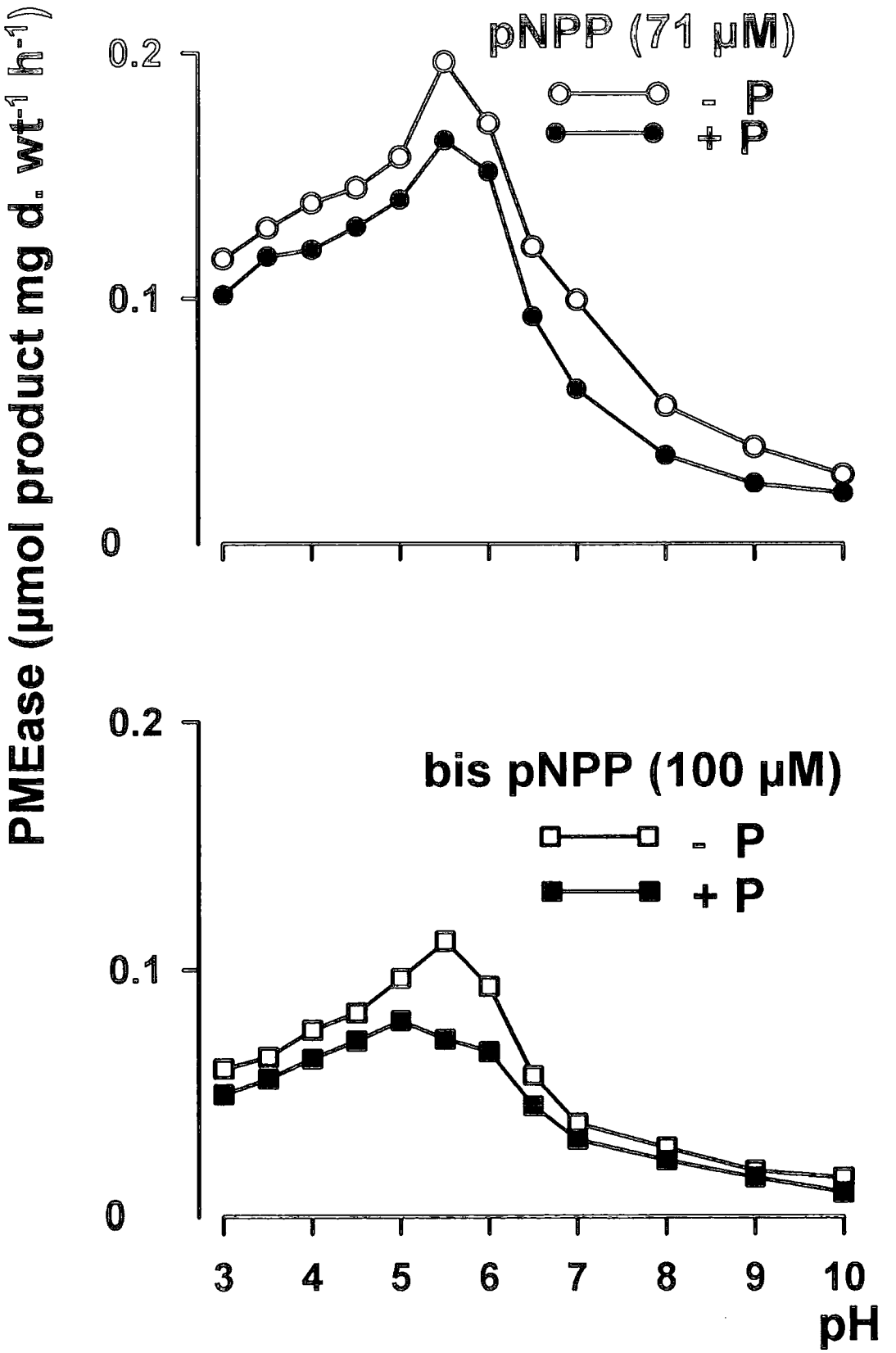
Table 4.12 Summary of statistical analyses of PMEase activity in three cotton cultivars with range of pH, grown without and with phosphorus added to the growth medium.

ANOVA	F (df)	Probability
Cultivars	27 (2)	** 0.001
pH	99 (11)	** 0.001
Conditions	564 (1)	** 0.001
Substrate	106 (1)	** 0.001
cv x pH	5 (22)	** 0.001
cv x condition	38 (2)	** 0.001
cv x substrates	17 (2)	** 0.001
cv x pH x condition	N.S	
cv x condition x substrate	11 (2)	** 0.001

4.16 Influence of pH on PMEase and PDEase activities of *cv* Qalandri

The results in Fig. 4.14 indicate that when pNPP was used as substrate the maximum PMEase activity was recorded at pH 5.5 in both growing conditions, the activity declined after pH 6.5. While bis-pNPP was used as substrate a similar trend of PDEase activities were observed. However, the rate of activity was lower than with pNPP at all pH values in both plant growing conditions. The ratio of optimum pH activity of

Fig.4.14 Influence of pH on PMEase & PDEase activity of roots of cv Qalandri grown without and with phosphorus added to the growth medium pNPP and bi-pNPP used as substrates



PMEase v/s PDEase 1.8 and 2.0 were recorded in the plants which were grown without and with phosphorus added to the growth medium respectively. There ratios were obtained even though the concentration of bis-pNPP was higher than the pNPP. The results indicate that PMEase activity is more dominant in cotton roots.

CHAPTER 5

5.1 PMEase activity in cotton seedling roots in relation to plant mineral content

5.11 Overview

The purpose of this study was to determine the concentrations of phosphorus, nitrogen, potassium and magnesium in the leaves, stem and roots of different cultivars of cotton seedlings. In the case of root tissues as well as mineral determination, PMEase activity was also assayed. To do this seedlings were grown in growth media where the element was either added or omitted.

5.12 Phosphorus content of individual parts of cotton seedlings

The phosphorus content of the leaves, stems and roots of cotton cultivars seedlings are shown in Table 5.1. In all cases the level of phosphate was higher in those seedlings which were grown in medium which contained the phosphorus. However, phosphate content of the different cultivars showed obvious differences.

For leaf tissue, *cv* Rehmani showed the highest amount of phosphorus (41.72 mg g^{-1}) whereas *cv* Qalandri showed the lowest amount (37.93 mg g^{-1}) for plants grown without added phosphorus. In contrast, where plants were grown with phosphorus added to the growth medium *cv* Reshami showed the highest concentration of phosphorus (86.62 mg g^{-1}) and *cv* TH-3/83 showed the lowest concentration of phosphorus (72.85 mg g^{-1}). Table 5.1 shows highly significant values for differences between the cultivars ($P \leq 0.001$).

There was a highly significant difference between the plants grown with and without phosphorus added to the growth medium ($P \leq 0.001$). There were also highly significant differences between the cultivars and the conditions ≤ 0.001).

Table 5.1 Phosphorus concentration (mg g^{-1} d. wt) in cultivar seedlings grown without and with phosphorus added to the growth medium (n = 4, ± SD) and PMEase activity (µmol mg d. wt^{-1} h^{-1}) for root tissues, using pNPP & 4-MUP as substrates.

Cultivars	Phosphorus content (mg g^{-1} d. wt)						PMEase activity (µmol mg d. wt^{-1} h^{-1})			
	Leaf		Stem		Root		pNPP		4-MUP	
	- P	+ P	- P	+ P	- P	+ P	- P	+ P	- P	+ P
Qalandri	37.9	76.03	14.98	41.98	16.36	54.06	0.141	0.085	0.104	0.061
	± 0.93	± 1.55	± 1.23	± 1.07	± 0.41	± 1.67	± 0.00	± 0.00	± 0.00	± 0.00
Rehmani	41.72	75.02	14.48	37.00	19.58	52.42	0.126	0.073	0.084	0.054
	± 1.82	± 1.25	± 1.04	± 1.89	± 0.96	± 1.87	± 0.00	± 0.00	± 0.00	± 0.00
Reshami	38.43	86.62	12.76	42.09	11.56	54.96	0.157	0.101	0.077	0.066
	± 1.64	± 2.07	± 1.64	± 2.20	± 1.81	± 1.58	± 0.00	± 0.00	± 0.00	± 0.00
TH-3/83	38.65	72.85	15.97	41.57	16.55	46.06	0.134	0.076	0.074	0.045
	± 2.11	± 1.43	± 0.45	± 2.09	± 1.55	± 1.13	± 0.00	± 0.00	± 0.00	± 0.00
ANOVA	F (df)	Prob.	F (df)	Prob.	F (df)	Prob.	F (df)	Prob.	F (df)	Prob.
Cultivars	20 (3)	0.001	9 (3)	0.05	15 (3)	0.001	129 (3)	0.001	108 (3)	0.001
Condition	3434 (1)	0.001	4412 (1)	0.001	4061 (1)	0.001	2393 (1)	0.001	1102 (1)	0.001
cv x con.	27 (3)	0.001	15 (3)	0.001	28 (3)	0.001	N.S	-	79 (3)	0.001

For stem tissues Table 5.1 shows that the highest phosphorus concentration, (15.97 mg g^{-1}), was detected in the *cv* TH-3/83 and the lowest concentration (12.76 mg g^{-1}) was noted in *cv* Reshami grown without phosphorus added to the growth medium. While with phosphorus added to the growth medium, the highest phosphorus concentration (49.09 mg g^{-1}) was recorded in *cv* Reshami and the lowest (37.00 mg g^{-1}) was noted in the *cv* Rehmani.

Table 5.1 shows significant differences between the cultivars ($P \leq 0.05$). Highly significant differences ($P \leq 0.001$) were observed between plants, from all cultivars, grown with and without phosphorus added to the growth medium. Highly significant differences ($P \leq 0.001$) were recorded for the interaction between cultivars and conditions.

In root tissues Table 5.1 indicates that when plants were grown without phosphorus added to the growth medium, the highest phosphorus concentration (19.58 mg g^{-1}) was noted in *cv* Rehmani, and the lowest phosphorus concentration (11.50 mg g^{-1}) was noted in *cv* Reshami. While plants grown with phosphorus added to the growth medium, the highest phosphorus concentration, (56.96 mg g^{-1}) was detected in *cv* Reshami. and the lowest phosphorus concentration (46.06 mg g^{-1}) was detected in *cv* TH-3/83.

Table 5.1 shows a highly significant differences ($P \leq 0.001$) for the cultivars and highly significant differences ($P \leq 0.001$) were recorded between the plants grown with and without phosphorus added to the growth medium. Highly significant differences ($P \leq 0.001$) were found in the interaction between plants grown in different conditions (with and without phosphorus added) and plants from different cultivars.

Table 5.1 shows a comparison of PMEase activity for different cultivars at the previously ascertained optimum of pH 5.5 for *cv* Qalandri when pNPP was used as the substrate. The highest PMEase activity of 0.157 µmol mg d. wt^{-1} h^{-1} was recorded in the *cv* Reshami, and the lowest activity, 0.122 µmol mg d. wt^{-1} h^{-1} was noted in the *cv* Rehmani, when phosphorus was not added to the growth medium. The data also show that the highest PMEase activity, 0.101 µmol mg d. wt^{-1} h^{-1} was observed in *cv* Reshami,

while the lowest PMEase activity, 0.073 μmol mg d. wt^{-1} h^{-1} was recorded in *cv* Rehmani when phosphate was added to the medium. In both treatments *cv* Reshami displayed the highest overall activity.

The data (Table 5.1) for the cultivars are highly significant ($P = \leq 0.001$). Highly significant differences ($P = \leq 0.001$) were noted in plants grown with and without phosphorus in the growth medium whereas non-significant differences were noted in the interaction between cultivars and conditions.

Table 5.1 indicates that when 4-MUP was used as substrate, the highest PMEase activity, 0.104 μmol mg d. wt^{-1} h^{-1} was observed in *cv* Qalandri and the lowest PMEase activity, 0.073 μmol mg d. wt^{-1} h^{-1} was in *cv* Rehmani, in growth medium lacking added combined phosphorus. The results show that when the plant is grown with combined phosphorus to the growth medium, the maximum PMEase activity, 0.066 μmol mg d. wt^{-1} h^{-1} was recorded in *cv* Reshami and the lowest PMEase activity 0.045 μmol mg d. wt^{-1} h^{-1} was noted in the *cv* TH-3/83.

The data (Table 5.1) for all the cultivars were highly significant ($P \leq 0.001$). Highly significant differences ($P \leq 0.001$) were noted in conditions. The highly significant differences ($P \leq 0.001$) were recorded for the interaction between cultivars and conditions.

5.2 Determination of macroelements in seeds of different cultivars

In order to assess their availability for seedlings the levels of various minerals were measured in seeds of different cultivars

The data shown in Table.5.2 indicate non-significant differences among cultivars for seed phosphorus levels. The highest seed phosphorus was 0.53 % in *cv* TH-3/83 and lowest was 0.49 % in *cv* Reshami. Any manipulation of phosphate levels in the growth media would therefore be independent of initial plant phosphate levels.

The results show highly significant differences ($P \leq 0.001$) for nitrogen between the cultivars. The highest nitrogen content, 3.43 % was recorded in the *cv* Qalandri and the lowest 1.14 % was noted in the *cv* Reshami.

The data (Table.5.2) indicate non-significant differences among cultivars for potassium. The highest seed potassium, 5.42 % was observed in *cv* Reshami and the lowest potassium, 5.11 % in *cv* Qalandri.

The results show that non-significant differences among the cultivars for magnesium content in the seed. The highest magnesium content, 1.20 % was recorded in *cv* Qalandri and lowest 1.14 % was recorded in *cv* Rehmani and TH-3/83.

Table 5.2 Phosphorus, nitrogen, potassium and magnesium contents of seeds of four different cultivars of cotton, data is expressed as a %, (n = 4, .± SD).

Cultivars	Phosphorus	Nitrogen	Potassium	Magnesium
Qalandri	0.52 ± 0.00	3.43 ± 0.13	5.11 ± 0.07	1.20 ± 0.09 %
Rehmani	0.50 ± 0.02	2.16 ± 0.11	5.15 ± 0.22	1.14 ± 0.03
Reshami	0.49 ± 0.03	1.14 ± 0.08	5.42 ± 0.48	1.15 ± 0.23
TH-3/83	0.53 ± 0.03	1.86 ± 0.17	5.25 ± 0.17	1.14 ± 0.03
ANOVA		F (df) Prob.		
Cultivar	N.S	198 (3) 0.001	N.S	N.S

5.3 Nitrogen content of individual parts of seedlings of different cultivars

Nitrogen content was determined in the individual parts of cotton seedlings when the plants were grown in conditions where nitrogen was added to or omitted from the growth medium Fig 2.2. Results of the analyses are shown in Table 5.3.

For leaf tissues Table 5.3 indicates that in plants grown without nitrogen added to the growth medium, the highest nitrogen concentration (84.85 mg g^{-1}) was noted in *cv* Reshami and the lowest concentration (48.02 mg g^{-1}) was noted in *cv* Rehmani. While in plants grown with nitrogen added to the growth medium, plants from the *cv* TH-3/83 had the highest (96.33 mg g^{-1}) concentration of nitrogen and the lowest concentration of nitrogen (56.87 mg g^{-1}) were found in the *cv* Rehmani.

Table 5.3 shows highly significant differences ($P \leq 0.001$) were found among the cultivars. Highly significant differences ($P \leq 0.001$) were noted in plants grown with and without nitrogen added to the growth medium. Non-significant differences were noted for the interaction between cultivars and plants grown in the two conditions (with and without nitrogen added to the growth medium).

For the stem tissues, Table 5.3 indicates that the plants grown without N added to the growth medium, the highest nitrogen concentration (65.00 mg g^{-1}) was recorded in *cv* Reshami and the lowest concentration (38.75 mg g^{-1}) was observed in *cv* Rehmani. When plants were grown with nitrogen added to the growth medium, the highest nitrogen concentration (68.99 mg g^{-1}) was noted in *cv* Reshami and the lowest concentration, (38.33 mg g^{-1}) was recorded in *cv* Qalandri.

Table 5.3 shows that highly significant differences ($P = 0.001$) were noted among the cultivars. Highly significant differences ($P \leq 0.001$) were found for the plants grown with and without nitrogen added to the growth medium. Non-significant differences were found in the interaction between cultivars and conditions.

For root tissues, Table 5.3 indicates that the plants grown without combined nitrogen in the growth medium, the highest (38.60 mg g^{-1}) was found in *cv* TH-3/83 and

the minimum, (17.56 mg g^{-1}) was obtained in *cv* Rehmani. When the plants were grown with combined nitrogen in the medium, the highest nitrogen (144.60 mg g^{-1}) was recorded in *cv* Reshami and the lowest (90.41 mg g^{-1}) was found in *cv* Rehmani.

Table 5.3 shows highly significant differences ($P \leq 0.001$) were noted among the cultivars. The highly significant differences ($P \leq 0.001$) were obtained in plants grown with and without combined nitrogen in the growth medium. The interaction between cultivars and conditions were highly significant ($P \leq 0.001$)

The results in Table 5.3 show a comparison of PMEase activity for different cultivars at the previously ascertained optimum of pH 5.5 when pNPP was used as the substrate, and plants grown without nitrogen added to growth medium. The maximum PMEase activity 0.203 µmol mg d. wt^{-1} h^{-1} was noted in *cv* Rehmani, and the lowest PMEase activity 0.142 µmol mg d. wt^{-1} h^{-1} was observed in *cv* Reshami. With the plants grown with nitrogen added to the growth medium the highest PMEase activity 0.129 µmol mg d. wt^{-1} h^{-1} was observed in *cv* Qalandri and the lowest PMEase activity 0.115 µmol mg d. wt^{-1} h^{-1} was recorded in *cv* Reshami. Highly significant differences ($P \leq 0.001$) were found among cultivars.

For the plants grown with and without nitrogen added to the growth medium differences were highly significant ($P \leq 0.001$). The interaction for cultivar and conditions were significant ($P \leq 0.05$).

The data in Table 5.3 indicate that when 4-MUP was used as a substrate and plants were grown without nitrogen added to the growth medium a PMEase activity of 0.100 µmol mg d. wt^{-1} h^{-1} was noted in *cv* Rehmani. The lowest recorded PMEase activity was 0.058 µmol mg d. wt^{-1} h^{-1} found in *cv* Reshami. When the plants were grown with N added to the medium, the highest PMEase activity of 0.074 µmol mg d. wt^{-1} h^{-1} was noted in the *cv* Rehmani and the lowest PMEase activity , 0.042 µmol mg d. wt^{-1} h^{-1} was recorded in the *cv* Reshami.

Table 5.3 Nitrogen concentration (mg g^{-1} d. wt) in cultivar seedlings grown without and with nitrogen added to the growth medium (n = 4, ± SD) and PMEase activity (µmol mg d. wt^{-1} h^{-1}) of root tissues.

