IDENTIFICATION OF PIGEON PEA GENOTYPES FOR HIGH PHOSPHORUS UPTAKE THROUGH ACID PHOSPHATASE ACTIVITY: A BIOCHEMICAL APPROACH

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Affectionately Dedicated
To My
Beloved Parents

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(Arun, K.)

Identification of Pigeonpea (*Cajanus cajana*) Genotypes for High Phosphorus Uptake through Acid phosphatase Activity: A Biochemical Approach

ABSTRACT

Availability of Phosphorus (P) is one of the major constraints limiting the productivity of crop plants, due to its fixation and binding with other elements in the soil. The bound P can be utilized by growing crop genotypes through increased organic acids exudation or by increasing the activity of acid phosphatase, both of which are known to solubilize the bound P.

Keeping this in view, thirty six pigeonpea genotypes were grown with normally available P, organically and inorganic bound P and without P under greenhouse conditions. Based on the leaf P content and enzyme activity, six contrasting genotypes were selected to investigate the physiological or biochemical basis of P uptake and utilization. It was observed that leaf P, shoot and root P, root volume, plant height, root and shoot biomass, R-S ratio and enzyme activity increased when P was provided in organic form and it was noticed that the P uptake efficiency increased under +P conditions whereas, the utilization efficiency increased under –P conditions. Selected genotypes like ICP3226, ICP8477 and ICP 12764 produced more root dry weight, root length and root volume, under P deficiency. There was an increase in root to shoot ratio and enzyme activity under P deficient condition. There was a considerable increase in root acid phosphatase activity in high P uptake types under organic P source and P under deficient conditions also . There was a strong correlation between root acid phosphatase activity and leaf P content in these contrasting types.

Place:
Bangalore

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Major Advisor

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INTRODUCTION

INTRODUCTION

Pulses have been cultivated as protein sources under low input agriculture for thousands of years. Among these pulses, pigeon pea (*Cajanus cajan* L. Millsp.) is a legume crop widely cultivated in tropics and semi arid tropics in soils with low P availability. This crop is generally observed to yield better than other crops in soils with low P or even without fertilizer (Barber, 1980). Possible reasons for this would include (i) an extensive rooting habit, (ii) strong mycorrhizal development, and (iii) the ability of pigeon pea to extract soil P normally unavailable to other crop plants through exudation of organic acids like piscidic acid and acidification of rhizosphere.

Phosphorus is one of the major nutrients required for the normal growth and development of plants. Pigeon pea also requires fairly high amount of phosphorus (50kg per hectare). Though soil contains high amount of phosphorus, its availability to plants is very low. One of the major reasons for non-availability to plants is its chelation or binding with other elements like iron, aluminium and calcium, in most of the acid soils and in calcareous soils respectively.

Soils contain nearly half of the total phosphorus (50-80%) in organic forms, mostly as complex organic esters, in which phosphorus (P) is bound to carbon via oxygen. The forms of organic P can vary with soil. The importance of soil organic P as a source of plant available P depends on its rate of solubilization and release as inorganic P in soil solution. Generally the type of organic phosphorus present within the soil or plant is phytic acid or phytate which is inosoital hexaphosphate the form of P present is phytic acid is easily solublize and made available to plants a group of enzymes called phosphatases which could be either acid phosphatase having the phytase activity.

Phosphatases play a specific role in hydrolysis of organic P compounds (Tabatabai, 1988), they are present in soils but their activities in rhizosphere soil may increase due to the direct release of extracellular phosphatases from the roots of growing plants and due to the release by the growing microbial biomass utilizing the root exudates

(Barber and Lynch, 1976). The rate-limiting step in the process of organic P mineralization might then be the availability of P-esters for hydrolysis and the activity of phosphatase in soil solution.

Phosphatases (P-ases), classified either as acid or alkaline, constitute an enzyme group which is presumed to catalyze the hydrolysis of several organic phosphatemonoesters, liberating available Pi, and occurring scattered in all tissue cells of plant organs (Juma & Tabatabai, 1988). Root-secreted phosphatase activity, named extracellular, is related to the plants ability to make soil P available for absorption. The intracellular acid phosphatases, present in the cytosol, plastids and vacuoles, are responsible for the P-hydrolysis from organic compounds, favoring P mobilization and translocation from senescent tissues (Duff et al., 1994). Therefore, this enzyme activity is a physiological characteristic related to plant efficiency in relation to P acquisition and utilization, and is genetically variable (Tadano et al., 1993). Plants usually secrete root acid Pases when P availability is low; however, plant species differ in secretion ability and enzyme activity (Yan et al., 2001).