Cultivar	Nitrogen content (mg g^{-1} d. wt)						PMEase activity (µmol mg d. wt^{-1} h^{-1})			
	Leaf		Stem		Root		pNPP		4-MUP	
	- N	+ N	- N	+ N	- N	+ N	- N	+ N	- N	+ N
Qalandri	63.18	77.06	45.53	38.33	27.19	130.26	0.151	0.129	0.075	0.057
	± 1.93	± 3.29	± 2.15	± 2.08	± 2.32	± 2.9	± 0.01	± 0.00	± 0.00	± 0.00
Rehmani	48.02	56.87	36.75	41.18	17.56	90.41	0.203	0.152	0.100	0.074
	± 4.02	± 4.76	± 1.62	± 1.74	± 3.35	± 7.27	± 0.01	± 0.01	± 0.00	± 0.00
Reshami	84.85	92.28	65.00	68.99	38.17	144.60	0.142	0.115	0.058	0.042
	± 3.90	± 4.55	± 1.84	± 2.46	± 1.96	± 5.00	± 0.01	± 0.00	± 0.00	± 0.00
TH-3/83	82.94	96.33	57.48	63.68	38.60	142.80	0.166	0.126	0.075	0.056
	± 4.46	± 2.11	± 1.61	± 2.00	± 2.05	± 4.90	0.01	± 0.01	± 0.00	± 0.00
ANOVA	F(df)	Prob.	F(df)	Prob.	F(df)	Prob.	F (df)	Prob.	F (df)	Prob.
Cultivars	452 (3)	0.001	278 (3)	0.001	155 (3)	0.001	130 (3)	0.001	272 (3)	0.001
Condition	183 (1)	0.001	32 (1)	0.001	4756 (1)	0.001	364 (1)	0.001	446 (1)	0.001
cv x con.	N.S		N.S		32 (3)	0.001	14 (3)	0.05	6 (3)	0.05

Highly significant differences ($P \leq 0.001$) were found between the cultivars. Highly significant differences ($P \leq 0.001$) were noted in the plants grown without nitrogen added to the growth medium. Significant differences ($P \leq 0.05$) were noted in the interaction between cultivars and conditions.

5.4 Potassium and phosphorus content in *cv* Qalandri seedling parts and their effect on root PMEase activity

The seedlings were grown without and with potassium added to the growth medium. In addition a study was made to investigate the potassium and phosphorus content (mg g-1 d. wt), and their effect on PMEase activity, of root tissues. For this experiment one ion, two substrates (pNPP, 4-MUP) and one cultivar were selected.

For leaf tissues, Table 5.4 indicates that the maximum potassium (213.25 mg g^{-1} d. wt) was observed in the plants grown with potassium added to the growth medium, while (206.25 mg g^{-1} d. wt) was noted in the plants grown without potassium added to the medium. When the influence of potassium was determined on plants grown with phosphorus the maximum phosphorus content (73.07 mg g^{-1} d. wt) was observed in the plants grown without potassium added to the growth medium. In contrast, a concentration of 72.61 mg g^{-1} d. wt was noted in the plants grown with potassium to the growth medium.

Table 5.4 shows that the plants grown without and with potassium in the growth medium had significant differences in their potassium levels ($P = \leq 0.05$). Highly significant differences ($P = \leq 0.001$) were found between the concentration of potassium and phosphorus in the leaves. Significant differences ($P = \leq 0.05$) were recorded between interactions of conditions and ions. However, no significant differences were seen in the phosphorus contents between the leaves of those plants grown without or with added potassium to the growth medium.

For the stem tissues, maximum potassium (63.25 mg g^{-1} d. wt) was recorded in the plants grown with potassium added to the growth medium and the lowest (51.5 mg g^{-1} d. wt) was obtained in the plants grown without potassium added to the growth medium. The maximum phosphorus (17.38 mg g^{-1} d. wt) was found in the plants which were grown with potassium added to the growth medium, and 13.81 mg g^{-1} d. wt phosphorus was noted in the plants grown without potassium added to the growth medium.

Table 5.4 shows that stems of plants grown without and with potassium added to the growth medium had highly significant differences in their potassium levels ($P = \leq 0.001$). Highly significant differences ($P = \leq 0.001$) were observed between the concentration of potassium and phosphorus in the tissues. Highly significant differences ($P = \leq 0.001$) were found between interaction of conditions and ions.

For root tissues, the maximum potassium concentration (16.75 mg g^{-1} d. wt) was recorded in the plants grown with potassium added to growth medium, and 9.5 mg g^{-1} d. wt potassium was recorded in the root of the plants grown without potassium added to the medium.

When plants were grown with added phosphorus the highest phosphorus (30.71 mg g^{-1} d. wt) was observed in the plants grown with potassium added to the growth medium, whereas a concentration of 16.21 mg g^{-1} d. wt was found in the plants grown without potassium added to the growth medium.

Table 5.4 shows that roots of plants grown without and with potassium added to the growth medium were highly significant for the levels of phosphorus ($P = \leq 0.001$). Highly significant differences ($P = \leq 0.001$) were found between the potassium and phosphorus levels. Highly significant differences ($P = \leq 0.001$) were noted for the interaction between conditions and ions. A significant difference was observed between the levels of root phosphorus for plants grown without or with added potassium.

Table 5.4 shows that when pNPP was used as a substrate the highest PMEase activity 0.08 μmol mg d. wt^{-1} h^{-1} was recorded in the root of plants grown without potassium added to the growth medium, and 0.04 μmol mg d. wt^{-1} h^{-1} was noted in roots

of plants grown with potassium added to the growing media. When 4-MUP was used as a substrate the maximum PMEase activity 0.05 μmol mg d. wt^{-1} h^{-1} was observed in roots of plants grown without potassium added to the medium and 0.03 μmol mg d. wt^{-1} h^{-1} was found in root of plants were grown with potassium added to the growth medium.

5.5 Magnesium and phosphorus content in *cv* Qalandri seedling parts and their effect on root PMEase activity

The seedlings were grown without and with magnesium added to the growth medium. In addition a study was made to investigate the magnesium and phosphorus content (mg g^{-1} d. wt) and their combined effects on PMEase activity of root tissues. For this experiment one ion, two substrates (pNPP and 4-MUP) and one pH were used .

Table 5.5 shows the magnesium concentration for plants grown without and with magnesium added to the growth medium were just significant ($P = \leq 0.05$) probably because magnesium pools in these plants are high. Highly significant differences ($P = \leq 0.001$) were found between the concentrations of magnesium and phosphorus, while non-significant differences were noted for interaction between condition and ions. However, the overall pattern of ion concentrations was not seen for all of the plant parts.

For the leaf tissues, Table 5.5 shows that the maximum magnesium content (31.45 mg g^{-1} d. wt) was recorded in leaves of plants grown with magnesium added to the growth medium, and 27.53 mg g^{-1} d. wt magnesium was obtained in leaves of plants grown without magnesium added to the growth medium.

When the influence of magnesium was determined on plants growth with phosphorus the highest phosphorus content (69.32 mg g^{-1} d. wt) was recorded in leaves of plants grown with magnesium added to the growth medium, and 64.86 mg g^{-1} d. wt phosphorus was observed in leaves of plants grown without magnesium added to the growth medium.

For the stem tissues, Table 5.5 shows that the maximum stem magnesium content (5.08 mg g^{-1} d. wt) was found in stems of plants grown without magnesium added to the medium and 4.63 mg g^{-1} d. wt magnesium was noted in stems of plants grown with magnesium added to the growth medium.

The maximum phosphorus content (14.44 mg g^{-1} d. wt) was recorded in stems of plants grown with magnesium added to the growth medium, and a phosphorus content of 12.43 mg g^{-1} d. wt was found in stems of plants grown without magnesium added to the medium.

Table 5.5 shows that stem of plants grown without and with magnesium added to the growth medium were significantly difference ($P = \leq 0.01$). Highly significant differences ($P = \leq 0.001$) were obtained between the magnesium and phosphorus content of stem. Significant differences ($P = \leq 0.05$) were found for the interaction between conditions and ions.

For the root tissues, Table 5.5 shows that the maximum magnesium (5.3 mg g^{-1} d. wt) was noted in roots of plants grown without magnesium added to the medium, and 2.93 mg g^{-1} d. wt magnesium was found in roots of plants which were grown with magnesium added to the growth medium. The maximum phosphorus (27.89 mg g^{-1} d. wt) was observed in roots of plants grown with magnesium added to the growth medium, with 17.90 mg g^{-1} d. wt phosphorus being recorded in roots of plants which were grown without magnesium added to the growth medium.

Table 5.5 shows that root of plants grown without and with magnesium added to the growth medium were significant ($P = \leq 0.05$). Highly significant differences ($P = \leq 0.001$) were observed between the magnesium and phosphorus content of root. Highly significant differences ($P = \leq 0.001$) were noted for the interaction between condition and ions. Table 5.5 shows that higher PMEase activity was detected with substrate pNPP and lower PMEase activity was found with 4-MUP substrate. However, no differences were found for both conditions, without and with added magnesium to the growth medium with both the substrates.

Table 5.4 Potassium and phosphorus concentration (mg g^{-1} d. wt) in different parts of cotton *cv* Qalandri seedlings grown without and with potassium added to the growth medium (n = 4, ± SD), and PMEase activity (µmol mg d. wt^{-1} h^{-1}) of root tissues

Ions	Element concentration (mg g^{-1} d. et)						PMEase activity (µmol mg d. wt^{-1} h^{-1})			
	Leaf		Stem		Root		pNPP		4-MUP	
	- K	+ K	- K	+ K	- K	+ K	- K	+ K	- K	+ K
Potassium	206.25	213.25	51.5	63.25	49.5	116.75	0.08	0.04	0.05	0.03
	± 0.95	± 0.50	± 0.57	± 0.50	± 0.50	± 0.50	± 0.00	± 0.00	± 0.00	± 0.00
Phosphorus	73.07	72.61	13.81	17.38	16.21	30.71				
	± 1.33	± 0.47	± 0.84	± 0.76	± 0.76	± 0.17				
ANOVA	F (df)	F. Prob.	F (df)	F.Prob.	F (df)	F.Prob.	F (df)	Prob.	F (df)	Prob.
Condition	90 (1)	* 0.05	2795 (1)	** 0.001	9033 (1)	** 0.001				
Ions	267 (1)	** 0.001	467 (1)	** 0.001	667 (1)	** 0.001				
Cond x ion	117 (1)	* 0.05	795 (1)	** 0.001	3796 (1)	** 0.001				

Table 5.5 Magnesium and phosphorus concentration (mg g^{-1} d. wt) in different parts of *cv* Qalandri seedlings grown without and with magnesium added to the growth medium (n = 4, ± SD), and PMEase activity (µmol mg d. wt^{-1} h^{-1}) of root tissues

Ions	Element concentration (mg g^{-1} d. wt)						PMEase activity (µmol mg d. wt^{-1} h^{-1})			
	Leaf		Stem		Root		pNPP		4-MUP	
	- Mg	+ Mg	- Mg	+ Mg	- Mg	+ Mg	- Mg	+ Mg	- Mg	+ Mg
Magnesium	27.53	31.45	5.08	4.63	5.3	2.93	0.06	0.06	0.3	0.03
	± 0.65	± 1.28	± 0.5	± 0.25	± 1.67	± 0.05	± 0.00	± 0.00	± 0.00	± 0.00
Phosphorus	64.86	69.32	12.43	14.44	17.90	27.89				
	± 0.95	± 1.11	± 0.72	± 0.71	± 0.63	± 0.01				
ANOVA	F (df)	F.Prob.	F (df)	F.Prob.	F (df)	F.Prob.	F (df)	Prob.	F (df)	Prob.
Condition	132 (1)	* 0.05	15 (1)	* 0.01	79 (1)	* 0.05				
Ions	207 (1)	** 0.001	1823 (1)	** 0.001	1919 (1)	** 0.001				
Condi. x Ion	N.S	-	38 (1)	* 0.05	208 (1)	** 0.001				

5.6 Influence of nitrogen on the PMEase activities of three cultivars of cotton

5.61 Overview

A study was made of the influence of nitrogen nutrition on PMEase activity of three cotton cultivars in order to assess any differential effect among them. A comparison of PMEase activity among the cultivars, was made using two substrates over a range of pH for roots of plants grow in media where phosphate was present but in the absence or presence of combined nitrogen.

A full statistical analysis for all cultivars and all conditions is shown in Table 5.6.

5.611 *cv* Qalandri

The results in Fig.5.1 show when pNPP was used as the substrate the pattern of activity for phosphate was the same as reported earlier (4.7,8). The PMEase activity of the plants grown without nitrogen added to the growth medium and with nitrogen added differed in a highly significant fashion ($P = \leq 0.001$). Plants grown without nitrogen added showed higher PMEase activity at all pH levels ranging from 0.023 (pH 10.0) to 0.224 µmol mg d. wt^{-1} h^{-1} (pH 6.0). In comparison the plants grown with nitrogen added had activities of 0.016 (pH 10.0) to 0.212 µmol mg d. wt^{-1} h^{-1} (pH 5.5). The ratio of PMEase activity between the two nitrogen states was recorded as 1.15 at optimum pH.

Highly significant differences ($P \leq 0.001$) were obtained between pH values. The highly significant differences ($P \leq 0.001$) were noted in interaction between pH and nitrogen status condition when plants with added nitrogen showed lower PMEase activity at all pH's values tested.

The results in Fig.5.2 show that when 4-MUP was used as the substrate the optimum of acid PMEase activity was at pH 5.5. Plants grown without nitrogen added to the growth medium and plants grown with nitrogen added showed highly significant

differences (P = ≤ 0.001) between their PMEase activity. The plants grown without nitrogen added gave higher PMEase activity at all pH levels ranging from 0.015 µmol mg d. wt^{-1} h^{-1} (pH 10.0) to 0.174 µmol mg d. wt^{-1} h^{-1} (pH 5.5) against plants grown with nitrogen added having 0.009 µmol mg d. wt^{-1} h^{-1} (pH 10.0) to 0.092 µmol mg d. wt^{-1} h^{-1} (pH 5.5). The ratio of PMEase activity was recorded 1.89 at optimum pH.

Highly significant differences (P = ≤ 0.001) were recorded for PMEase activity between all pH values. Highly significant differences (P = ≤ 0.001) were obtained for the interaction between pH and nitrogen status conditions

5.612 *cv* Rehmani

Fig 5.1 shows that with pNPP the PMEase activity was detectable between pH 3.0 to 8.0 with a optimum at pH 5.0. The differences in PMEase activity between plants grown without nitrogen added and plants grown with nitrogen added differed highly significantly (P = ≤ 0.001). Plants grown without nitrogen added showed higher PMEase activity at all pH levels ranging from 0.024 µmol mg d. wt^{-1} h^{-1} (pH 10.0) to 0.226 µmol mg d. wt^{-1} h^{-1} (pH 5.0) against plants grown with N added having an activity of 0.011 µmol mg d. wt^{-1} h^{-1} (pH 10.0) to 0.196 µmol mg d. wt^{-1} h^{-1} (pH 4.0). The ratio of PMEase activity at the pH optima for plants without nitrogen v/s with nitrogen was 1.15.

Highly significant differences (P = ≤ 0.001) were recorded for PMEase activity between all pH values. Highly significant differences (P = ≤ 0.001) were obtained for interaction between pH and nitrogen conditions.

The data in Fig.5.2 show that when 4-MUP was used as the substrate optimum activity was reached at pH 5.5. The plants grown without nitrogen added to the growth medium and the plants grown with nitrogen added were highly significantly different (P = ≤ 0.001) with regard to PMEase activity. The plants grown without nitrogen added yielded higher PMEase activity at all pH levels ranging from 0.005 (pH 10.0) to 0.081 µmol mg d. wt^{-1} h^{-1} (pH 5.5) in comparison to the plants grown with nitrogen added

having activity of 0.004 (pH 10.0) to 0.065 μmol mg d. wt^{-1} h^{-1} (pH 4.0). The ratio of PMEase activity was noted 1.23 at optimum pH.

Highly significant differences ($P = \leq 0.001$) were noted for PMEase activity between all pH values. The highly significant differences ($P = \leq 0.001$) were obtained for the interaction between pH and nitrogen status conditions.

5.613 *cv* Reshami

The results in Fig. 5.1 show PMEase activity with pNPP reached its optimum at pH 5.5. The level of PMEase activity between plants grown without nitrogen added and plants grown with nitrogen added in the growth medium showed highly significant differences ($P = \leq 0.001$). Plants grown without nitrogen added gave higher PMEase activity at all pH levels ranging from 0.014 (pH 10.0) to 0.185 μmol mg d. wt^{-1} h^{-1} (pH 5.5) in comparison to the plants grown with nitrogen added having 0.009 (pH 10.0) to 0.146 μmol mg d. wt^{-1} h^{-1} (pH 5.0). The ratio of PMEase activity was recorded as being 1.26 at the pH optima.

Highly significant differences ($P = \leq 0.001$) were noted for PMEase activity between all pH values. Highly significant differences ($P = \leq 0.001$) were obtained for the interaction between pH and nitrogen status conditions.

The results in Fig.5.2 show that when 4-MUP was used the acid PMEase activity in this cultivar reached its optimum at pH 5.5. Again, PMEase activity of plants grown without nitrogen added and the plants grown with nitrogen added were highly significantly different($P = \leq 0.001$). The plants grown without nitrogen added gave higher PMEase activity at all pH levels ranging from 0.017 μmol mg d. wt^{-1} h^{-1} (pH 10.0) to 0.103 μmol mg d. wt^{-1} h^{-1} (pH 5.5) against plants grown with nitrogen added with 0.008 μmol mg d. wt^{-1} h^{-1} (pH 10.0) to 0.069 μmol mg d. wt^{-1} h^{-1} (pH 5.5). The ratio of PMEase activity was recorded as 1.46 at optimum pH.

Highly significant differences ($P = \leq 0.001$) were noted for PMEase activity between all pH values. The highly significant differences ($P = \leq 0.001$) were recorded to the interaction between pH and nitrogen status conditions.