The possibility of exploiting genotype differences for improving nutrient efficiency of crop plants has received increased attention in recent years (Baligar and Duncan, 1990). Phosphorus (P) efficient genotypes can be useful for maintaining high productivity in low input agriculture. From mineral nutrition point of view, a genotype is more efficient than others if it absorbs and mobilizes more P from soils (P acquisition efficiency) and/or makes better use of the absorbed P to produce biomass (P use efficiency). Improvement of phosphorus efficiency of crop plants seems possible (Caradus, 1994). Additional breeding of new crop genotypes with improved P efficiency may be a supplementary alternative for reducing the traditional amendments of soils by the applications of fertilizers (Batten, 1992). For successful exploitation of such alternative approaches, the knowledge on the extent of genetic variation among the existing genotypes appears to be a primary step (Mahon, 1983).

The identification and development of plant varieties, which can scavenge phosphorus more efficiently from the soil, will be one of the major steps in increasing agricultural production. Development of novel varieties through classical breeding is slow and laborious process and some time unpredictable in terms of durability. Under such circumstances, alternative tools would be to identify types through a simple screening technique.

With this background, one of the best approaches is to enhance phosphorus uptake under phosphorus deficient conditions by to identify and develop genotypes, which have high phosphorus uptake and utilization efficiency. Hence the specific objectives of this investigation are as follows

1. To evaluate genotypes for phosphorus uptake and to identify the contrasting type
2. To study the activity of acid phosphatase in these genotypes and to co-relate this with the uptake of phosphorus
3. Identify genotypes have high acid phosphatase activity and high P uptake

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

One of the most important, yet least available mineral nutrients for plant growth is phosphorus (P). It plays a vital role in energy transfer and metabolic regulation and is a component of many biological molecules, such as DNA and RNA. Consequently, P assimilation, storage, and metabolism are critical to plant growth and development (Duff et al., 1994).

Phosphorus is required in larger quantities but its availability in the soils is invariably low warranting attention towards its uptake and use efficiency. Phosphorus efficient genotypes can be useful for maintaining high productivity in low input agriculture. The mechanism by which specific plant genotypes are able to grow and produce under mineral stress conditions, while others do not, differs with the mineral element and plant species or by which one genotype is able to accumulate or concentrate higher amounts of an element compared to another. As for as P is concerned plants adopt several mechanisms to acquire more P under P deficient conditions. One such mechanism is the increased activity of acid phospahtase activity which helps in solubilising organically bound P in the soil.

With this background, one of the best approaches to enhance phosphorus uptake under phosphorus deficient conditions would be to identify and develop genotypes, which have high phosphorus uptake and utilization efficiency. Hence the specific objective of this investigation was to identify pigeon pea genotypes with high p uptake through acid phosphatase activity. Keeping this objective in mind a brief review of the work was done and discussed in this chapter. Hence in the chapter a brief review of all the aspects related to this topic are given

Phosphorus efficiency

Phosphorus efficiency is a multifaceted trait, which is influenced by a range of environmental factors. Generally factors that contribute to phosphorus efficiency can be divided into two components.

(i) The efficiency of phosphorus acquisition from the soil-P (acquisition efficiency).

(ii) The efficiency with which phosphorus in the plant is utilized to produce yield (P-use efficiency).

From mineral nutrition point of view, a genotype is more efficient than others if it mobilizes and absorbs more P from soils (P acquisition efficiency) and/or makes better use of the absorbed P to produce biomass (P use efficiency). Improvement of phosphorus efficiency of crop plants by selection seems possible (Caradus, 1994). Additional breeding of new crop genotypes with improved P efficiency may be a supplementary alternative for reducing the traditional amendments of soils by the applications of fertilizers (Batten, 1992). For successful exploitation of such alternative approaches, the knowledge on the extent of genetic variation among the existing genotypes appears to be a primary step (Mahon, 1983).

Plant characteristics considered to be of primary importance for P acquisition efficiency are,

(1) Size and distribution of root system (Nielson, 1979), root hairs (Fohse. et. al.1991) and thereby Modification of soil exploration by roots through increased absorptive area.

(2) Kinetic uptake parameters (Nielsen and Barbar, 1978)

(3) Better symbiosis with soil & microbes

(4) Modification of rhizosphere to increase nutrient availability

- Root induced pH changes (Gahoonia and Nielsen, 1992a)

-Root exudation (Bar-Yosef, 1991).

-Secretion of Acid phosphatases

P Utilization efficiency depends on key factors such as

(1) Partitioning of P among plant parts,

(2) Remobilization of P from vegetatative parts to reproductive plant organs and yield structure.

The possibility of exploiting genotype differences for improving nutrient efficiency of crop plants has received increased attention in recent years (Baligar and Duncan, 1990). Phosphorus (P) efficient genotypes can be useful for maintaining high productivity in low input agriculture.

The reviews related to exploiting genetic variation for P uptake and utilisation are updated under the following headings

a. **Soil Phopshorus and Genetic variability for P uptake and utilization**
b. **Phosphorus uptake affected by root and soil characters**
c. **Adaptation of plants to low P availability**
d. **Acid phosphatases under P deficiency**

Soil Phosphorus and Genetic variability for P uptake and utilization

Soils contain nearly half of the total phosphorus (50-80%) in organic forms, mostly as complex organic esters, in which phosphorus (P) is bound to carbon via oxygen. The forms of organic P can vary with soil. The importance of soil organic P as a source of plant available P depends on its rate of solubilization and release as inorganic P in soil solution.

Soils contain nearly half of the total phosphorus in organic forms, mostly as complex organic esters, in which phosphorus (P) is bound to carbon via oxygen.

The forms of organic P can vary with soil. Hawkes et al. (1984) reported that an old grass land soil (pH 4.6) without fertilizer and lime application for 125 years contained inorganic orthophosphate (22% of extracted P), orthophosphate monoester (49%), orthophosphate

diesters (14%), phosphonates (3%), pyrophosphate (4%). Application of 35 kg P ha- 1 annually for 121 years affected only slightly the organic P forms in the soil.

The importance of soil organic P as a source of plant available P depends on its rate of solubilization and release as inorganic P in soil solution. Phosphatase may play a specific role in hydrolysis of organic P compounds (Brown and Tabatabai, 1978; Eivazi and Tabatabai, 1977; Juma and Tabatabai, 1988; Sharpley, 1985). Soil contains always considerable amount of total phosphatase which is protected by different soil components (Burns, 1982), whereas the activity of soluble enzymes in bulk soil are mostly very low (Asmar, 1992; Asmar et al., 1992).

Phosphatases are thus present in soils but their activities in rhizosphere soil may increase due to the direct release of extracellular phosphatases from the roots of growing plants and/or due to the release by the growing microbial biomass utilizing the root exudates (Barber and Lynch, 1976). Earlier studies (Rogers et al., 1940) showed mineralizing action of corn and tomato roots on organic bound phosphorus. Rape roots induced pH changes in the rhizosphere which influenced the depletion of inorganic phosphate whereas the depletion of labile organic P was maintained unaffected (Gahoonia and Nielsen, 1992b). Wheat and clover differed in their ability to induce phosphatase activities, which significantly correlated ($r = 0.97$) with the depletion of organic P in their rhizosphere (Tarafdar and Jungk, 1987). Hydrolysis of organic P and production of inorganic P for plant uptake may occur more effectively when organic P and/or phosphatase are in soil solution. The rate limiting step in the process of organic P mineralization might then be the availability of P-esters for hydrolysis and/or the activity of phosphatase in soil solution. These steps are important for the ultimate release of inorganic P in soil solution for plant uptake, but at the present state of knowledge, their separation seems difficult.

Genetic variability for P uptake and utilization

In order to improve adaptation of a crop to low P conditions, it is important to explore genotypic variation for all these characteristics. It is known for a long time that crops/varieties differ in the pools of soil P they exploit. Understanding of the effects of such differences on the cropping systems is still rudimentary.

In general breeding for Phosphorus use efficiency (PUE) is considered easy in a crop which has wide genotypic variation in PUE and which is stable in adaptation in terms of responses to low P availability in different soils. Sustainable diversity in PUE parameters was found in maize, rice, field bean, (Baliger.*et.al.*1987). The presence of genetic variation in P uptake from Fe-P is essential to decide breeding strategies for the development of appropriate genetic stocks that could utilize Fe-P more efficiently than the present pigeon pea varieties.