Fig.5.1 Influence of pH on PMEase activity of roots of three cotton cultivars grown without and with nitrogen added to the growth medium using pNPP as a substrate

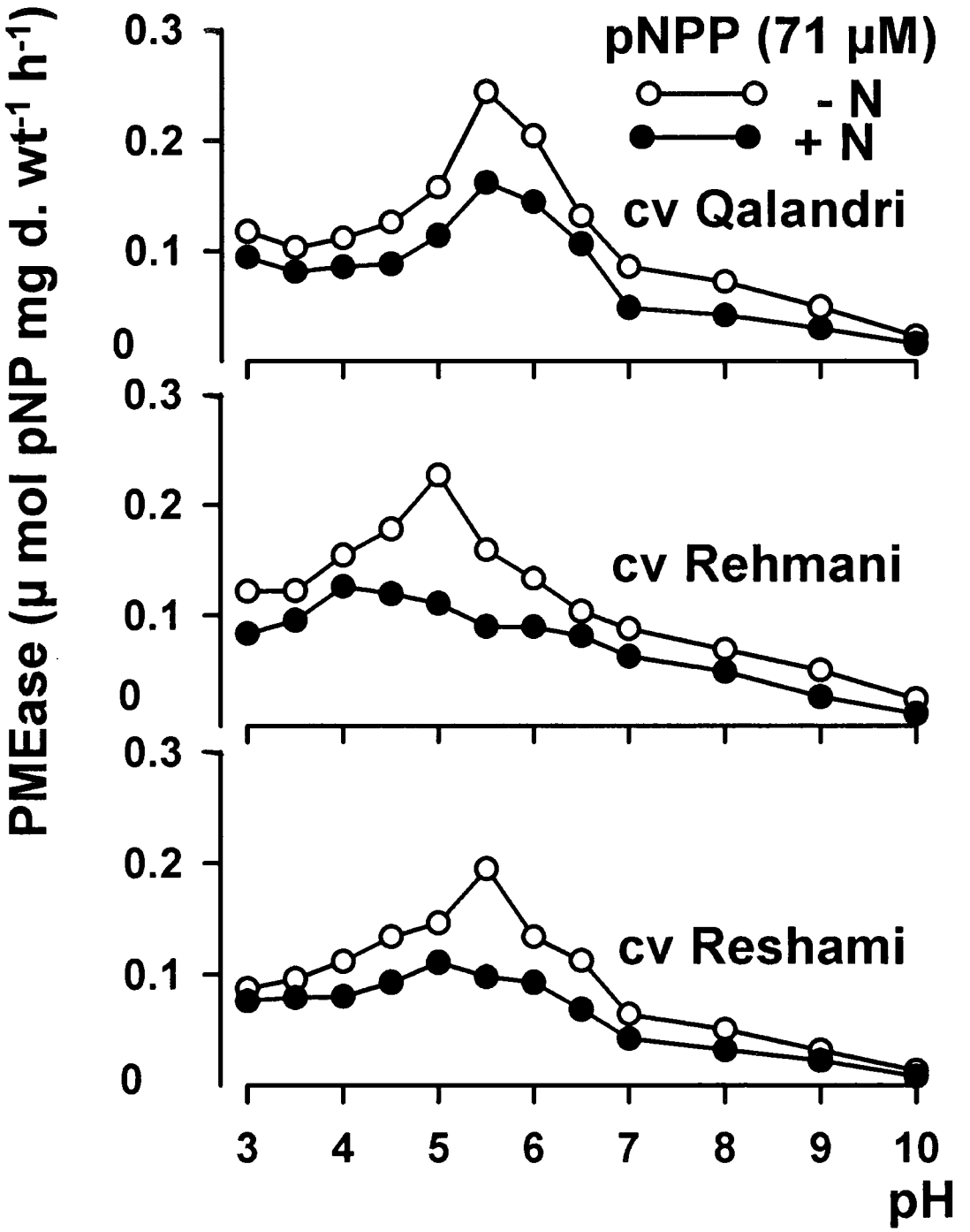


Fig.5.2 Influence of pH on PMEase activity of roots of three cotton cultivars grown without and with nitrogen added to the growth medium 4-MUP used as substrate

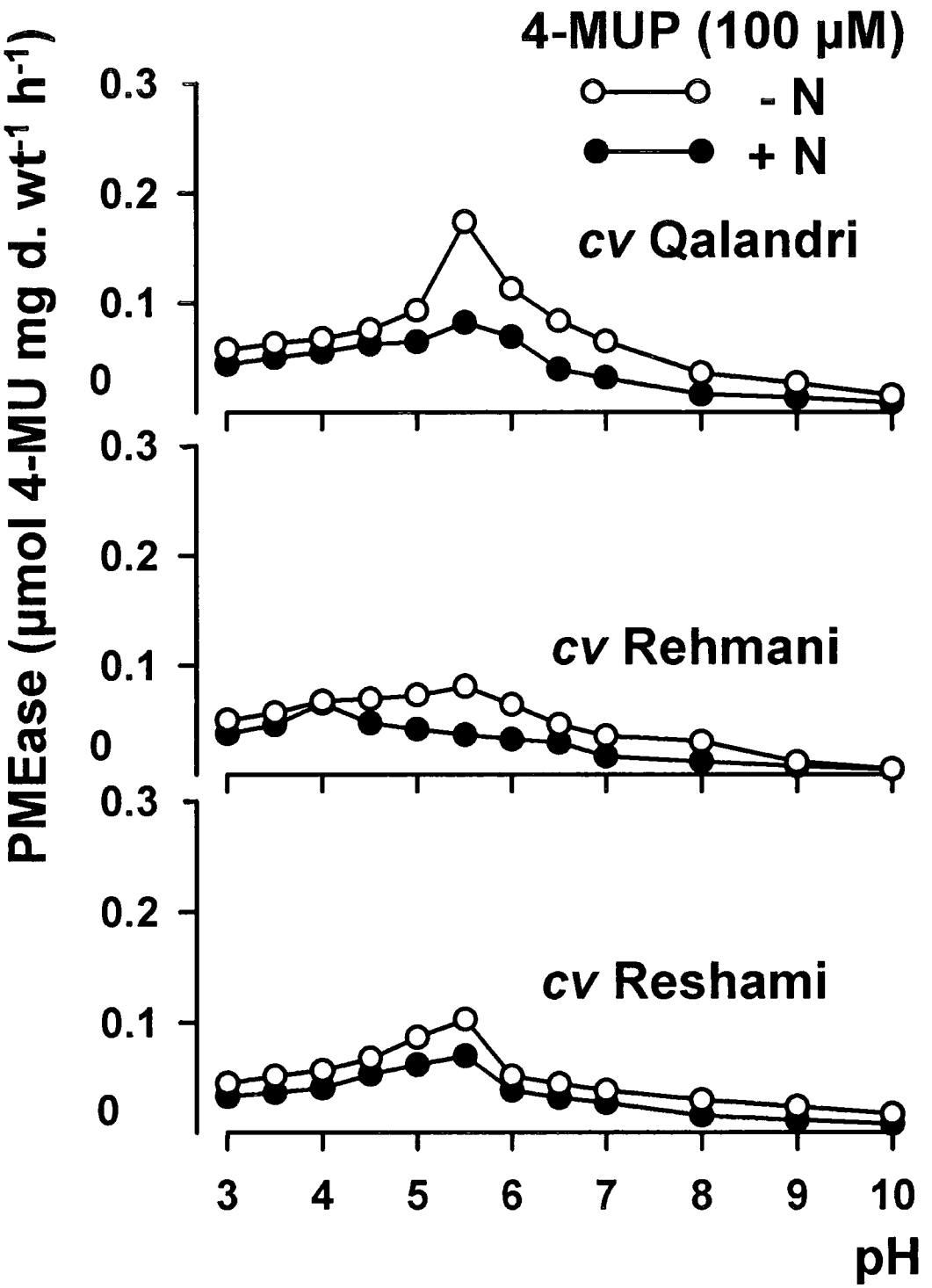


Table 5.6 Summary of statistical analyses for the plants grown without and with nitrogen added to the growth medium.

Cultivars	pH		condition		pH x condition		Substrate
	F(df)	P	F(df)	P	F(df)	P	
Qalandri	1715 (11)	≤ 0.001	869 (1)	≤ 0.001	9 (11)	≤ 0.001	pNPP
Qalandri	1942 (11)	≤ 0.001	4709 (1)	≤ 0.001	191 (11)	≤ 0.001	4-MUP
Rehmani	153 (11)	≤ 0.001	352 (1)	≤ 0.001	71 (11)	≤ 0.001	pNPP
Rehmani	1786 (11)	≤ 0.001	4070 (1)	≤ 0.001	185 (11)	≤ 0.001	4-MUP
Reshami	2823 (11)	≤ 0.001	1700 (1)	≤ 0.001	49 (11)	≤ 0.001	pNPP
Reshami	2027 (11)	≤ 0.001	3053 (1)	≤ 0.001	47 (11)	≤ 0.001	4-MUP

5.7 Split root systems and PMEase activity

Initial experiments using a split root system indicated that phosphate content within the plant was partitioned and that this was related to PMEase activity. A similar set of experiments were conducted to investigate this effect in different cultivars.
For this experiment four cultivars, two substrates and two phosphorus growth conditions were used.

The results in Table 5.7 show that when pNPP was used as the substrate the highest PMEase activity, 0.213 μmol mg d. wt^{-1} h^{-1} was noted in *cv* Reshami, and lowest PMEase activity 0.152 μmol mg d. wt^{-1} h^{-1} was observed in *cv* Rehmani, for the plant roots grown without phosphorus added to the growth medium. When half of the plant roots grown with phosphorus added to the growth medium, the maximum PMEase activity, 0.081 μmol mg d. wt^{-1} h^{-1} was recorded in *cv* Qalandri and the minimum PMEase activity 0.058 μmol mg d. wt^{-1} h^{-1} was found in *cv* Rehmani. Overall *cv* Rehmani gave lowest PMEase activity in both conditions.

When 4-MUP was used as the substrate the highest PMEase activity was also found in *cv* Reshami, and the lowest activity was noted in *cv* Rehmani, in the roots which were grown without phosphorus added to the growth medium. Where plant roots were grown with phosphorus added to the growth medium, the highest activity was recorded in *cv* TH-3/83 and the minimum 0.033 μmol mg d. wt^{-1} h^{-1} was noted in *cv* Reshami.

The data in Table 5.7 show that highly significant differences ($P = \leq 0.001$) were recorded in activity of cultivars, while pNPP was used as the substrate. Highly significant differences ($P = \leq 0.001$) were noted for conditions. Highly significant differences ($P = \leq 0.001$) were observed for interaction of cultivars and conditions. While 4-MUP was used as the substrate N.S results were noted in cultivars and conditions.

The results (Table 5.7) indicate that the highest leaf phosphorus was observed in *cv* Reshami, and the minimum was noted in *cv* TH-3/83. Highly significant differences ($P = \leq 0.001$) were noted among the cultivars.

Table 5.7 Phosphorus content (mg g^{-1} d. wt) and PMEase activity (µmol mg d. wt^{-1} h^{-1}) in root halves of a split root system. Cotton seedlings were grown without and with phosphorus added to one half of the split root system (n = 4, ± SD).

Cultivars	Phosphorus content (mg g^{-1} d. wt)				PMEase activity (µmol mg d. wt^{-1} h^{-1})			
-	Leaf	Stem	Root		pNPP		MUP	
-	-	-	- P	+ P	- P	+ P	- P	+ P
Qalandri	19.44	6.99	8.02	13.92	0.195	0.081	0.080	0.034
Rehmani	21.77	6.27	7.52	11.79	0.152	0.058	0.074	0.039
Reshami	24.61	5.83	4.49	15.35	0.213	0.066	0.085	0.033
ANOVA	F(df)	F(df)	F(df)	Prob.	F(df)	Prob.	F(df)	
Culti.	567 (3)	5(3)	9(3)	* 0.01	33(3)	** 0.00	N.S	
Condi.	-	-	680(1)	** 0.00	2124 (1)	** 0.00	N.S	
cv x cond	-	-	26(3)	** 0.00	16(3)	** 0.00	N.S	
Prob.	** 0.001	* 0.01						

The maximum stem phosphorus was detected in *cv* Qalandri, and the lowest was found in *cv* Reshami. Significant differences (P = ≤ 0.01) were noted among the cultivars.

The plant roots grown with phosphorus added to the growth medium showed highest root phosphorus in *cv* Reshami, and lowest was noted in *cv* Rehmani. Where plants were grown without phosphorus added to the growth medium, the highest root phosphorus was recorded in *cv* Qalandri and the lowest was recorded in *cv* Reshami.

Significantly differences (P = ≤ 0.01) were noted among cultivars. Highly significant differences (P = ≤ 0.001) were noted for the conditions. Highly significant differences (P = ≤ 0.001) were obtained between interaction of cultivar and condition.

CHAPTER 6

6.1 Effect of salinity on PMEase activity of roots of *cv* Qalandri

As was indicated in Chapter 1 salinity is an important factor in mineral nutrition in Pakistan. The purpose of this study was to investigate the response of increasing concentrations of NaCl on acid PMEase activity of the roots of cotton *cv* Qalandri, and to determine what level of salinity is detrimental.

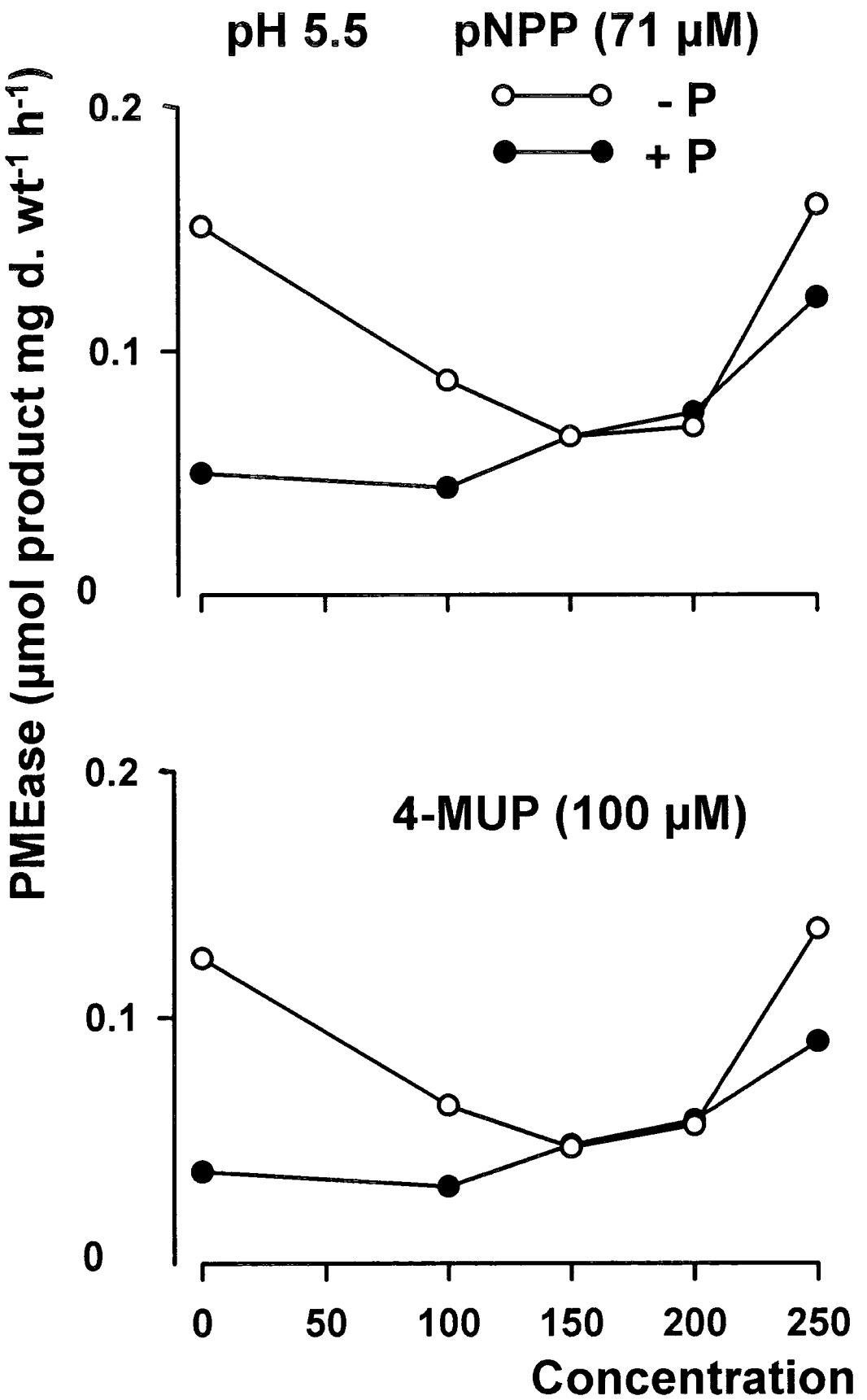
Plants were grown as described in Materials and Methods except that NaCl was added into the growth medium at concentrations ranging from 100 mM-300 mM. 300 mM was found to be toxic to the extent that the plants died, therefore the highest concentration used for PMEase manipulation was 250 mM.

For this experiment five concentrations of NaCl, two substrates (pNPP, 4-MUP) and one cultivar were used. The enzyme activity was assayed at the previously determined pH optimum of 5.5

The results of assay using pNPP and 4-MUP as substrates are shown in Fig. 6.1. For both substrates the responses to increased NaCl concentration in the growth medium are essentially the same. For plant grown without phosphate present, there was a gradual decline in PMEase activity up to 150 mM NaCl. However , from 200 mM to 250 mM there was a marked increase in activity. These responses to NaCl are highly significant ($P = \leq 0.001$) and indicate that surface phosphatase activity may be suppressed by increasing NaCl. At high concentrations of salt, however, PMEase activity is increased and this may be due to a release of cellular PMEase activity in response to salt injury to the root tissues.

When phosphate was added to the plant growth medium the levels of PMEase activity were initially lower than with phosphate omitted, but then increased with increasing salt concentrations. This response also was highly significant ($P = \leq 0.001$) and may reflect the fact that NaCl interferes with phosphate uptake into the roots with the result that PMEase activity increases. Alternatively a combination of phosphate and NaCl could induce the release of cellular PMEase onto the root surfaces. The second

Fig. 6.1 Effect of NaCl concentrations on PMEase activity of roots of cv Qalandri grown without and with phosphorus added to the growth medium



explanation may be more valid since the activity of PMEase actually increases and appears unaffected by salt in contrast to the situation where P was not present in the growth medium.

Highly significant differences ($P = \leq 0.001$) were observed in plants grown with and without phosphorus added to the growth medium. Highly significant differences ($P = \leq 0.001$) were found in the interaction between concentration and conditions.

Table 6.1 Summary of statistical analyses for PMEase activity under salinity treatments

Source	PNPP	Probability	4-MUP	Probability
Condition	198 (1)	** 0.001	1739 (1)	** 0.001
Concentration	130 (4)	** 0.001	1014 (4)	** 0.001
Condition x concentration	57 (4)	** 0.001	432 (4)	** 0.001

6.2 Effect of growth in the presence of specific metallic ions on PMEase activity in *cv* Qalandri roots

The objectives of this investigation were to study the PMEase activity of cotton cv Qalandri roots which had been grown without or with specific metallic ions present. Plants were grown for 12 days with or without 0.02 mM of Zn, Fe, Mn, Al or Ni in the growth media; duplicate treatments were set up to which phosphate was added or omitted. For the assay of PMEase activity pNPP and 4-MUP were used as substrates at pH 5.5.

Table 6.2 shows that with both substrates, and both phosphate levels, the presence of all of the metal ions acted to reduce PMEase activity. The data show that the differences are highly significant in the rate of activity ($P = \leq 0.001$) among the heavy metals. The greatest reduction in activity was seen for Al and Ni with both substrates and phosphate treatments. In all cases highly significant differences ($P = \leq 0.001$) were noted for interactions between heavy metals and phosphorus conditions with least activity when

phosphorus was present. The data also indicated highly significant ($P = \leq 0.001$) interactions among heavy metals, phosphorus conditions and substrates.

Table 6.2 Effect of cations on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown without and with phosphorus added to the growth medium in the presence of Zn, Fe, Mn, Al, Ni. pNPP & 4-MUP were used as substrates ($n = 4 \pm SD$)

Salt added	PMEase activity (μmol mg d. wt^{-1} h^{-1})			
	pNPP		4-MUP	
	- P	+ P	- P	+ P
$ZnSO_4$	0.101	0.083	0.074	0.057
$FeSO_4$	0.090	0.076	0.067	0.045
$MnSO_4$	0.111	0.106	0.093	0.075
$Al_2(SO_4)$	0.064	0.057	0.052	0.031
$NiSO_4$	0.054	0.046	0.043	0.033
Control	0.144	0.128	0.112	0.092
ANOVA	F value	-	Probability	-
Cation	304 (5)	**	0.001	-
Condition	5030 (1)	**	0.001	-
Substrate	212 (1)	**	0.001	-
Cation x condition	56 (5)	**	0.001	-
Cation x substrate	204 (5)	**	0.001	-
Condition x substrate	N.S	-	-	-
Metal x condition x substrat	38 (5)	**	0.001	-

6.3 Effect of different concentrations of ions on the PMEase activity of *cv* Qalandri

A study was carried out to investigate the individual influence of ion concentrations on PMEase activity. To do this plants were grown under conditions where phosphate was added to or omitted from the growth medium. A series of cultures were set up using a range of concentrations of different ions as indicated to the two conditions described above see Fig.2.3 and 2.4 (Chapter 2). The levels of PMEase activity detected following these treatments are shown in Table 6.3.

Sodium

No discernible trend was seen for the effects of added sodium at low concentration either with cultures from which phosphate was omitted or added. The highest activity 0.201 µmol mg d. wt^{-1} h^{-1} was noted at 1.0 mM and the lowest activity 0.160 µmol mg d. wt^{-1} h^{-1} was recorded at 0.1 mM of sodium.

Potassium

A trend was seen for the effects of increased potassium in the plant growth medium where increase of the ion caused a decreases in activity. For cultures without phosphate the maximum activity (132 µmol mg d. wt^{-1} h^{-1}) was noted at 10 mM potassium. A similar trend was seen when potassium was added to cultures which contained phosphate.