Sumio (1987) reported that at high solution concentrations of P soybean and maize were able to take up P more rapidly than chickpea and pigeon pea (as the Imax is higher). At low concentrations of P chickpea and pigeon pea could take up more rapidly than maize. These root absorption characteristics may help to explain whey chickpea and pigoenpea are less responsive than maize to fertilizer P application. Chickpea usually grows well without P application in vertisol with such low soil solution P levels suggesting that other factors contribute to the enhancement of P uptake from low P concentration in soil solution. These could include extensive root hair development, role of mycorrhizae and the ability to solubilise the soil P.

Based on the evaluation of several crop species for their ability to utilize bound form of Fe-P, it was found that pigeon pea is one of the few crop species that utilizes Fe-P very efficiently (Ae *et al*, 1990a, 1993) like other crop species such as lupin (*Lypinus albus* L.), alfalfa (*Medicago sativa* L.) are reported to have the ability to utilize P from Fe-P (Gardner *et al* 1982). It remains to be determined whether pigeon pea can obtain its entire P requirements for growth from Fe-P. Cultivars of crops differ genetically with

respect to magnitude of uptake, translocation, accumulation and use of mineral nutrients (Chapin, 1982)

Clark (1996) reported the plant genotype differences in uptake, translocation, accumulation and use of mineral nutrients. Subbarao et al (1997) while assessing the genotypic variation in P uptake from Fe-P in pigeon pea reported that P use efficiency varied substantially among genotypes. Dry matter production was positively correlated with P uptake both under Fe-P and Ca-P treatments. Further they have reported a positive correlation was found between P uptake and root dry matter production.

Adu-Guamfi *et al* (1989) reported the effect of P supply on absorption and utilization efficiency of P in relation to dry matter production and dinitrogen fixation in pigeonpea and soybean cultivar under field conditions. The leaf area per plant of pigeonpea increased With P application up to 100kg P ha^{-1}. At low P applications of 5 and 25 kg P ha^{-1}, the medium and long duration cultivars recorded larger leaf area than the short duration cultivars. At low P rates the short duration cultivars distributed more biomass in roots and nodules than the medium and long duration ones. All the pigeonpea cultivars showed an increase in P amount with increasing amount of P applied up to 100 kg P ha^{-1} regardless of P rates, the short duration cultivars absorbed very little amount of P whilst the absorption in medium and long duration cultivars was comparatively high. The P distribution percentage varied tremendously among plant part of pigeonpea with values being 54, 30, 10 and 6 in leaf stem root and nodule respectively.

Krishna (1997) evaluated the genetic variation for P uptake and utilization among peanut (*Arachis hypogea* L.) genotypes. He reports that genotypes of similar physiological maturity differed significantly in their P efficiency ratio in terms of total p per plant, root P, shoot P, and seed P. Significant differences among genotypes for total seed and shoot P contents and for seed P to shoot P ratio were also observed. Several traits contribute to the total P efficiency of the genotype including root length, rate of P uptake per unit root lengths, leaf and pod characters such as P accumulation and dry matter/yield produced per unit P absorbed (i.e. P efficiency ratio).

Weineke (1990) found higher efficiency of P absorption in SC 33-9-8-E (a maize cutlivar) and attributed it to higher allocation of photosynthates to roots and also higher remobilization capacity of the plants from root and leaves. Helal (1990), while attributing the differences in P uptake to root characteristics found variety Daisy as an efficient cultivar in French bean, when P is supplied from a inorganic source.

Tara Singh *et al* (1996) assessed the extent of variation in phosphorus acquisition efficiency of some winter wheat *(Triticum aestivum* L.), winter and spring barley *(Hordeum vulgare* L.) genotypes, depletion of inorganic phosphorus (P) extractable with 0.5 M NaHCO3 (NaHCO3-Pi) from the rhizosphere. The winter wheat, winter barley and spring barley genotypes differed significantly ($p>0.05$) in their efficiency to acquire NanCO3-Pi from the rhizosphere soil.. The winter barley and spring barley genotypes also showed significant differences in their P depletion profiles near roots. The rhizosphere pH remained unchanged, suggesting that additional mechanisms such as root hair formation and root exudates play a significant role in causing variation in P acquisition among the genotypes.