Table 6.3 Effect of concentrations of various ions in the plant growth medium on PMEase (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown without and with phosphorus added to the growth medium, 4-MUP was used as substrate (n = 4, ± SD)

Concentration	K		Na		Mg	
(mM)	- P	+ P	- P	+ P	- P	+ P
10.0	0.132 ± 0.01	0.043 ± 0.00	0.162 ± 0.03	0.152 ± 0.01	0.125 ± 0.00	0.118 ± 0.01
1.0	0.144 ± 0.00	0.066 ± 0.00	0.201 ± 0.00	0.147 ± 0.01	0.164 ± 0.01	0.148 ± 0.00
0.1	0.152 ± 0.01	0.089 ± 0.01	0.160 ± 0.00	0.157 ± 0.02	0.147 ± 0.02	0.137 ± 0.01
0.01	0.152 ± 0.04	0.076 ± 0.00	0.169 ± 0.01	0.154 ± 0.01	0.140 ± 0.02	0.135 ± 0.01
0.001	0.159 ± 0.00	0.080 ± 0.01	0.189 ± 0.01	0.166 ± 0.00	0.138 ± 0.02	0.133 ± 0.01
Control	0.165 ± 0.00	0.085 ± 0.00	0.198 ± 0.02	0.183 ± 0.01	0.132 ± 0.00	0.128 ± 0.01
ANOVA	Source	Ions	Concentration	Condition	Ion x concen.	Ion x condition
	F value	141 (4)	17 (5)	N.S	6 (20)	34 (4)
	Probability	** 0.001	** 0.001	-	** 0.001	** 0.001

Table 6.3 cont. Effect of concentrations of various ions in the plant growth medium on PMEase (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown without and with phosphorus added to the growth medium 4-MUP was used as substrate (n = 4, ± SD)

Concentration	Ca		Zn	
(mM)	- P	+ P	- P	+ P
10.0	0.134 ± 0.00	0.119 ± 0.00	0.009 ± 0.00	0.008 ± 0.00
1.0	0.129 ± 0.01	0.114 ± 0.00	0.017 ± 0.00	0.088 ± 0.00
0.1	0.120 ± 0.00	0.109 ± 0.01	0.056 ± 0.00	0.090 ± 0.00
0.01	0.111 ± 0.00	0.101 ± 0.02	0.084 ± 0.01	0.087 ± 0.00
0.001	0.109 ± 0.01	0.096 ± 0.01	0.093 ± 0.03	0.084 ± 0.00
Control	0.099 ± 0.01	0.072 ± 0.00	0.110 ± 0.04	0.082 ± 0.00
ANOVA	Concen. x condi.	Ion x con x cond		
	N.s	3 (20)		
	-	* 0.01		

Magnesium

A partial trend of increased PMEase activity with increased magnesium for plants grown without phosphate was seen. However, in the presence of phosphate there was a marked stimulation of PMEase activity by magnesium up to 1 mM.

The highest PMEase activity 0.148 μmol mg d. wt^{-1} h^{-1} was noted at 1.0 mM and lowest activity 0.118 μmol mg d. wt^{-1} h^{-1} was obtained at 10.0 mM. The data (Table 6.3) indicate that for different ions the differences were highly significant ($P = \leq 0.001$). Highly significant differences ($P = \leq 0.001$) were obtained for concentrations. Highly significant differences ($P = \leq 0.001$) were recorded between interactions of ions and conditions. Highly significant differences ($P = \leq 0.001$) were recorded between interactions of ions and concentrations. Non-significant results were obtained for interactions between concentrations and conditions.

Calcium

The results (Table 6.3) showed that in plants grown without phosphorus added to the growth medium the highest PMEase activity (0.134 μmol mg d. wt^{-1} h^{-1}) was obtained at 10.0 mM concentration of calcium, and lowest activity 0.099 μmol mg d. wt^{-1} h^{-1} was recorded in plants which were grown without added calcium in the medium. A consistent increase in PMEase, both without and with added phosphorus, was noted for increased concentrations of calcium.

Zinc

Zinc, at all concentrations, was shown to be highly inhibitory to the build up of phosphatase activity in roots of plants with or without added phosphate. Activity at 10 mM zinc was more than 100 times less than in the controls where phosphate was not added. Where phosphate was added to the growth medium at 10 mM was highly inhibitory, effects at lower concentrations were not seen.

CHAPTER 7

7.1 Comparison of PMEase activity of different plant species

As a part of an understanding of the relative levels of PMEase activity in cotton a comparison between the *cv* Qalandri and other crop plants was made. The seedlings of all the species used were grown with and without phosphorus added to the growth medium. For this experiment wheat, rice and cotton plant were used with two substrates pNPP, 4-MUP. In addition an assay pH range was utilized.

Cotton

When pNPP was used as the substrate (Fig. 7.1) the peak of PMEase activity was noted at pH 5.5. The activity increased after pH 4.0 to the maximum at pH 5.5 and started to decrease from pH 6.5 to 10.0. The data showed (Table 7.1) that the plants grown with and without phosphorus added to the growth medium, had highly significant differences in activity ($P = \leq 0.001$). The plants grown without phosphorus added to the growth medium showed more PMEase activity at all pH levels, the range of PMEase activity was 0.011 (pH 10.0) to 0.195 µmol mg d. wt^{-1} h^{-1} (pH 5.5) in comparison with the plants grown with phosphorus added to the growth medium where the range of PMEase activity was 0.005 (pH 10.0) to 0.160 µmol mg d. wt^{-1} h^{-1} (pH 5.5).

When 4-MUP was used as the substrate (Fig.7.2) the maximum PMEase activity was noted at pH 5.5. The activity increased after pH 4.0 to the peak at pH 5.5 and started to decrease from pH 6.0 to 10.0. The data showed (Table 7.1) that the plants grown with and without phosphorus added in the growth medium, highly significant differences ($P = \leq 0.001$) between their activities. The plants grown without phosphorus added to the growth medium showed more PMEase activity at all pH levels, the range of PMEase activity 0.004 (pH 10.0) to 0.153 µmol mg d. wt^{-1} h^{-1} (pH 5.5) was recorded, in comparison with the plants grown with phosphorus added to the growth medium, where the range of PMEase activity 0.003 (pH 10.0) to 0.132 µmol mg d. wt^{-1} h^{-1} (pH 5.5) was noted.

Wheat

Wheat plants (Fig.7.1) showed maximum PMEase activity at pH 6.0. The activity increased after pH 3.5 reached its optimum at pH 6.0 and started decline from pH 6.5 to 10.0. The data showed (Table 7.1) that for the plants grown with and without phosphorus added to the growth medium had highly significant differences in activity ($P = \leq 0.001$). The plants grown without phosphorus added to the growth medium, yielded more PMEase activity at all pH levels, the lowest PMEase activity was at pH 10.0 with the highest at pH 6.0. Plants grown with phosphorus added to the growth medium, showed lower PMEase activity at pH 10.0 but the pattern of phosphatase to pH was the same as for when phosphate was omitted.

Fig.7.2 shows the peak PMEase activity was found at pH 5.0. The activity increased after pH 3.5, reached its peak at pH 5.0 and started to decrease from pH 5.5 to 10.0. The results show (Table 7.1) that the plants grown with and without phosphorus added to the growth medium highly significant differences ($P = \leq 0.001$) were recorded. The plants grown with no phosphorus added to the growth medium gave more PMEase activity at all pH levels. The lowest PMEase activity was at pH 10.0 with the highest at pH 5.0 when plants grown with phosphorus added to the growth medium, where the plants were grown without phosphorus added to the medium the same trend of PMEase activity was also observed.

Rice

Rice plants showed maximum PMEase activity at pH 5.0. The activity increased from pH 3.5, reaching its maximum at pH 5.0 and started to decrease from pH 5.5 to 10.0. The data showed (Table 7.1) that the plants grown with and without phosphorus in the growth medium had highly significant differences ($P = \leq 0.001$) between them in activity. The plants grown without phosphorus added to the growth medium, showed more PMEase activity at all pH levels, the lowest PMEase activity was at pH 10.0 and the highest at pH 5.0 in the plants grown without phosphorus added to the growth medium,

Fig.7.1 Comparison of PMEase activity in different plant species grown without and with phosphorus added to the growth medium pNPP used as substrate

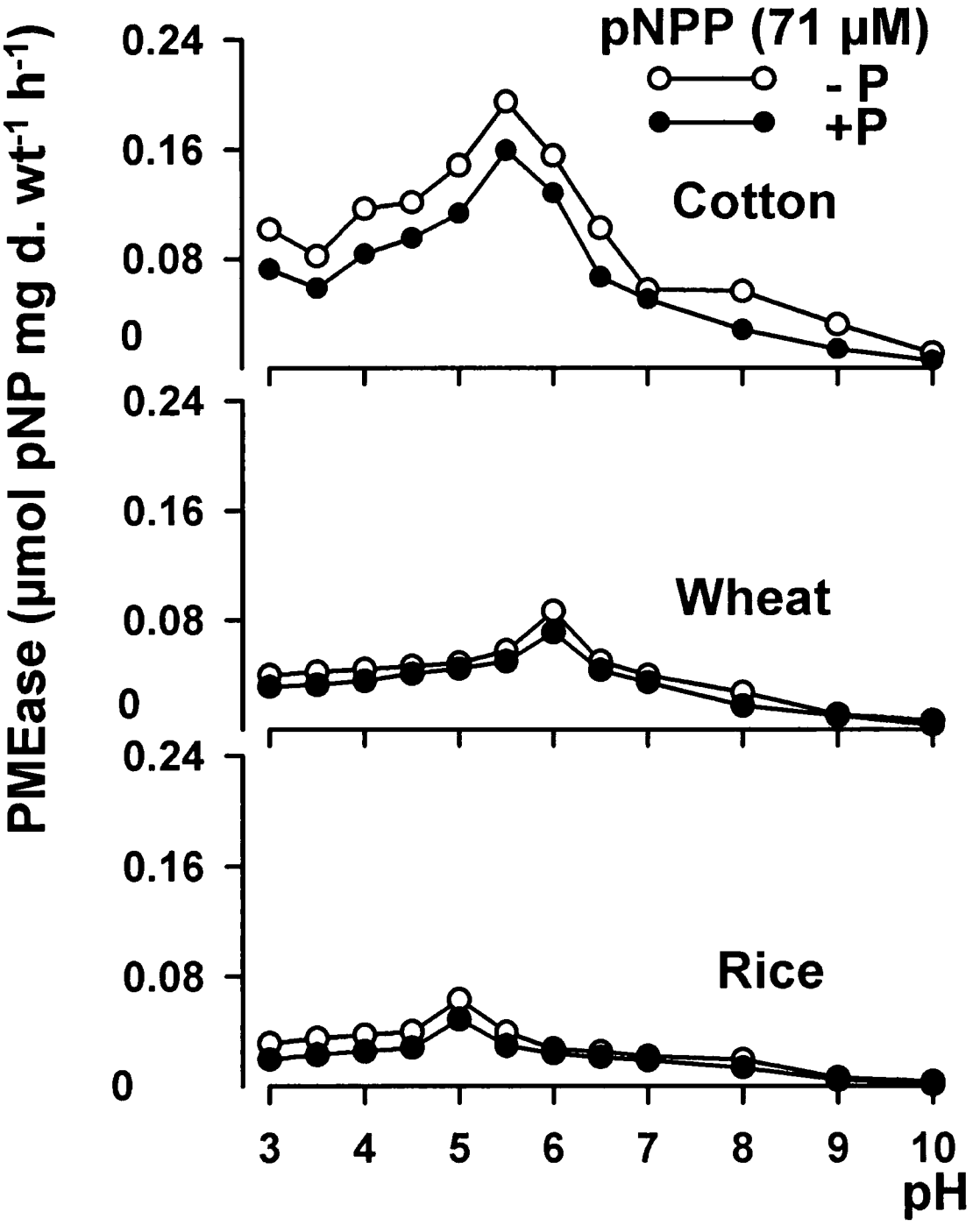
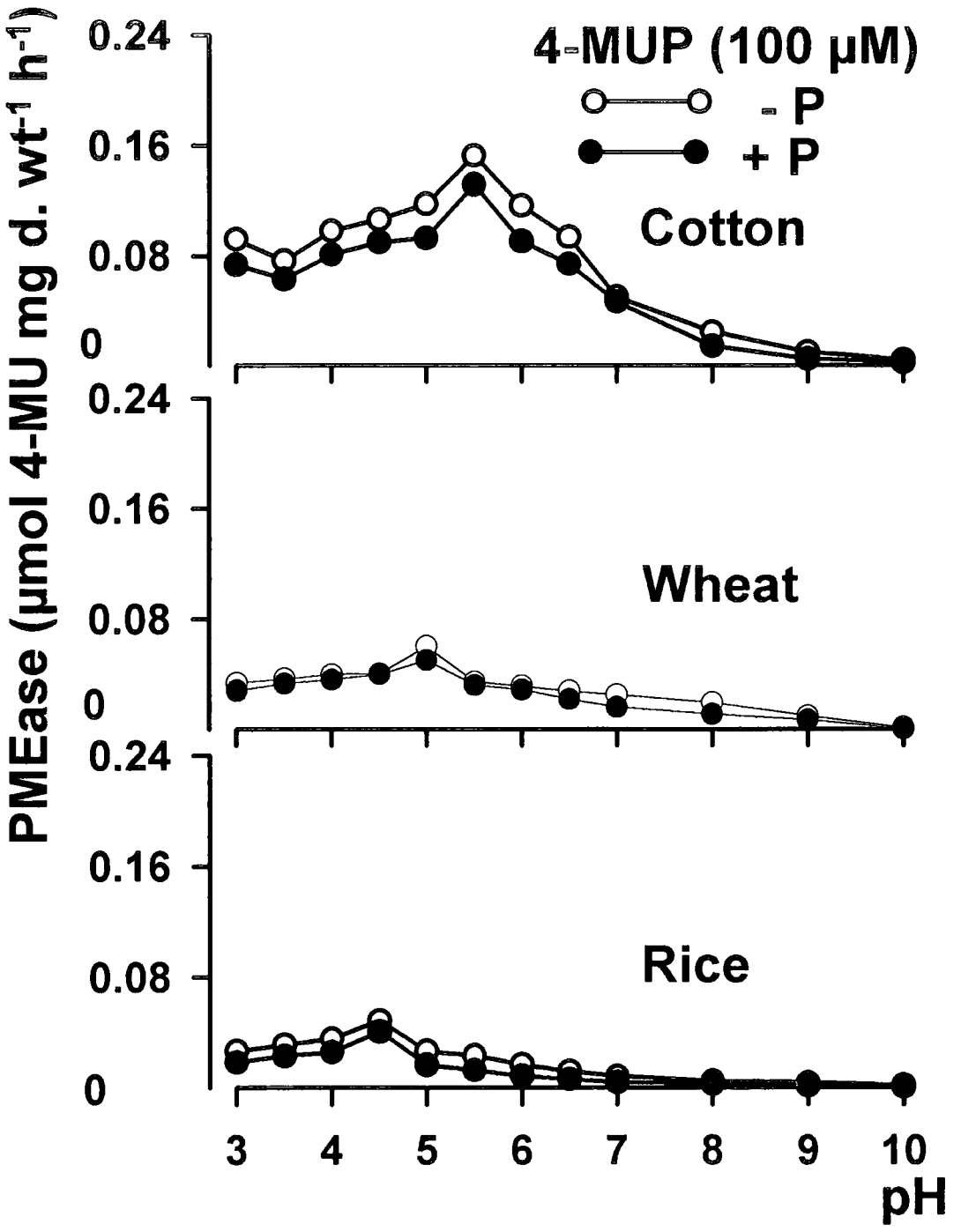


Fig.7.2 Comparison of PMEase activity in different plant species grown without and with phosphorus added to the growth medium 4-MUP used as substrate



while the same pattern of activity observed when the plants were grown with phosphorus added to the growth medium.

At all pH values it was noted that rice had higher PMEase activity when phosphorus was omitted from or added to the plant growth medium.

In the case of rice plants (Fig.7.2) the peak PMEase activity was found to be at pH 4.5. The activity increased after pH 3.5 to the maximum at pH 4.5 and started to decrease from pH 5.0 to 10.0. The data showed (Table 7.1) that the plants grown with and without phosphorus added to the growth medium were highly significantly different in their activities ($P = \leq 0.001$). Highly significant differences ($P = \leq 0.001$) were noted for pH's.

The differences in PMEase activity between species were also highly significant ($P = \leq 0.001$). The highly significant differences ($P = \leq 0.001$) were also noted in activity for the two substrates. The data (Table 7.1) indicate highly significant differences between the interaction for species and substrates ($P = \leq 0.001$). The results showed highly significant differences ($P = \leq 0.001$) were noted for the interaction of pH, conditions and species. The data also showed highly significant differences ($P = \leq 0.001$) , for interaction of conditions, species and substrates.

When a comparison was made between the PMEase activity for the different species using pNPP with and without phosphorus added to the growth medium, it was found that cotton was 68 % and 69 % higher than rice respectively. Also the values for cotton were 55 % and 56 % higher than for wheat. When 4-MUP was used as the substrate cotton was found to have PMEase activity which was 68 % and 70 % higher then rice and 61 %, 62 % higher then wheat. As with pNPP, the PMEase activity in cotton plants was higher than that for wheat or rice.

Table 7.1 Summary of statistical analyses of PMEase activity of roots of different plant species grown without and with phosphorus added to the growth medium.

Source	F value	Probability
pH	1249 (11)	** 0.001
Condition	908 (1)	** 0.001
Species	8814 (2)	** 0.001
Substrate	892 (1)	** 0.001
pH x condition	9 (11)	** 0.001
pH x species	301 (22)	** 0.001
pH x substrate	85 (11)	** 0.001
Condition x species	188 (2)	** 0.001
Condition x substrate	32 (1)	** 0.001
Species x substrate	17 (2)	** 0.001
pH x species x condition	4 (22)	** 0.001
Condi.x species x substrate	16 (2)	** 0.001

7.2 Influence of pH on PMEase activity in roots of *Primula farinosa* and algae

In order to establish the methodology preliminary experiments were carried out on *Primula farinosa* and algae (which had been already tested in the laboratory at Durham), before detailed studies started on cotton root PMEase activity. The purpose was to investigate the PMEase activity of these organisms with range of pH values used 71 μM pNPP and 100 μM 4-MUP as substrates.

The results in Fig.7.3 show that when *Primula farinosa* was assayed with narrow range of buffer pH 4-7, acid PMEase activity was observed. The maximum activity

Fig.7.3 Influence of pH on PMEase activity of *Primula farinosa* using pNPP as substrate

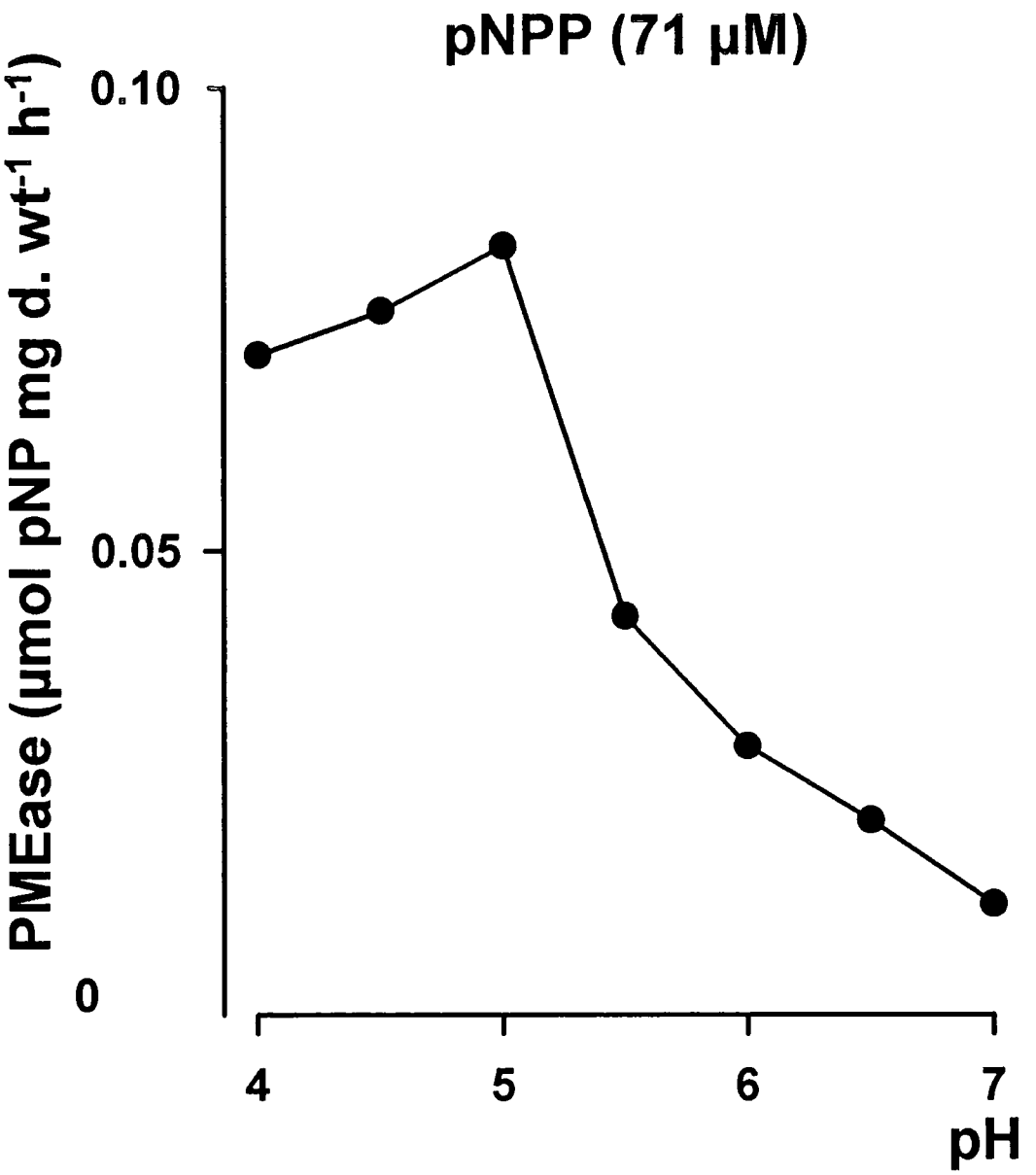
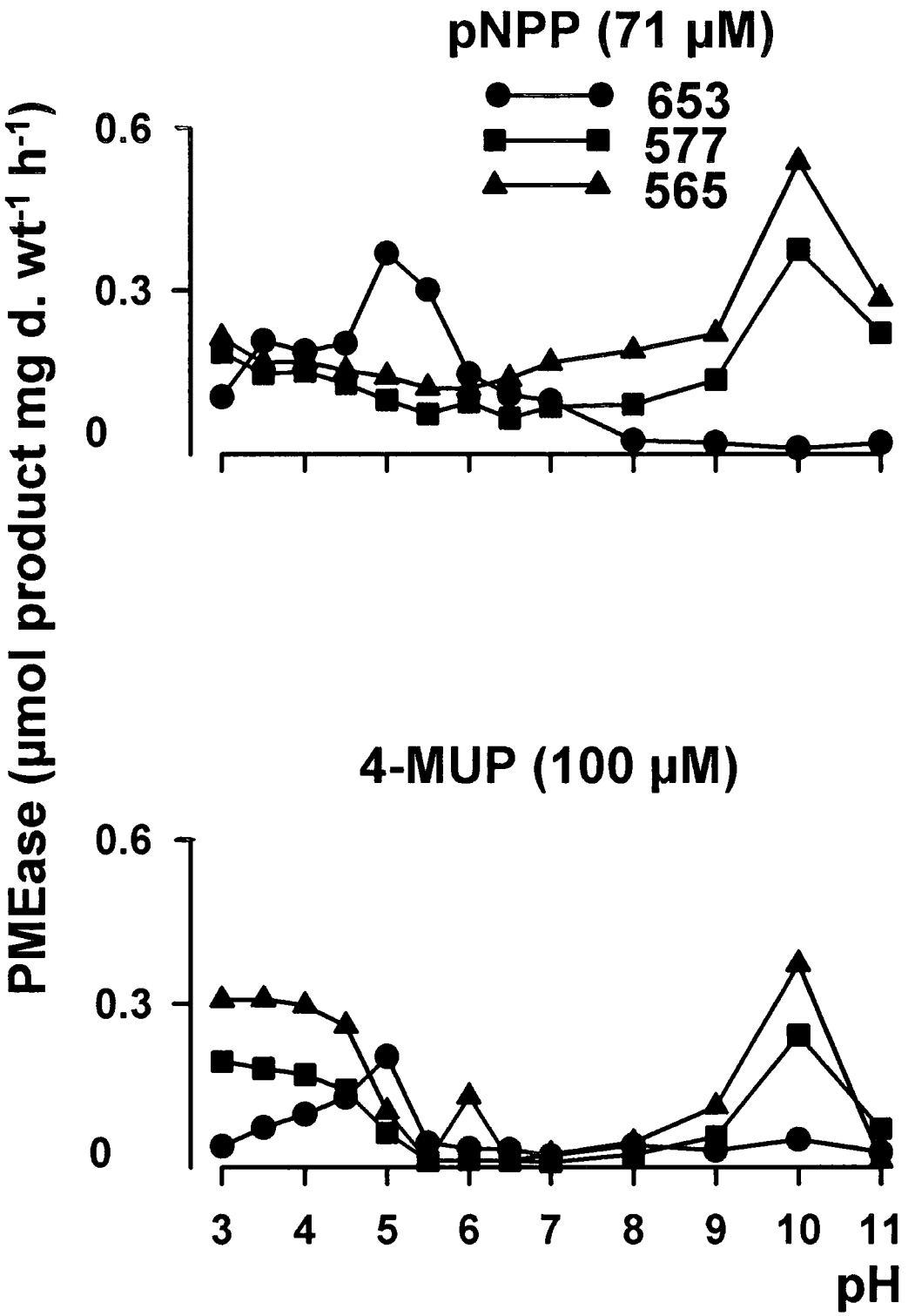


Fig.7.4 Influence of pH on PMEase activity of two strains of *Stigeoclonium* (565 and 577 one strain of *Draparnaldia* (653) using pNPP and 4-MUP as substrates



0.083 μmol mg d. wt^{-1} h^{-1} was recorded at pH 5.0, and after pH 5.5 PMEase activity declined.

In the case of algae the results in Fig.7.4 indicate that when pNPP was used as substrate alkaline PMEase activity was observed in both the strains of *Stigeoclonium* and the maximum activity was noted at pH 10.0. While acid PMEase activity was observed in the strain of *Draparnaldia* 653 at pH 5.0.

Similar results were obtained when 4-MUP was used as substrate.

CHAPTER 8
DISCUSSION

Soil conditions of cotton growing area of Sindh (Pakistan)
Soil pH

Soil pH is an important factor in determining the solubilities of elements that tend to equilibrate with a solid phase. Soil pH is a factor affecting the growth of crops and pastures, and the distribution of native plant species (Epstein, 1972). This is particularly important when considering minerals which may be limiting in agricultural systems. The results presented in this work, Chapter 3 (3.41), indicate that the soil pH's of cotton growing ecological zones of Sindh (Pakistan) vary considerably from site to site and depth to depth. It was noted that soils studied had values above pH 8.0. Cotton tolerates a wide range of soil acidity and alkalinity; the optimum pH for growth lies between 5.0-8.0.

Robinson *et al.*, (1969) reported that for the Gezira and Sabi Valley soils (Zimbabwe) the soil pH was 8.5 in cotton growing regions and produced up to 5600 kg ha^{-1} seed cotton, which is a higher than average yield. Data from experiments in the Lake Victoria region of Tanzania showed that soil pH was mostly between 5.0-6.0 in the cotton growing region (Le Mare, 1974). In the same region, however, the application of lime to a long-term fertilizer trial increased the seed yield from 400-1200 kg ha^{-1}, while raising the pH from 4.5 to 6.2 (Le Mare, 1972). Comparing the results of early workers with the present study, it is reasonable to believe that the soils of the cotton growing zones of Sindh are within the most suitable pH range for growing cotton crops. It could therefore be recommended that most probably pH is not an absolute limiting factor responsible for relative low yields in Sindh province. However, it needs detailed study by conducting yield trials on different soil pH levels which is not within the scope of this study. Of importance to this study in the fact that it has been reported that the availability of soil

phosphorus to the plant may be influenced by the root enviroments eg. soil pH (Murrmann & Peech, 1969)

Soil organic matter

Soil organic matter is a major component in the biochemical cycling of major nutrient elements and the quantity and quality of soil organic matter both reflects and constrains primary productivity (Burke *et al.*, 1989). The data presented in Chapter 3 (3.43) demonstrate that across the districts of the cotton growing zones of Sindh, depths 1, 2, 3 have an average organic matter of 1.12, 0.94 and 0.75 % respectively. These soil organic content levels could be classified as being in range of adequate to medium levels. The trend of organic matter decreased with increasing depth of soil profile. Cotton is a deep-rooted plant, its root penetration in the soil is up to 300 cm and it may be possible that the deep level of soil, where new root growth takes place, may be deficient in organic matter and this may in turn be the cause of yield losses. This is particular possible since young roots are mostly responsible for nutrient uptake.

It is widely reported in the literature that continuous cropping of land leads to depletion of organic matter (Haas *et al.*, 1957; Burke *et al.*, 1989). The extent of soil organic matter depletion depends upon the same variables as those controlling soil organic matter formation, such as parent material, time, climate and biota (Jenny, 1980) and this in turn would lead to a depletion of inorganic mineral availability.

Soil phosphorus

80-90 % of soils from arid and semi arid regions of the World, including Pakistan, are deficient in phosphorus (Memon *et al.*, 1992), eventually it needs to be supplemented to obtain the required yield. The results in Chapter 3 (3.45) indicate that the phosphorus status of the soils of cotton growing areas of Sindh can be regarded as

being deficient, also they vary considerably. For cotton crops the levels of phosphorus present in the soil are probably such as to limit growth significantly. Soil solution phosphorus is the immediate source for plants (Holford, 1989), this is regarded as available phosphorus. The results of this study show that maximum soil phosphorus levels were noted at the surface of the soil (0-15 cm). These results are in agreement with the findings of Tarafdar *et al.*, 1988 who also found that surface soil (0-15cm) had significantly higher available phosphorus. Soils vary greatly in the amount of fertilizer phosphorus required to provide an adequate supply of available phosphorus to plants and plants also vary in the phosphorus requirements for optimum growth (Memon & Fox, 1983; Fox 1981). Phosphorus plays an important role in promoting early growth and establishment of cotton in Pakistan. The general recommendations for the phosphorus in the Sindh soils are 67 kg ha^{-1} for the maximum yield expectation (Saleem *et al.*, 1986). This amount of phosphorus supplementation is economically-demanding and, therefore, the ability of cotton plants to mobilize organic phosphorus becomes an important consideration. Of particular importance are the relative abilities of different cultivars to mobilize organic phosphates which makes them suitable for growing in soils with variable available phosphate contents.

Soil nitrogen content

Nitrogen availability indices of the soil are a measure of the potential of a soil to supply N to plants, when conditions are ideal for mineralization. The results reported in this study, Chapter 3 (3.44), show that the average N % in the cotton growing soils of Sindh is variable. It varies considerably from site to site with the lowest at average rooting depth being present in Hyderabad and the highest in Sanghar. The overall levels, however, are considered to be low when compared with soil from other agricultural systems. Hearn (1981) reported the minimum nitrogen level in the soil 10 ppm below which deficiency symptoms are likely to appear. There is also considerable variation in

nitrogen content with soil depth at any one site. The quantity of N % generally varies with season or year to year depending upon the amount and type of plant residue applied and environmental conditions. Bramer (1965b) and Stevenson (1982) reported that between 0.08 and 0.4 % of total soil nitrogen is mostly in the organic form in surface soils. Birch (1964) has shown that alternate wetting and drying cycles in tropical soils cause the release of mineral nitrogen from organic matter. This may be adequate for the cotton crop, but Jones (1976) has shown that in a wet season the mineral nitrogen may be leached down beyond the root zone before the plant can take it up and use it. Globally soils are deficient in nitrogen and are supplemented with artificial fertilizers. Similarly Sindh soils are also supplied with nitrogen fertilizer for sustained high yields. The added nitrogen requirement for Sindh soils varies from 84-112 kg $ha.^{-1}$ (Saleem *et al.*, 1986). Ramig and Rhoades (1963) working on the fine sandy loam soil samples drawn from 7 depths (0-183 cm) concluded that the nitrogen drastically decreased at increasing depth levels and was only available up to the depth of 61 cm. Since cotton has the capacity for rooting down to 300 cm this indicates that these plants are physiologicaly acting below their full potential. Since the nitrogen supply will be limiting this could have implications for phosphate availability.

Soil potassium

According to Baligar and Bennett (1986), for tropical soils, the efficiency of applied potassium is anywhere from 20-40 %. According to Tisdale and Nelson (1966) only a small proportion, 2-10 %, of the total potassium in the soil is available to plants. It has been suggested that potassium deficiency is not likely to develop until after a number of years of arable cropping (Stephens, 1969; Foster, 1972). As indicated in this study Chapter, 3 (3.46), the average exchangeable potassium ranges from 151.10 (DI) to 103.16 (DIII) in the cotton growing soils of Sindh. The potassium in crop residues is held in the soil in a readily available form after decomposition or burning. Hearn (1981) quotes

figures ranging from 3-6 kg per bale of lint for the potassium lost by the removal of seed cotton at harvest. As the present results indicate Sindh soils are rich in potassium content, therefore it is unlikely that they will be K-deficient. However, in light textured soils applications of 30-50 kg ha $^{-1}$ K_2O at planting are recommended (Saleem *et al.*, 1986). It seems unlikely therefore that potassium availability should be have a significant an effect on plant potassium content and on availabiblity of other minerals.

Mineral content of cotton seeds

The levels of minerals within a seed are of great significant because they will determine the success of seedling establishment follow germination and prior to the seedling becoming self-sufficient. Variation in cultivar success could therefore be due to differences in seed mineral content.The results presented in this work show that non-significant differences were noted among cultivars for phosphorus, potassium and magnesium contents so it is unlikely that these are important in determining cultivars' differences. In terms of the minerals estimated, potassium was present in the highest percentage in all cultivars. In contrast, phosphorus was present in the lowest amounts and so may reflect a limitation for this mineral. However, very marked and significant differences were noted for nitrogen content in seeds from cultivars. These differences may reflect variation in the level of stored nitrogen present as protein, however, individual chemical components were not analysed. The results are partially in agreement with the findings of Bassett *et al.*, 1970 for cotton seed who observed that the nitrogen was most abundant and that phosphorus content was the lowest . The contradiction in the results with regard to nitrogen v/s potassium could be due to several reasons. The above workers used fresh seed from mature plants, while the seed used in the present studies were three years old and it may be that nutritional value of old seeds diminishes over the time period with respect to organic nitrogen content.

Since seeds were similar in the overall phosphorus content this indicates that the seedlings were dependent on absorbed phosphorus from the medium. In the growing plants differences were seen in phosphorus content therefore these differences indicate varying phosphate uptake and utilization by the different cultivars.

Phosphorus in cotton seedlings.

The distribution of phosphorus within a plant is important in order to maintain physiological activity which will be specific to specific tissues. The results of this study indicate that the highest phosphorus levels were detected in the leaf followed by the root and then the stem. The results support the findings of Mullins and Burmerster (1990) who reported the maximum phosphorus distribution in the cotton plant to be in the leaves. These results are consistent with those of Bassett *et al.*, (1970) who provided the evidence that tissues can be listed in order of increasing phosphorus concentration as: roots, stem, mature leaf, immature leaf fruiting parts and finally seed. Working on different varieties of field-grown cotton Mullins and Burmerster (1990) did not find any consistent differences among the varieties studied, these findings are contrary with the results of the present study, where significant differences were observed among cultivars. The differences in results to those of Mullins and Burmerster (1990) could be due to the different plant genotypes used in both sets of studies. However, unlike the study by Mullins and Burmerster (1990), the cultivars used here were all grown in the same nutrient systems and therefore ecological variables were discounted. This indicates that cultivars differ in their activity to absorb and distribute phosphorus..

As may be expected the results indicate that the amount of phosphorus was higher in all plants grown with phosphorus added to the growth medium than the plants grown without phosphorus added to the growth medium. These results are in agreement with the findings of other workers (Coupe, 1984; Khasawnch & Copeland, 1973; Sharplley &

Reed, 1982; Stelley & Morris, 1953) who found that phosphorus concentration in cotton plants may be increased by phosphorus fertilization.

In greenhouse and culture trials with cotton, an increase in phosphorus in the culture solutions had a direct effect on the level of phosphorus in the plants (Ergle & Eaton, 1957). However, other research has not been able to demonstrate such a relationship (Janat & Strochlein 1986).

Nitrogen in cotton seedlings

The results presented here show that the plants of all the cultivars, when grown with N added to the growth medium, showed higher nitrogen concentrations in their parts than plants grown without nitrogen added to the growth medium. These results are in agreement with the findings of Hearn, (1981) who stated that the concentrations of total-nitrogen in leaves and petioles of low-nitrogen plants were in the deficient range. Thompson *et al.*, (1976), while working with cotton, reported that the nitrogen concentrations declined with age in vegetative organs of the plant. They further stated that changes in tissue concentrations of total-N in low-N control plants due to ageing and depletion of soil nitrogen reserves were not as pronounced as in plants from the high-N controls as found in earlier work. The results collected here show that 130, 77, and 48 mg g^{-1} d.wt nitrogen was observed in the roots, leaves and stem respectively. The results are in agreement with the findings of Mullins and Burmester (1990) while working with cotton plants who observed similar results.

Roots of cotton reduce only sufficient $NO^{-}_{3}N$ for their requirements and export the rest to the shoot (Radin, 1977; Radin, 1978). The relationship between growth rate and plant nitrogen concentrations provide valuable information about the way in which plants utilized nitrogen and the efficiency with which they convert it into dry matter (Greenwood *et al.*, 1991).

The nitrogen status of a plant also has been an effect upon the uptake and utilization of other mineral elements. Joham (1951) found that nitrogen fertilizer increased magnesium levels, that phosphate had no effect on magnesium levels and that potassium fertilization decreased leaf magnesium levels.

Cole *et al.*, (1963) found that pretreatments with nitrogen stimulated phosphorus uptake to more than a tenfold increase in phosphorus concentration in the root system. Blanchar and Caldwell (1966) found that ammonium fertilizer increased phosphorus uptake in the absence of significant increases in root growth or water soluble phosphate and concluded that metabolic stimulation was the operating mechanism.

Nitrogen is most effective in increasing phosphorus absorption when the fertilizers are placed together in soil (Miller & Ohlrogge, 1958). Ammonium fertilizer, which are not easily leached, frequently caused greater phosphorus uptake in soil studies than nitrate nitrogen (Blanchar & Caldwell, 1966; Leonce & Miller, 1966; & Olsen & Dreier, 1956).

Potassium in cotton seedlings

All plant parts contained higher potassium concentrations when plants were grown with potassium added to the growth medium than the plants grown without potassium added to the growth medium. The results are in agreement with the findings of Mullins *et al.*, (1994) who also obtained similar results in the leaves, stem and burs of cotton plant.

Magnesium in cotton

The results presented here show that the plants grown with magnesium added to the growth medium yielded higher magnesium concentration in the leaves, stem and roots than the plants grown without magnesium added to the growth medium. Phosphorus

levels in the plant appeared not to be influenced by the levels of magnesium in the growth medium.