Song *et al* (2001) studied large variation in phosphorus-(P) acquisition efficiency exists among maize inbred and hybrid genotypes. The patterns and levels of change in APase activities in B73 and Mo17 were not sufficiently different to account for the diverse growth response of these genotypes in low-P conditions. The results suggest that APases may not be a major mechanism for scavenging or acquiring P and changes in APases may reflect a state of P stress in both varieties. Other factors such as root architecture, secretion of low-molecular weight carboxylates and microbial interactions might explain the difference between these two genotypes.

Cynthia *et al* (2004) characterized variation among maize genotypes in relation to phosphorus (P) uptake and utilization efficiency, two experiments were set up to measure phosphatase (P-ase) activity in intact roots of six local and improved maize varieties and two sub-populations. Plants were grown at one P level in nutrient solution (4 mg L-1) and the P-ase activity assay was run using 17-day-old plants for varieties and 24-day-old plants for subpopulations. Shoot and root dry matter yields and P concentrations and

contents in plant parts were determined, as well as P-efficiency indexes. Root P-ase activity differed among varieties, and highest enzimatic activities were observed in two local varieties

Phosphorus uptake is influenced by root and soil characters

Wissuwa (2003) showed genotypic differences in P uptake from P deficient soils may be due to high root growth or high external root efficiency. Increasing root fineness or high external root efficiency for root dry matter production by 22% was sufficient to increase P uptake. The direct effect of increasing external root efficiency accounted for little over 10% of increase in P uptake. The remaining 90% was due to enhanced root growth as a result of higher P uptake per unit root size. These demonstrate that large genotype differences in P uptake from a P deficient soil can be caused by rather small changes in tolerance mechanism.

Sun et al. (2002) studied morphology of root systems of different wheat (*Triticum aestivum* L.) genotypes under low P stress was studied to determine, effect of external factors. The length of the root axis and root system and number of lateral roots were sharply increased under P stress. The number and length of root axis were significantly different under different levels of P supply. This implied that two traits were controlled by genotypes and external factors and they showed traits of lateral roots mainly depended on external factors.

Differences in root morphology and physiology among maize genotypes were related to difference in P accumulation by plants in nutrient solutions or soils(Cyanthia *et al* (2003)). Genotypes with larger and longer root system presented high dry matter yield of shoot and root (Furlani, 1988).Significant differences in root surfaces area of field grown maize genotypes positively correlated with shoot and root dry matter yield at flowering stage (Schenk and Barbar, 1980). While, Clark and Brown (1974) recorded differences among maize genotypes for high and low P accumulation properties and attributed the same to root size, the ability to compete effectively for P and the ability to tolerate harmful effects of other elements like aluminium. Relative growth rate and

concentration of P in shoot were also considered important in addition to root length as the parameter influencing P absorption in white clover (Carandus, 1994).

A major factor contributing to the increased P uptake in tomato was thought to be an apparent greater affinity of the absorbing sites for H2PO4 on the roots (Cress *et al.*, 1979). The efficient tomato lines produced almost 100 per cent more dry matter than inefficient lines though the latter had higher mobility for P with in the plant under phosphorus stress (Clarkson and Scattergood, 1982).

It was observed in tomato cultivars (Itoh and Barber, 1983) that P uptake of efficiency was influenced by the amount of root surface per plant, rate of P uptake, kinetics of roots in addition to root hairs. In a similar study by Foehse and Jungk (1988) it was found that root hair length was closely related to the P content of the root and shoot but formation of root hairs did not depend directly on P concentration at the root surface but on the P content of the plant. It was found that under different conditions, contribution to P uptake by root hairs was upto 90% of total uptake as revealed by the study of Foechse *et al.* (1991).

Bates *et al.* (1996) determined the efficiency of root hairs in phosphorus acquisition at low and high P availability. They observed that under high P availability root hairs did not have any effect on plant P uptake and plant P content of three-week-old *Arabidopsis.* Under low P availability, wild type Arabidopsis had greater total root surface area and high P per unit length. He concluded response of root hairs to low P availability is an efficient strategy for P acquisition. Bates *et al.* (2000) observed Arabidopsis root hairs grow longer and denser in response to low P availability. At low P, all plants were small and showed severe P stress symptoms. They concluded that the response of increased root hair growth under low P availability is important in increasing P acquisition under low P conditions.