Phosphatase in cotton

Since phosphate nutrition is of such importance to plants in general, and cotton in particular, the studies reported here on root surface phosphatase activities are of significance in an agro-economic context. These enzyme systems are important in maintaining the phosphorus status of plants in soils and conditions where phosphate levels are limiting. In addition, as has been shown, many environmental factors associated with growing cotton eg., other ions, have marked effects on the levels of phosphate in cotton, therefore, it was important to investigate the effect of these also on phosphatase activity. Initial experiments were performed with a single *cv* of cotton, Rehmani, in order to establish the presence of the phosphatase enzyme systems. Further in-depth studies involved use of other cultivars. The laboratory studies were carried out on phosphatase activity, to characterise the properties, the influence of environmental factors on phosphatase activity, and the field ecological investigations were also made to understand the original nutritional situations of the plant area from which the cultivars derived.

As has been reported for other higher plant species (McLachlan, 1980) roots of cotton have associated with them phosphatase activty. This can be detected in whole roots which indicates that it is surface cell-bound. The results presented in Table 4.1 indicate that the phosphatase activity was present in the root of cotton *cv* Rehmani.

The results in Chapter 4 (Table 4.2) indicate that some of the phosphatase activity was detected in the cut cells as well as on the surface of the roots. However, experiments designed to wash away contaminant cellular phosphatases indicated that less than 1 % of the activity appeared to be cellular. However, Luff (1993) found retention of pNP by roots of *Typha latifolia.*

The data collected suggest that the injured root released some quantity of phosphatase activity. The results are not fully in agreement with Chang and Bandurski

(1964) who reported that any injury to a root releases considerable phosphatase activity. Other workers have stated that activities measured with excised roots might bear little relation to activities in the root which may affect the external solution. Activity is mediated only by enzymes shown histochemically to be attached to the surfaces of outer cells of the root (Estermann & McLaren, 1961; Hall, 1969).

Washing of the roots did not reduce significantly the associated phosphatase, as would be expected had their micro-flora accounted for the enzyme activity. Further indications that the phosphatase derives from the root are discussed where staining of roots was employed.

Tarafdar and Claassen (1988) found acid phosphatase activity in clover, barley, oats and wheat. Helal and Saucerbeck (1984) also found acid phosphatase activity in maize roots. Roma, Bhargava and Sachar (1983), while working with 48-hour old germinated cotton embryos found acid phosphatase activity. Further they also noted strong inhibition in the activity by the addition of phosphate . Plekhanova et al. (1991) while working with highly purified microsome fraction of primary roots of 3-day cotton seedling (*Gossypium hirsutum* L.), detected significant ATPase activity.

Most higher plants appear to have phosphatase activity associated with their roots. Besford, (1979 b) observed acid phosphatase activity in seven higher plant species in sand culture. Tarafdar and Claassen (1988) while working with clover, barley, oats and wheat also found that phosphatase activity is associated with the roots of tested species. It has been reported that most plants are rich in non-specific and specific acid phosphatase, but do not contain any appreciable alkaline phosphatase activities (McComb *et al.*, 1979; Lee, 1988; Adam, 1989; Gabbrielli *et al.*, 1989). It has been reported that many plant tissues contain acid phosphatase activity in their roots. McLachlan (1976) reported that differences in phosphatase activity of roots between plant genera have been demonstrated and related to the ability of the plants to take up phosphorus from low available phosphorus situations. Soil phosphorus may also be present as organic phosphates, and

phosphatase activity at the surface of some roots has been shown to catalyse the hydrolysis of these to other forms including orthophosphate (Bartlett & Lewis, 1973).

The result presented here show PMEase activities of different species of higher plants; cotton, *Primula*, rice and wheat. The highest root PMEase activity was observed in cotton plants as compared to the other species. It was also noted that with both substrates and with both plant-growing conditions, without or with phosphorus added to the growth medium, the rate of PMEase activity was higher. This is consistent with the findings of other workers (Bieleski, 1973; Boutin *et al.*, 1981; McLachlan & DeMarco, 1982; Dracup *et al.*, 1984) who observed maximum phosphatase activity under conditions of phosphate depletion. In higher plants phosphatases associated with the cell surface have been shown to increase in activity during phosphate starvation (Reid & Bieleski, 1970; Bieleski, 1974; Clark, 1975).

Localization of phosphatase in roots

In order to establish that the activity was associated with the surface of roots, and not with contaminating organisms it was necessary to perform phosphatase staining experiments. The results described in Chapter 4.(Fig. 4.2) show that BCIP staining of phosphatase activity was observed at the root surface, especially in the cell walls. The plant roots grown without phosphorus added to the growth medium exhibited strongly stained tissues. In contrast, plants grown with added phosphorus showed minimal staining.

The results presented here are in agreement with the findings of Dracup *et al.* (1984) who reported that the phosphatase activity was localized in phosphorus deficient clover root cell walls. Also Preston (1974) stated that considerable amounts of enzymes from higher plants are associated with the cell wall.

The results are also in agreement with the findings of Hall and Davie (1971), who while working with *Zea mays* L. root tips, found that, by observations using electron

microscopy, the highest activity for β-glycerophosphatase was found in the cell wall and associated with the vacuoles. They also concluded that the staining for β-glycerophosphatase of fixed root sections showed highest activity in the cell walls of cortical cells.

Lamport and Northcote (1960), using cultured sycamore cells, and Nakagawa *et al.*, (1974), using tomato and cultured rice cells, showed that large amounts of acid phosphatases were tightly bound to the cell wall fraction and not released easily by chemical or physical treatment. Bieleski (1973) suggested that the large increases in phosphatase activity associated with phosphorus deficiency were not associated with increases in the turn-over of cytoplasmic phosphate-ester, since phosphatases appear to be located either in cell walls or in the vacuoles.

Other workers also reported that the enhanced phosphatase activity appears to be localised in the cell wall whereas most of the activity in cells adequate in phosphorus activity is located only at particulate sites in the cytoplasm (Malamy & Horecker, 1961; McLellan & Lampen, 1963; Weimberg & Orton, 1964; Sommer & Blum, 1965).

Given the specific staining of root cells it appears that any microbial contamination was not an important problem. In addition, microscopic observation on root systems of plants grown without and with phosphate did not show visually obvious fungal mycelia or bacterial contamination.

Time course and substrate concentrations

The results presented in this studies show that with both substrates and both plant growing conditions the maximum enzyme activity was found at highest concentrations of the substrates. The results also indicates that up to 50 min, the rate of enzyme activity was linear. At the lower concentrations of the substrate the enzyme activity increased over the time.

The results presented here indicate that the enzyme in cotton roots hydrolized 354 μM pNPP, up to maximum rate of activity, above this concentration the rate of activity was similar with both plants growing conditions without and with phosphorus added to the growth.

Similarly, 250 μM 4-MUP was hydrolyzed at the highest rate of PMEase activity, after this concentration the enzyme activity was not seen to increase. It could be suggested that at high concentrations the enzyme limitation may be occurring with both substrates and both conditions. During the course of this study it was found that an incorrect value had been used for the determination of the pNPP substrate. This resulted from the fact that no allowance was made for the presences of water of crystallization, this also was not indicated on the literature provided with the chemical itself. It is therefore not possible to make a direct comparison between pNPP and 4-MUP activity since whilst it was assumed that both were used at the same concentrations the levels of pNPP was in fact approximately 25 % lower.

Temperature optima

A number of studies have shown that phosphatase enzymes are particularly heat stable and that their optimum temperatures are higher than normal growth temperatures of plants. In these study PMEase activity in roots of cotton *cv* Qalandri seedlings was tested over a wide range of temperatures (Chapter 4, Fig.4.6). The optimum temperature was found to be 55° C with both of the substrates used, pNPP and 4-MUP. These results were obtained with the optimum pH, for roots of plants grown in nutrient solution with either phosphate added or omitted. These results show that in cotton phosphatases are particularly stable enzymes. The optimum is higher than those found for yeast (Watorek *et al.*, 1977) where it was reported that maximum phosphatase rate of activity of *Rhodotorula rubra* was seen at 50° C. However, Lane and Puckett (1979) found maximum phosphatase activities in the lichen *Cladonia rangifera* at 61° C. Al-Shehri,

(1992) working with the moss, *Hydrogonium fontanum*, found an optimum temperature of 60° C for PMEase activity and 65 °C for PDEase.

Similar observations of high temperature optima for phosphatases were made on *Calothrix parietina* (Grainger *et al.*, 1989) and *Calothrix* strain D 764 (Islam & Whitton, 1991).

Effect of ions in the phosphatase assay medium

The results presented indicate that when specific ions, Na, Ca, Mg and K, were omitted from the assay medium the rate of cotton PMEase activity was reduced. The highest PMEase activity was recorded in the control plants where all the ions were present. It was also noted that there was no significant differential reduction in PMEase activity among the ions. As in previous results the plants grown without phosphorus added to the growth medium showed greater PMEase activity.

Effect of ions in the plant growth medium

The results presented here indicate that Na ion had no significant effects on PMEase activity of *cv* Qalandri up to 10 mM, either with cultures from which phosphate was omitted or added. Potassium had effects the PMEase activity of *cv* Qalandri, the increased potassium in growth medium decreases PMEase activity in both the conditions where plants were grown without or with phosphorus added to the growth medium. This may indicate enhanced uptake of phosphate or redistribution within the plant.

Calcium stimulated slightly the PMEase where plants were grown without phosphorus added to the medium in both conditions except 10 mM calcium which is reduced slightly the activity in the plant roots grown with phosphorus added to the growth medium. The results presented are in agreement with the findings of other workers

(Healey, 1973; Doonan & Jenson, 1980; Grainger *et al.*, 1989) who reported that calcium had stimulatory effects on alkaline phosphatase activity of Cyanobacteria species.

Zinc at all concentrations inhibited highly the PMEase activity in both growing conditions without or with phosphorus added to the growth medium. The results presented are in agreement with the findings of Grainger *et al.*, (1989) and Whitton *et al.*, (1990) who stated that zinc at 10 mM had a marked inhibitory effect on PMEase activity in a blue-green algae.

Hasegawa *et al.* (1976) while working with wheat reported that zinc inhibited wall enzymes 23-32 % and showed a 36-39 % inhibition towards cytoplasmic enzymes. Zinc is an important micro-nutrient for growth metabolism in plants (Price *et al.*, 1972).

Influence of plant age on phosphatase activity

As plants age and develop their requirements for specific minerals can change. Szabo *et al.*, (1987) showed that the total phosphorus level in plants increased with increasing phosphate concentration in the growth medium, however, the overall level decreased with plant age. In relation to the phosphate levels it has also been shown that phosphatase activity is not always the same at all ages of plant. At one level the activity of phosphatase builds up to a peak of activity following depreciation of phosphate. McLachlan (1980), working with wheat plants, found that the phosphatase activity increased within the first four days of the plants being deprived of phosphorus and reached its maximum in 8 days before declining. These results were essentially the same as those of Takijima (1953) who found that on the 3rd day after P-limited treatment of wheat roots, phosphatase activity was 20 times higher than just before the treatment and remained high for about 9 days followed by a decline.

In this present study on cotton PMEase activity increased from 13-day old seedlings to19-day old seedlings and then decreased in 25-day old seedlings. The low phosphatase activity in a 13-day old plant in comparison to a 19-day old plants could be

due to high concentrations of phosphorus in the roots of the younger plants and low concentration of phosphorus in the roots of the 19 day-old plants. The decline in the 25-day old plants could be due to senescence of the roots after this time period. It appears, therefore, that phosphatase activity is greatest during the early stages of seedling growth.

The capacity for plants to show phosphatase activity also has been shown to change as the plant ages. Onofeghara and Koroma (1974) reported that acid phosphatase and alkaline phosphatase activity also increased with plant age.

Similarly Barrett-Lennard and Greenway (1982) reported that the total P level in plants increased with increasing phosphate concentration in the growth medium solution, however, levels inside the plant decreased with age. Alternatively Greaves and Webley (1965) concluded that older plants growing in soil would lose considerable amounts of root material including phosphatase to the soil where they could stimulate the growth of micro-organisms, so that the age of the plant could also be important in retention of enzyme activity.

The amount of the Na-soluble wall acid phosphatases probably depends on such factors as plant species, tissues and age (Stafford & Bravinder-Bree, 1972).

Influence of plant growth conditions on phosphatase

Whilst levels of phosphate in the cotton root growth medium have been shown clearly to influence the amount phosphatase activity it is unclear as to what is the influence of internal phosphate status on the activity of the enzyme. Pakarinen (1978) has suggested that the concentration of elements in the plant tissues provide an accurate record of the nutritional status of the environment and hence acid phosphatase activity may be related to the tissue phosphorus concentration. Roux (1970) showed that the hydrolysis of phosphate complexes is unconnected to the absorption of released Pi by studying the hydrolysis of potassium tripolyphosphate by tomato roots at 10° C, or in the presence of 2, 4-dinitrophenyl: in both cases the absorption of Pi is inhibited but the

hydrolysis continues. Whole phosphate-starved roots show an increasing surface phosphatase activity (Roux, 1971 ; Bieleski & Johnson, 1972), and the renewal of the phosphorus supply involves a decrease in the surface phosphatase activity. Within a short term, phosphate status is restored by the increased phosphate influx and decreased phosphate efflux which resulted in a declined phosphatase activity (Bieleski & Ferguson 1983). Bouton *et al.*, (1981) while working with tomato roots reported that the surface phosphatase in different parts depends on phosphorus status during their primary growth

Results presented here for cotton indicate that the phosphatase activity build up in the plants under phosphate limitation conditions is maintained when plants are returned to high phosphate status (Chapter 4, 4.13). Since the phosphatase level increased in the older plants this indicates that phosphatase level is related to the age of the plant rather than the phosphatase limitation in the growth medium.

The results also suggested that the phosphatase activity is not closely connected with the levels of phosphate inside the root system.

Split root system

In order to further distinguish between the phosphate status within a plant and the phosphate levels in the medium a phosphatase activity split root experiment was performed. Here one half of a root system could be supplied with phosphate and the other without phosphate but the phosphate status of the plant should be regulated independently of the roots. The results presented here show that the roots grown without phosphate added to the growth medium gave higher surface phosphatase activity than the roots grown with phosphate added to the growth medium. This indicates that the presence of external phosphate in the medium decreases the phosphatase activity of the roots even through the plant itself may have sufficient phosphorus.

The results are in agreement with the findings of Boutin *et al.* (1981) who, working with tomato roots, reported that split root systems show increases in surface

phosphatase activity locally, i.e. only in the parts of the root system which are phosphorus deficient. They further stated that under phosphorus deficiency conditions, the root surface phosphatase activity depends only on the tissue phosphorus concentrations i.e. the root does not bear any cell wall phosphatase which would be activated when the medium become phosphorus deficient.

Similar results (Bieleski, 1973) concluded that when a plant is grown in a phosphorus deficient substrate, the intracellular and cell wall phosphatase activities increase as the tissue phosphorus concentration decreases.

Excised tomato roots grown in a -P medium show a negative linear relation between their surface phosphatase activity and their tissue concentration (Boutin & Roux, 1973). Brouder and Cassman (1993) while working with split roots of cotton reported that K acquisition is strongly influenced by the quantity and distribution of NO_3^-N in the root zone through its effects on root proliferation, and that distinct culture differences associated with crop performance on low potassium soils can be detected in short term solution culture growth system. Zinc deficiency depressed whole plant dry matter, enhanced P concentration in whole plants and enhanced P concentration in leaves to toxic levels (Webb & Loneragan *et al.*, 1988; Christensen & Jackson 1981; Loneragan *et al.*, 1982).

Webb and Loneragan (1990) while working with wheat reported that when the supply of zinc to the wheat shoots was adequate, omitting zinc from the environment of only half the root system did not affect dry matter or enhance the phosphorus concentration or accumulation in the shoot, or shoot parts; however the translocation of zinc to its paired root system not supplied with zinc may have been adequate to maintain all root functions.

Influence of pH on PMEase activity of three cotton cultivars

Phosphatase activity in plants has been detected over a wide range. However, most higher plants exhibit acid phosphatase activity in their root systems. Hall and Davie (1971) reported phosphatase activity in maize roots, also Ridge and Rovira (1971) showed that most crops plant roots have associated phosphatase activity. In cotton it was similarly found that the activity was associated with the range of acidic buffers used in the assays. Most activity was found in the pH range 4-6. The optimum pH of activity for the cultivars studied varied slightly from 5.5 in *cv* Qalandri to pH 6.0 in Rehmani and pH 4.5 for *cv* Reshami. These results indicate clearly that cotton roots have predominating acidic phosphatase activity. However, some slight activity was also detected in the basic range of pH's. In all except one *cv* there was no difference in pH optimum of phosphatase activity between those plants grown without and with addition of phosphate. The exception was Reshami when pH optimum changed from 4.5 to 5.0 on the addition of phosphate. No difference was seen in pH optimum when either of the two substrates were used. The results are in conformity with earlier workers who described that phosphorus deficiency in the growth medium can produce increased acid phosphatase activity (Soumalainen *et al.*, 1960; Nye, 1967). To play an effective role in increasing inorganic phosphorus supply in P-deficient plants, phosphatase should be capable enough for the operation at the pH of the environment in which their associated organism commonly functions.

The results are in disagreement with the findings of Bieleski (1974), who using the pNPP substrate, showed the presence of both acid and alkaline phosphatases in *Spirodela* in homogenates and a marked shift in favour of alkaline phosphatase activity with phosphorus deficiency in plants. His test in intact plants showed relatively greater alkaline than acid phosphatase activity in both conditions and phosphorus deficient plants having more of both forms present under phosphorus deficient conditions.

The results reported here for cotton are supported by the studies of McLachlan (1980) while working with roots of rye, wheat, buckwheat and subterranean clover plants

who concluded that optimum pH for phosphatase activity lay in the range of pH 5-6. P-deficient plants had greater activity than the P-sufficient ones. The results are also in agreement with Besford (1979) who observed that an increase in leaf acid phosphatase activity associated with phosphorus deficiency in terrestrial plants.

Effect of salinity on phosphatase activity

One of the major problem in the cotton growing regions of Pakistan is the accumulation of salt in the soil as a direct result of irrigation and the high evaporative rate. This accumulation of salt causes marked reduction in cotton production. Mehta and Desal (1959) observed marked reductions in cotton yield under salinization conditions. As a part of this study on phosphatase the effect of salinity on the enzyme system was investigated. The phosphatase activity was investigated in the intact root of cotton seedlings *cv* Qalandri grown with and without added phosphorus to the growth medium in relation to the addition of different concentrations of NaCl.

Results in Chapter 6 (6.1) indicate that salinity has a marked effect on the acid phosphatase activity of cotton roots. However, this effect is complex in that 100-200 mM NaCl reduced phosphatase activity in the plant roots grown without added phosphorus to the growth medium. These levels of NaCl obviously have a deleterious effect on phosphate metabolism. However, at higher concentrations of salt a stimulation of phosphatase activity was noted. This effect could be due these levels of NaCl causing cellular damage with a subsequent leaching out of cellular phosphatase onto the root surface. 250 mM NaCl showed enhanced phosphatase activity with both of the substrates used (pNPP and 4-MUP).

In the case of roots of plants grown with added phosphorus to the growth medium similar effects were seen. It can be hypothesised that increased NaCl concentrations decrease phosphate uptake into cotton plants particularly through an inhibition of organic phosphate mobilization.

The results are in agreement with the findings of Kumar and Tarafdar (1989) who suggested that the acid and alkaline phosphatase activity in the rhizospheres of sunflower was many times higher on saline than in control soils. Similar results were reported by Rai and Abraham (unpublished), for *Anabaena doliolum*, where they observed that extracellular alkaline phosphatase activity increased proportionally with increased NaCl level in the growth medium.

The overall effects on phosphate status of cotton are in agreement with Martinez and Lauchli (1991) who while working with cotton, found an inhibition of phosphorus transport from root to shoot due to salinity.

Effect of nitrogen on phosphatase activity

It has been reported that soil nitrogen was able to stimulate phosphorus uptake more than tenfold and increased the phosphorus concentration in the root zone (Cole *et al.*, 1963). Interactions have been observed between elements when consideration has been made of their uptake into root system. Stimulatory effects on the uptake of one element have been noted in the presence of an other. However, the exact mechanism behind this is unclear and may involve direct or stimulation of uptake increased availability. Leonce and Miller, (1966) suggested that ammonium increased the dissociation rate of the phosphate-carrier complex at the xylem where NH_4 + ions were shown to give an increased phosphorus concentration in corn. Blanchar and Caldwell (1966) found ammonium increased fertilizer phosphorus uptake in the absence of significant increases in root growth or water soluble phosphorus and concluded that metabolic stimulation was the operating mechanism. Ammonium fertilizers, which are not easily leached, frequently caused greater phosphorus uptake in soil studies than did nitrate nitrogen (Blanchar, 1966; & Olsen & Dreier, 1956).Due to the increased yield of biomass on high nitrate, the demand for phosphate will be increased, which is the conditions in the bacterium *Escherichia coli* favouring high phosphatase activity (Torriani, 1960). Growth of

Anabaena flos-aquae at high concentrations of nitrate results in nitrogen rich cells (Bone, 1971) and high phosphatase activity.

In this study the influence of combined nitrogen on phosphate nutrition of cotton was studied with particular reference to stimulation of availability. As has been shown in Chapter 5.(5.3), the inclusion of combined nitrogen in the plant growth medium had the effect of decreasing phosphatase activity in cotton root systems. This was seen in all the cultivars studied.

These results indicate that the overall nutritional status of plants influences the activity to mobilize and absorb another particular ion. In this case decreases of phosphatase was observed by increased combined nitrogen. It is unclear, however, whether this was a direct effect on the enzyme activity or on indirect effect on protein and enzyme synthesis due to the enhanced nitrogen status of the plant. These results are of particular interest if the phosphatase activity is the result of a stimulation of the genes for enzyme synthsis. It would appear that there is a non-specific induction system operating.

Effect of cations on phosphatase

A more extensive study of the effects of ions on phosphatase activity was made using manipulation of the levels of Zn, Fe, Mn, Al and Ni. In all cases these ions had inhibitory effects on phosphatase activity of cotton roots from plants grown without and with phosphorus added to the culture medium.

A number of other studies have been made on the effects of ions on phosphatase activity. Naganna *et al.* (1955) observed that As (v), Mo (vi) and W (vi) are competitive inhibitors of acid phosphatase of potato. Studies by Juma and Tabatabai, 1977 showed that many elements, including heavy metals, inhibited acid phosphatase activity of soil. Other studies by Roberts (1956) on wheat leaves found that Cu (ii), Zn (ii), Ni (ii), Co(ii) and Fe(iii) were strong inhibitors of acid phosphatase activity.

In particular the response of the acid phosphatase activity to zinc seems to be greatly dependent on the tissue, organ and plant species and also on the assay condition (Cox & Thurman, 1978 ; Lane & Puckett, 1979 ; Mathys, 1980). Zinc acts as a non-competitive inhibitor of the cell wall acid phosphatase in the root of *Anthoxanthum odoratum* (Cox & Thurman, 1978). This effect is not always consistent since acid phosphatase is regarded as being fairly tolerant of zinc (Mathys, 1980). Juma and Tabatabai (1988) found that using 250 μM final metal concentrations of Zn, Fe, Ni, Mn, and Al had marked 21 to 62 % inhibitory effects on acid phosphatase activity in roots. However, these inhibitions were species specific and variable.

The results presented in this work on cotton showed 34, 40, 17, 53, and 61 % inhibition of phosphatase activity roots of cotton by Zn, Fe, Mn, Al and Ni respectively, when MUP was used as the substrate. Similar trends of inhibition were seen when pNPP was used as the substrate. Clearly these ions are not all equally active at the same range in inhibiting phosphatase. Zinc has a medium to low effect whereas Ni is the most effective.

Phosphatase of higher plants and algae

Whilst phosphatase activity was shown to be a component of cotton roots, particularly in the absence of phosphate in the plant growth medium, it was essential that this activity be related to that in other plants. A comparison was therefore made between cotton and a range of other species; *Primula farinosa,* rice, wheat and two species of algae *Stigeoclonium* and *Draparnaldia*. In all cases phosphatase was found to be associated with these organisms, with roots of the higher plants and the whole organism in the case of the algae. Acid phosphatase activities were detected with a pH range 4-6 in all except *Stigeoclonium* where the activity was optimum at pH 10.0.

These results are in disagreement with those of Bieleski (1974) who, showed using the same substrate the presence of both acid and alkaline phosphatases in *Spirodela*

homogenates and a marked shift in favour of alkaline phosphatase activity with phosphorus deficiency in the plant.

Wynne (1977) also reported an increase in alkaline phosphatase activity in algal cells with a decrease in phosphorus supply to them, and others (Lien & Knutson, 1973) have shown similar effect with phosphorus deficiency in a culture of micro-organisms. However, phosphorus deficiency in the growth medium have also been seen to produce increased acid phosphatase activity (Soumalamen *et al.*, 1960 ; Heredia *et al.*, 1963; Nye, 1967).

Higher phosphatase activity was noted in cotton roots than in wheat and rice plants at all pH levels, this may be due to nutrient uptake capacity of the plant roots. The results described here show different rate of phosphatase activity in the species examined, this could be due to the different phosphate content in plant tissues. Plant tissues often contain high activity non-specific phosphatases (Bieleski, 1973), and the activity of the enzymes has been shown to be related to the phosphate nutrition of the organism.

The results reported are also in agreement of the findings of Pakarinen (1978) who, concluded that the concentration of elements in the plant tissue provide an accurate record of the nutritional status of the environment and hence acid phosphatase activity may be related to the tissue phosphorus concentration. Clearly the phosphatase status of cotton is higher than the other species of higher plant studied. This indicates that the cotton crop has the capacity to utilize organic phosphates in soils to a relatively efficient level.

The results presented here showed that the roots *Primula farinosa* had acid surface phosphatase activity with maximum at pH 5.0, like other higher plants. The results are in agreement with the findings of other workers who found the phosphatase activity at range of pH from 4.0-6.0. Woolhouse (1969) while working with *Agrostis tenuis* roots found acid phosphatase bound to cell walls. Suzuki and Sato (1973) found acid phosphatase derived from walls of cultured cells of tobacco. Igaue *et al.* (1976) while working with rice also found phosphatase activity in the same range of pH.

In the case of *Stigeoclonium* results indicate both the strains (565, 577) showed alkaline phosphatase activity with optimum at pH 10.0. The results are in agreement with the findings of (Martin *et al.*, 1987) who stated that the pH optimum of three *Stigeoclonium* strains was near 10.0. The results showed that the optimum pH 5.0 was noted for the strain 653 of *Draparnaldia*.

The results of this study are important with regard to the phosphate nutrition of cotton, and the economic aspect of this, in Pakistan. Phosphate deficiency in soils of Pakistan is a major factor in contributing to low crop yields in many areas. Deficiency may arise because of lack of phosphate in the soil and /or its unavailability to plants.

Selection of cotton cultivars for enhanced ability to mobilize organic phosphate is therefore very important. Currently this could be achieved by screening for maximum phosphatase activity in present-day cultivars. In future, however, it should be possible to enhance this capacity through selective breeding programmes. These selections may be made by conventional genetic selection or, once genes for phosphatase have been identified, by selective genetic transformation protocols.

This project should also be continued as an investigation into the phosphatase activity of cotton cultivars no longer in use. Many of these cultivars were used extensively prior to the application of modern phosphate fertilizers and therefore may have retained their capacities for mobilization of organic soil phosphates. These cultivars may well serve as a source of important genomic material. Current cultivars from a wide range of soil types could also be screened. This could be very useful to the Breeder or Agronomist for zoning of specific cultivars for particular phosphate-deficient soils according to the phosphatase capacities of the cultivars.

SUMMARY

1) Seeds of cotton were brought from Sindh Pakistan and were used as a source of seedlings for laboratory studies. A soil survey at the sites of seed origin was carried out in 1992 as a part of this study. It was found all the cotton zones of Sindh had alkaline soil pH, above 8.0. Conductivity of soil varied from 263 to 451 $\mu S\ cm^{-1}$ within the districts. The soil organic matter also varied from 1.47 ppm at Khairpur to 0.29 ppm at Hyderabad.

2) The soil nitrogen did not differ significantly in each district , whereas soil phosphorus was found to vary markedly; both of the elements were found to be at limiting levels for plant growth. Potassium content of the soils was sufficient, although marked differences were observed among the districts, and soil depths. In relation to the phosphate nutritional levels of the plants phosphatase activity of seedling roots was investigated.

3) The roots of cotton cultivars were tested over a range of pH values; all the cultivars showed acid phosphatase activity in the optimum pH range of 4-6. The plants grown without phosphorus added to the growth medium yielded higher phosphatase activity than the plants grown with phosphorus added to the growth medium.

4) Two substrates were used for measurement of PMEase activity, pNPP and 4-MUP. No direct comparison between the rates was possible due to different concentrations being used. It was observed that pNPP gave higher phosphatase activity than 4-MUP even though it was used at a lower concentration.

5) Histological studies were carried out with the roots of cotton *cv* Qalandri grown without and with phosphorus added to the growth medium. The plant roots grown without phosphorus added to the growth medium exhibited strong PMEase staining activity with BCIP, at the root surface, and in the cell walls. Fast Garnet GBC salt and β-naphthyl phenyl phosphate was used and it was found that whole tissues stained indicating PDEase. Staining and a marginal change in phosphatase activity on thorough washing of roots indicated that the PMEase activity was from the roots not from contaminant micro-organism. Microscopy revealed no significant contamination.

6) A range of temperatures were tested for cotton root phosphatase activity, the optimum was observed at 55°C with two substrates pNPP 71 µM and 4-MUP 100 µM, in both growing conditions; without and with phosphorus added to the growth medium. It was noted the exclusion of Na ,Ca, Ma, and K, from the assay medium reduced the PMEase activity of roots of cv Qalandri.

7) Three ages (13, 19, and 25 days) of *cv* Qalandri roots were tested for PMEase activity and it was found that the phosphatase activity increased from 13-day old plants to reach its peak in 19-day old plants and decreased in 25-day old plants. This was seen with both substrates and both growing conditions, without and with phosphorus added to the growth medium.

8) Phosphorus, nitrogen, potassium and magnesium mineral contents were individually studied, in the different parts of cotton cultivars. In all cases the level of phosphate was found higher in those seedlings which were grown with phosphorus added to the growth medium than the plants grown without phosphorus added to the growth medium. A similar trend was observed with the other minerals tested, nitrogen, potassium and magnesium. However, the PMEase activity was found to be higher in roots of plants which were grown without phosphorus or nitrogen added to the growth medium.

9) It was found that omitting magnesium from the plant growth medium had no effect on PMEase activity of *cv* Qalandri roots, whereas roots of plants grown without potassium added to the growth medium showed stimulated PMEase activity in comparison to the plant grown with potassium added to the growth medium.

10) Experiment were performed where the concentrations of ion in the growth medium were increased and the influence on phosphatase activity was limited. It was found up to 10 mM sodium added to the growth medium had no effect on PMEase activity *cv* Qalandri roots, potassium showed marked reduction of PMEase activity, whereas calcium stimulated the activity on lower concentrations, but 10 mM calcium reduced the PMEase activity. Zinc inhibited highly the PMEase activity.

11) Phosphatase activity, increased in the plants under phosphate limitation conditions, was maintained when plants were returned high phosphate status. It was observed phosphatase increased in the older plants, this showed phosphatase level was related to the age of the plant. Use of split root growth conditions indicated that the phosphorus status of the whole plant was not important in regulation of root phosphatase activity. Levels of phosphate at the individual roots were regulating, and particularly at their surfaces.

12) A comparison of different higher plants indicated that cotton was able to maintain a relating high activity of phosphatase activity in its roots.

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APPENDIX A

The raw data for phosphatase assays carried out and reported in the body of this dissertation are included here. Appendix A indicates figure data for Chapter 4.

Fig 4.1 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown with and without phosphorus to the growth medium (n = 4, ± SD)

pH	pNPP		4-MUP	
	- P	+ P	- P	+ P
3.0	0.096 ± 0.004	0.051 ± 0.003	0.058 ± 0.001	0.035 ± 0.001
3.5	0.107 ± 0.001	0.056 ± 0.002	0.087 ± 0.001	0.046 ± 0.001
4.0	0.117 ± 0.003	0.057 ± 0.001	0.091 ± 0.001	0.051 ± 0.002
4.5	0.119 ± 0.014	0.060 ± 0.004	0.093 ± 0.002	0.057 ± 0.002
5.0	0.127 ± 0.003	0.066 ± 0.003	0.096 ± 0.002	0.058 ± 0.001
5.5	0.148 ± 0.004	0.080 ± 0.001	0.104 ± 0.002	0.068 ± 0.001
6.0	0.105 ± 0.003	0.060 ± 0.009	0.091 ± 0.002	0.051 ± 0.002
6.5	0.091 ± 0.003	0.031 ± 0.005	0.084 ± 0.001	0.035 ± 0.001
7.0	0.053 ± 0.001	0.019 ± 0.001	0.035 ± 0.001	0.023 ± 0.000
8.0	0.029 ± 0.004	0.008 ± 0.001	0.029 ± 0.001	0.005 ± 0.000
9.0	0.014 ± 0.001	0.004 ± 0.000	0.008 ± 0.000	0.003 ± 0.000
10.0	0.009 ± 0.001	0.003 ± 0.000	0.003 ± 0.000	0.001 ± 0.000

Fig.4.2 Effect of phosphate on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of 12-day *cv* Qalandri grown with different P concentrations added to the growth medium pNPP & 4-MUP was used as substrates (n = 4, ± SD)

Concentration	pNPP	4-MUP
1 g-1	0.108 ± 0.001	0.086 ± 0.001
2 g-1	0.085 ± 0.000	0.073 ± 0.001
3 g-1	0.076 ± 0.001	0.064 ± 0.001
4 g-1	0.064 ± 0.000	0.056 ± 0.001
5 g-1	0.059 ± 0.000	0.051 ± 0.001
7.5 g-1	0.028 ± 0.000	0.009 ± 0.000
10.0 g-1	0.006 ± 0.000	0.001 ± 0.000
Control	0.142 ± 0.001	0.107 ± 0.001

Fig 4.3 Influence of different concentrations of pNPP substrates on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown without and with phosphorus added to the growth medium

Concentrations	- P	+ P
18 μM	0.078 ± 0.005	0.029 ± 0.003
35 μM	0.101 ± 0.009	0.045 ± 0.003
53 μM	0.145 ± 0.008	0.066 ± 0.001
71 μM	0.162 ± 0.006	0.073 ± 0.001
177 μM	0.264 ± 0.013	0.141 ± 0.004
354 μM	0.490 ± 0.007	0.249 ± 0.003
532 μM	0.486 ± 0.003	0.247 ± 0.000
710 μM	0.485 ± 0.026	0.247 ± 0.008

Fig 4.3 Influence of different concentrations of 4-MUP substrate on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown without and with phosphorus added to the growth medium

Concentrations	- P	+ P
0.5 μM	0.006 ± 0.000	0.003 ± 0.000
5.0 μM	0.010 ± 0.001	0.006 ± 0.000
10.0 μM	0.026 ± 0.000	0.021 ± 0.001
50.0 μM	0.071 ± 0.004	0.062 ± 0.004
100.0 μM	0.142 ± 0.028	0.083 ± 0.009
250.0 μM	0.223 ± 0.003	0.166 ± 0.026
500.0 μM	0.222 ± 0.023	0.192 ± 0.013
750.0 μM	0.222 ± 0.002	0.186 ± 0.031
1000.0 μM	0.221 ± 0.079	0.183 ± 0.006

Fig 4.9 Influence of temperature on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown with and without phosphorus added to the growth medium (n = 4, ± SD)

Temperature	pNPP		4-MUP	
	- P	+ P	- P	+ P
25	0.129 ± 0.001	0.096 ± 0.003	0.087 ± 0.002	0.061 ± 0.003
35	0.137 ± 0.001	0.111 ± 0.001	0.132 ± 0.003	0.096 ± 0.003
45	0.167 ± 0.003	0.137 ± 0.001	0.134 ± 0.002	0.114 ± 0.001
55	0.177 ± 0.003	0.144 ± 0.001	0.162 ± 0.005	0.126 ± 0.000
65	0.151 ± 0.002	0.117 ± 0.002	0.144 ± 0.005	0.110 ± 0.001
75	0.133 ± 0.002	0.104 ± 0.003	0.127 ± 0.002	0.096 ± 0.004

Fig 4.10 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of 13-day *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium using pNPP as substrate (n =4, SD ±)

pH	- P	+ P
3.0	0.099 ± 0.000	0.081 ± 0.001
3.5	0.076 ± 0.001	0.054 ± 0.000
4.0	0.086 ± 0.001	0.061 ± 0.001
4.5	0.088 ± 0.001	0.065 ± 0.000
5.0	0.090 ± 0.000	0.069 ± 0.001
5.5	0.092 ± 0.000	0.072 ± 0.001
6.0	0.096 ± 0.001	0.079 ± 0.001
6.5	0.049 ± 0.001	0.034 ± 0.001
7.0	0.026 ± 0.000	0.011 ± 0.000
8.0	0.009 ± 0.001	0.005 ± 0.000
9.0	0.004 ± 0.000	0.002 ± 0.000
10.0	0.002 ± 0.000	0.001 ± 0.000

Fig 4.10 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1})of 19-day *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium using pNPP as substrate (n = 4, ± SD)

pH	- P	+ P
3.0	0.109 ± 0.001	0.082 ± 0.000
3.5	0.095 ± 0.001	0.068 ± 0.001
4.0	0.123 ± 0.001	0.088 ± 0.001
4.5	0.137 ± 0.001	0.112 ± 0.001
5.0	0.150 ± 0.001	0.127 ± 0.003
5.5	0.161 ± 0.000	0.137 ± 0.005
6.0	0.194 ± 0.004	0.168 ± 0.001
6.5	0.099 ± 0.005	0.072 ± 0.001
7.0	0.079 ± 0.001	0.040 ± 0.001
8.0	0.063 ± 0.001	0.019 ± 0.001
9.0	0.037 ± 0.001	0.011 ± 0.001
10.0	0.011 ± 0.001	0.004 ± 0.000

Fig 4.10 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1})of 25-day *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium using pNPP as substrate (n = 4, ± SD)

pH	- P	+ P
3.0	0.071 ± 0.000	0.051 ± 0.001
3.5	0.049 ± 0.000	0.025 ± 0.001
4.0	0.077 ± 0.001	0.053 ± 0.001
4.5	0.080 ± 0.000	0.058 ± 0.000
5.0	0.084 ± 0.000	0.061 ± 0.000
5.5	0.085 ± 0.000	0.065 ± 0.001
6.0	0.090 ± 0.001	0.075 ± 0.001
6.5	0.046 ± 0.000	0.028 ± 0.001
7.0	0.021 ± 0.001	0.010 ± 0.000
8.0	0.007 ± 0.000	0.003 ± 0.000
9.0	0.003 ± 0.000	0.001 ± 0.000
10.0	0.001 ± 0.000	0.001 ± 0.000

Fig 4.11 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of 13-day *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium using 4-MUP as substrate (n = 4, ± SD)

pH	- P	+ P
3.0	0.086 ± 0.001	0.067 ± 0.002
3.5	0.075 ± 0.001	0.058 ± 0.001
4.0	0.072 ± 0.000	0.050 ± 0.001
4.5	0.073 ± 0.001	0.042 ± 0.001
5.0	0.076 ± 0.001	0.059 ± 0.001
5.5	0.082 ± 0.002	0.064 ± 0.001
6.0	0.066 ± 0.002	0.055 ± 0.002
6.5	0.047 ± 0.002	0.031 ± 0.001
7.0	0.032 ± 0.001	0.011 ± 0.001
8.0	0.011 ± 0.000	0.005 ± 0.000
9.0	0.003 ± 0.000	0.002 ± 0.000
10.0	0.001 ± 0.000	0.001 ± 0.000

Fig 4.11 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of 19-day *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium using 4-MUP as substrate (n = 4, ± SD)

pH	- P	+ P
3.0	0.096 ± 0.001	0.080 ± 0.001
3.5	0.085 ± 0.001	0.069 ± 0.002
4.0	0.100 ± 0.001	0.086 ± 0.001
4.5	0.106 ± 0.001	0.091 ± 0.001
5.0	0.122 ± 0.000	0.101 ± 0.002
5.5	0.157 ± 0.000	0.131 ± 0.003
6.0	0.122 ± 0.000	0.108 ± 0.001
6.5	0.098 ± 0.001	0.056 ± 0.001
7.0	0.052 ± 0.001	0.034 ± 0.001
8.0	0.025 ± 0.001	0.012 ± 0.000
9.0	0.009 ± 0.000	0.005 ± 0.000
10.0	0.003 ± 0.000	0.001 ± 0.000

Fig 4.11 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1})of 25-day *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium using 4-MUP as substrate (n = 4, ± SD)

pH	- P	+ P
3.0	0.057 ± 0.002	0.050 ± 0.000
3.5	0.043 ± 0.001	0.033 ± 0.001
4.0	0.062 ± 0.002	0.054 ± 0.001
4.5	0.066 ± 0.000	0.058 ± 0.000
5.0	0.067 ± 0.001	0.062 ± 0.001
5.5	0.078 ± 0.000	0.068 ± 0.001
6.0	0.063 ± 0.000	0.054 ± 0.001
6.5	0.032 ± 0.001	0.012 ± 0.001
7.0	0.010 ± 0.001	0.003 ± 0.000
8.0	0.003 ± 0.000	0.001 ± 0.000
9.0	0.001 ± 0.000	0.001 ± 0.000
10.0	0.001 ± 0.000	0.001 ± 0.000

Fig 4.12 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1})of *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium, pNPP was used as the substrate (n = 4, ± SD)

pH	-P	+P
3.0	0.143 ± 0.004	0.086 ± 0.002
3.5	0.147 ± 0.005	0.088 ± 0.003
4.0	0.159 ± 0.010	0.097 ± 0.001
4.5	0.166 ± 0.012	0.126 ± 0.005
5.0	0.185 ± 0.011	0.133 ± 0.006
5.5	0.216 ± 0.006	0.152 ± 0.011
6.0	0.192 ± 0.008	0.133 ± 0.005
6.5	0.128 ± 0.003	0.098 ± 0.002
7.0	0.044 ± 0.001	0.036 ± 0.001
8.0	0.032 ± 0.003	0.015 ± 0.003
9.0	0.015 ± 0.001	0.007 ± 0.000
10.0	0.009 ± 0.000	0.002 ±0.000

Fig 4.13 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium 4-MUP was used as the substrate (n = 4, ± SD)

pH	-P	+P
3.0	0.118 ± 0.006	0.071 ± 0.010
3.5	0.128 ± 0.017	0.077 ± 0.002
4.0	0.141 ± 0.012	0.104 ± 0.003
4.5	0.147 ± 0.005	0.116 ± 0.002
5.0	0.169 ± 0.004	0.135 ± 0.012
5.5	0.184 ± 0.006	0.138 ± 0.004
6.0	0.163 ± 0.003	0.133 ± 0.016
6.5	0.119 ± 0.001	0.103 ± 0.003
7.0	0.055 ± 0.001	0.053 ± 0.001
8.0	0.046 ± 0.002	0.027 ± 0.001
9.0	0.006 ± 0.001	0.003 ± 0.001
10.0	0.005 ± 0.000	0.002 ± 0.000

Fig 4.12 Influence of pH on PMEase activity (µmol mg d. wt^{-1} h^{-1}) of *cv* Rehmani seedlings grown with and without added phosphorus to the growth medium pNPP was used as the substrate (n = 4, ± SD)

pH	-P	+P
3.0	0.164 ± 0.013	0.068 ± 0.002
3.5	0.163 ± 0.012	0.075 ± 0.002
4.0	0.172 ± 0.004	0.079 ± 0.009
4.5	0.169 ± 0.012	0.068 ± 0.003
5.0	0.194 ± 0.018	0.068 ± 0.009
5.5	0.187 ± 0.009	0.065 ± 0.003
6.0	0.267 ± 0.029	0.062 ± 0.004
6.5	0.117 ± 0.005	0.042 ± 0.003
7.0	0.084 ± 0.012	0.041 ± 0.001
8.0	0.084 ± 0.002	0.022 ± 0.002
9.0	0.082 ± 0.010	0.022 ± 0.002
10.0	0.063 ± 0.012	0.012 ± 0.006

Fige 4.13 Influence of pH on PMEase activity (µmol mg d. wt^{-1} h^{-1}) of *cv* Rehmani seedlings grown with and without added phosphorus to the growth medium 4-MUP was used as the substrate (n = 4, ± SD)

pH	-P	+P
3.0	0.092 ± 0.003	0.086 ± 0.005
3.5	0.098 ± 0.011	0.086 ± 0.002
4.0	0.106 ± 0.019	0.099 ± 0.004
4.5	0.108 ± 0.028	0.066 ± 0.002
5.0	0.125 ± 0.004	0.056 ± 0.001
5.5	0.157 ± 0.004	0.071 ± 0.001
6.0	0.149 ± 0.017	0.082 ± 0.011
6.5	0.085 ± 0.004	0.058 ± 0.009
7.0	0.057 ± 0.007	0.032 ± 0.003
8.0	0.026 ± 0.000	0.012 ± 0.001
9.0	0.005 ± 0.001	0.002 ± 0.000
10.0	0.002 ± 0.000	0.001 ± 0.000

Fig 4.12 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Reshami seedlings grown with and without added phosphorus to the growth medium pNPP was used as the substrate (n = 4, ± SD)

pH	-P	+P
3.0	0.174 ± 0.011	0.069 ± 0.009
3.5	0.181 ± 0.008	0.074 ± 0.004
4.0	0.189 ± 0.012	0.079 ± 0.009
4.5	0.245 ± 0.018	0.099 ± 0.011
5.0	0.223 ± 0.011	0.101 ± 0.007
5.5	0.204 ± 0.004	0.082 ± 0.005
6.0	0.203 ± 0.037	0.049 ± 0.006
6.5	0.183 ± 0.027	0.048 ± 0.003
7.0	0.156 ± 0.016	0.046 ± 0.007
8.0	0.154 ± 0.011	0.036 ± 0.005
9.0	0.096 ± 0.010	0.023 ± 0.003
10.0	0.081 ± 0.007	0.024 ± 0.007

Fig 4.13 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Reshami seedlings grown with and without added phosphorus to the growth medium 4-MUP was used as the substrate (n = 4, ± SD)

pH	-P	+P
3.0	0.118 ± 0.010	0.075 ± 0.004
3.5	0.110 ± 0.002	0.065 ± 0.003
4.0	0.133 ± 0.006	0.077 ± 0.001
4.5	0.162 ± 0.010	0.089 ± 0.006
5.0	0.171 ± 0.011	0.071 ± 0.009
5.5	0.162 ± 0.008	0.074 ± 0.005
6.0	0.196 ± 0.007	0.099 ± 0.008
6.5	0.107 ± 0.004	0.048 ± 0.003
7.0	0.079 ± 0.006	0.038 ± 0.002
8.0	0.040 ± 0.001	0.009 ± 0.000
9.0	0.005 ± 0.000	0.003 ± 0.000
10.0	0.003 ± 0.000	0.001 ± 0.000

APPENDIX B

Indicates the raw figure data for Chapter 5.

Fig.5.1 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown with and without nitrogen in the growth medium using pNPP as substrate (n = 4, ± SD)

pH	-N	+N
3.0	0.117 ± 0.001	0.094 ± 0.010
3.5	0.103 ± 0.00 4	0.081 ± 0.002
4.0	0.102 ± 0.004	0.085 ± 0.003
4.5	0.105 ± 0.002	0.087 ± 0.004
5.0	0.137 ± 0.001	0.114 ± 0.003
5.5	0.164 ± 0.005	0.144 ± 0.006
6.0	0.244 ± 0.010	0.211 ± 0.005
6.5	0.131 ± 0.004	0.106 ± 0.006
7.0	0.085 ± 0.00 3	0.048 ± 0.002
8.0	0.072 ± 0.001	0.041 ± 0.001
9.0	0.049 ± 0.001	0.030 ± 0.001
10.0	0.023 ± 0.003	0.016 ± 0.002

Fig.5.2 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Qalandri seedlings grown with and without nitrogen combined in the growth medium using 4-MUP as substrate (n = 4, ± SD)

pH	-N	+N
3.0	0.063 ± 0.001	0.050 ± 0.006
3.5	0.057 ± 0.003	0.040 ± 0.001
4.0	0.067 ± 0.001	0.044 ± 0.003
4.5	0.075 ± 0.000	0.045 ± 0.001
5.0	0.093 ± 0.011	0.063 ± 0.001
5.5	0.174 ± 0.005	0.092 ± 0.006
6.0	0.113 ± 0.004	0.069 ± 0.003
6.5	0.083 ± 0.002	0.039 ± 0.002
7.0	0.065 ± 0.001	0.031 ± 0.001
8.0	0.036 ± 0.003	0.016 ± 0.001
9.0	0.026 ± 0.001	0.013 ± 0.001
10.0	0.015 ± 0.001	0.008 ± 0.000

Fig.5.3 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Rehmani seedlings grown with and without nitrogen combined in the growth medium using pNPP as substrate (n = 4, ± SD)

pH	-N	+N
3.0	0.122 ± 0.002	0.084 ± 0.002
3.5	0.113 ±0.006	0.076 ± 0.001
4.0	0.134 ± 0.004	0.196 ± 0.008
4.5	0.138 ± 0.005	0.119 ± 0.004
5.0	0.226 ± 0.007	0.111 ± 0.004
5.5	0.159 ±0.000	0.090 ± 0.005
6.0	0.133 ± 0.004	0.089 ± 0.002
6.5	0.104 ± 0.004	0.081 ± 0.003
7.0	0.088 ± 0.003	0.063 ± 0.007
8.0	0.069 ± 0.003	0.049 ± 0.001
9.0	0.051 ± 0.003	0.026 ± 0.003
10.0	0.024 ± 0.001	0.011 ± 0.001

Fig.5.2 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Rehmani seedlings grown with and without nitrogen combined in the growth medium using 4-MUP as substrate (n = 4, ± SD)

pH	-N	+N
3.0	0.050 ± 0.002	0.037 ± 0.002
3.5	0.057 ± 0.001	0.046 ± 0.002
4.0	0.068 ± 0.002	0.065 ± 0.002
4.5	0.069 ± 0.001	0.047 ± 0.001
5.0	0.073 ± 0.002	0.042 ± 0.001
5.5	0.081 ± 0.002	0.036 ± 0.002
6.0	0.064 ± 0.001	0.033 ± 0.001
6.5	0.046 ± 0.001	0.029 ± 0.001
7.0	0.036 ± 0.001	0.017 ± 0.001
8.0	0.030 ± 0.011	0.012 ± 0.001
9.0	0.012 ± 0.001	0.008 ± 0.000
10.0	0.005 ± 0.000	0.005 ± 0.000

Fig.5.1 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Reshami seedlings grown with and without nitrogen combined in the growth medium using pNPP as substrate (n = 4, ± SD)

pH	-N	+N
3.0	0.064 ± 0.002	0.077 ± 0.001
3.5	0.088 ± 0.002	0.080 ± 0.001
4.0	0.096 ± 0.004	0.081 ± 0.001
4.5	0.102 ± 0.002	0.083 ± 0.001
5.0	0.116 ± 0.001	0.091 ± 0.003
5.5	0.134 ± 0.001	0.098 ± 0.001
6.0	0.185 ± 0.001	0.146 ± 0.010
6.5	0.112 ± 0.001	0.089 ± 0.002
7.0	0.065 ± 0.000	0.043 ± 0.002
8.0	0.051 ± 0.001	0.033 ± 0.001
9.0	0.032 ± 0.001	0.023 ± 0.001
10.0	0.014 ± 0.000	0.009 ± 0.000

Fig.5.2 Influence of pH on PMEase activity (μmol mg d. wt^{-1} h^{-1}) of *cv* Reshami seedlings grown with and without nitrogen combined in the growth medium using 4-MUP as substrate (n = 4, ± SD)

pH	-N	+N
3.0	0.045 ± 0.001	0.033 ± 0.004
3.5	0.051 ± 0.001	0.036 ± 0.002
4.0	0.057 ± 0.004	0.040 ± 0.001
4.5	0.067 ± 0.001	0.053 ± 0.010
5.0	0.087 ± 0.002	0.062 ± 0.005
5.5	0.103 ± 0.003	0.070 ± 0.004
6.0	0.052 ± 0.002	0.038 ± 0.003
6.5	0.045 ± 0.001	0.032 ± 0.002
7.0	0.038 ± 0.001	0.027 ± 0.001
8.0	0.030 ± 0.001	0.015 ± 0.002
9.0	0.024 ± 0.001	0.012 ± 0.002
10.0	0.017 ± 0.001	0.008 ± 0.001

APPENDIX C

Indicates the raw figure data for Chapter 6.

Fig.6.1 Effect of NaCl on PMEase activity (µmol mg d. wt^{-1} h^{-1}) of cotton *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium used pNPP substrate (n = 4, ± SD)

Concentrations	- P	+ P
100 mM	0.088 ± 0.013	0.044 ± 0.001
150 mM	0.065 ± 0.001	0.065 ± 0.002
200 mM	0.069 ± 0.001	0.075 ± 0.014
250 mM	0.160 ± 0.003	0.122 ± 0.001
Control	0.151 ± 0.021	0.050 ± 0.002
ANOVA	F (df)	F.Probability
Concentrations	130.40	** 0.001
Conditions	198.21	** 0.001
Con x Condi	56.80	** 0.001

Fig.6.2 Effect of NaCl on PMEase activity (µmol mg d. wt^{-1} h^{-1}) of cotton *cv* Qalandri seedlings grown with and without added phosphorus to the growth medium used 4-MUP substrate (n = 4, ± SD)

Concentrations	- P	+ P
100 mM	0.064 ± 0.003	0.031 ± 0.001
150 mM	0.047 ± 0.001	0.048 ± 0.002
200 mM	0.056 ± 0.001	0.058 ± 0.004
250 mM	0.136 ± 0.004	0.090 ± 0.010
Control	0.124 ± 0.002	0.037 ± 0.001
ANOVA	F (df)	F.Probability
Concentrations	1014.08 (4)	** 0.001
Conditions	1739.44 (1)	** 0.001
Con x Condi	432.54 (4)	** 0.001

APPENDIX D

Indicates the raw figure data for Chapter 7.

Fig.7.1 Comparison of phosphatase activity (μmol mg d. wt^{-1} h^{-1}) of different species of higher plant seedlings grown without and with phosphorus added to the growth medium, pNPP used as substrates (n = 4, ± SD)

pH	Cotton		Wheat		Rice	
	- P	+ P	- P	+ P	- P	+ P
3.0	0.101 ± 0.000	0.073 ± 0.001	0.040 ± 0.001	0.031 ± 0.002	0.031 ± 0.000	0.020 ± 0.000
3.5	0.082 ± 0.001	0.059 ± 0.003	0.042 ± 0.001	0.033 ± 0.002	0.035 ± 0.000	0.023 ± 0.001
4.0	0.117 ± 0.001	0.083 ± 0.001	0.044 ± 0.002	0.036 ± 0.001	0.038 ± 0.001	0.026 ± 0.001
4.5	0.122 ± 0.003	0.095 ± 0.001	0.046 ± 0.002	0.041 ± 0.001	0.040 ± 0.001	0.028 ± 0.001
5.0	0.148 ± 0.001	0.113 ± 0.002	0.049 ± 0.000	0.044 ± 0.000	0.063 ± 0.002	0.050 ± 0.001
5.5	0.156 ± 0.001	0.128 ± 0.002	0.058 ± 0.001	0.050 ± 0.001	0.040 ± 0.001	0.030 ± 0.002
6.0	0.195 ± 0.003	0.160 ± 0.002	0.087 ± 0.002	0.071 ± 0.001	0.027 ± 0.001	0.024 ± 0.002
6.5	0.103 ± 0.002	0.067 ± 0.001	0.050 ± 0.001	0.043 ± 0.001	0.025 ± 0.001	0.022 ± 0.002
7.0	0.058 ± 0.001	0.051 ± 0.003	0.040 ± 0.002	0.035 ± 0.001	0.022 ± 0.001	0.019 ± 0.000
8.0	0.056 ± 0.001	0.028 0.002±	0.027 ± 0.002	0.017 ± 0.001	0.020 ± 0.000	0.014 ± 0.001
9.0	0.032 ± 0.003	0.014 ± 0.001	0.011 ± 0.001	0.010 ± 0.001	0.006 ± 0.000	0.004 ± 0.001
10.0	0.011 ± 0.001	0.005 ± 0.000	0.006 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.001 ± 0.000

Fig.7.2 Comparison of phosphatase activity (μmol mg d. wt^{-1} h^{-1}) of different species of higher plant seedlings grown without and with phosphorus added to the growth medium, 4-MUP used as substrate (n = 4, ± SD)

pH	Cotton		Wheat		Rice	
	- P	+ P	- P	+ P	- P	+ P
3.0	0.092 ± 0.002	0.074 ± 0.003	0.034 ± 0.002	0.028 ± 0.005	0.027 ± 0.001	0.019 ± 0.002
3.5	0.077 ± 0.003	0.063 ± 0.000	0.037 ± 0.002	0.033 ± 0.000	0.031 ± 0.001	0.023 ± 0.003
4.0	0.098 ± 0.002	0.081 ± 0.001	0.040 ± 0.004	0.036 ± 0.003	0.036 ± 0.002	0.026 ± 0.001
4.5	0.107 ± 0.002	0.090 ± 0.002	0.041 ± 0.005	0.040 ± 0.001	0.049 ± 0.002	0.040 ± 0.001
5.0	0.118 ± 0.001	0.093 ± 0.003	0.060 ± 0.001	0.050 ± 0.002	0.027 ± 0.001	0.017 ± 0.000
5.5	0.153 ± 0.002	0.132 ± 0.002	0.034 ± 0.000	0.032 ± 0.000	0.023 ± 0.002	0.013 ± 0.003
6.0	0.117 ± 0.001	0.091 ± 0.001	0.031 ± 0.000	0.028 ± 0.000	0.017 ± 0.001	0.009 ± 0.001
6.5	0.094 ± 0.001	0.074 ± 0.000	0.028 ± 0.002	0.022 ± 0.003	0.012 ± 0.000	0.006 ± 0.001
7.0	0.050 ± 0.002	0.047 ± 0.001	0.025 ± 0.003	0.016 ± 0.000	0.008 ± 0.000	0.004 ± 0.001
8.0	0.025 ± 0.002	0.014 ± 0.000	0.019 ± 0.002	0.011 ± 0.001	0.005 ± 0.001	0.003 ± 0.001
9.0	0.009 ± 0.000	0.005 ± 0.000	0.009 ± 0.001	0.007 ± 0.000	0.004 ± 0.000	0.002 ± 0.001
10.0	0.004 ± 0.000	0.003 ± 0.001	0.002 ± 0.001	0.004 ± 0.000	0.002 ± 0.000	0.001 ± 0.000