Root length, radius, surface area, root to shoot dry weight ratio and root hair density are important morphological parameters of root for uptake of any nutrient and also for P. These root characteristics may be affected by soil properties (Prummel, 1979). The greater contribution of root hairs to P uptake is partly due to their surface area, which is similar to that of root cylinder. However, the main reason for high uptake efficiency of root hairs was their small radius and their perpendicular growth into the soil form the root

axis. Because of the small radius compared to root axes. P concentration at root hair surfaces decreased at a slower pace and therefore P influx remains higher. The main reasons for differences found in P response among species were the size of root and number and length of root hairs. In a soil, hairs were able to satisfy a higher proportion of their P demand required for maximum growth.

Lopez-brucio *et al.* (2002) studied the effect of P availability on development of root system in *Arabidopsis.* They found that under P-limiting conditions (< 50 μm), the *Arabidopsis* root system undergoes major architectural changes in terms of lateral root number, lateral root density and primary root length.A primary adaptation to low P availability involves post-embryonic developmental changes in root system, which are directed toward enhancing patterns of total root length, root hair elongation, and lateral root formation (Butes and Lynch, 1996, Borch *et al,* 1997).

Root hairs and lateral roots assist the acquisition of P by exploring a greater soil volume and by increasing the absorptive surface of the root. The formation of a highly branched root system, in response to nutrient starvation, may be a consequence of canalization of carbon and energy resources to produce a root system capable of exploring large areas of upper soil layer, where nutrient rich patches are normally present (Stitt and Rudigh-Scheible, 1998).

Fohse. *et. al* (1991) indicated the importance of root hairs for P uptake in soils low in P and report that it is based on three features (a) increase of absorbing surface (b) very low radius (c) Accessibility of larger root volume. They have also showed that surface area of root hairs varies among the species. For bean plants it is about 40%, for wheat, ryegrass and rape about equal and for spinach almost twice that of the root cylinder. An increased absorbing surface means a lower required influx per unit surface area. For plant species like spinach, root hairs increased the absorbing surface by 200%, although influx per cm of root increased by as much as 700%.

Nielson *et al* (2001) assessed the importance of increased allocation of carbon to roots for adaptations of plants to low P availability in common bean. P-efficient genotypes

allocated fraction of their biomass to root growth especially under low P conditions. Efficient genotypes had lower rates of root respiration than inefficient genotypes, which enabled them to maintain greater biomass allocation than inefficient genotypes without increasing overall root carbon costs.

Plant P utilization

Different species and cultivars within a species vary widely in their ability to thrive in nutrient- deficient environments, i.e. plants differ greatly in their nutrient efficiencies (Chen and Gabelman, 1995; Cakmak, et.al, 1997; Trehan and Claassen, 1998).

Genotypes that can acquire and use scarce P resources more efficiently from low-P soils could improve and stabilize agricultural production (Friesen et al., 1997; Rao et al., 1999c). Genotypical differences in nutrient efficiency are related to differences in efficiency of acquisition by the roots or in use by the plant, or both (Sattelmacher et al., 1994; Horst et al., 1996; Rao et al., 1999a). Phosphate acquisition efficiency (PAE) is defined in terms of total uptake per plant. It is related to morphological root characteristics such as root system size, fine roots which means an increased root surface per unit of root weight and root hairs which allows for efficient nutrient scavenging of a larger soil volume. The second component is the root physiological activity such as differing uptake kinetics i.e. I_{max}, K_m and C_{Lmin}, which results in different nutrient uptake rates per unit root and time (Steingrobe and Claassen, 2000). Other mechanisms affecting the uptake efficiency include symbiosis with mycorrhizal fungi (Wilcox, 1991; Tarafdar and Marschner, 1994; Marschner, 1995) and chemical mobilization of nutrients by root exudates of protons, of complexing or chelating substance or of enzymes in the rhizosphere (Uren and Reisenauer, 1988; Raghothama, 1999; Jones and Farrar, 1999).

Plant P use efficiency (PUE) is the dry matter produced per unit P taken up. It depends on the available amount of nutrient into the plant (Godwin and Blair, 1991; Marschner, 1995). The PUE is equivalent to the reciprocal of the nutrient concentration in the entire plant, often termed as the nutrient efficiency ratio (Gourley et. al., 1994).

The plants' P efficiency could be also assessed by other terms like the "external" and "internal" P requirements for plant growth and yield under limited P availability in soil. Genetic variation in plant adaptation to low-P soils may be related to external and internal P requirements.

The internal requirement is the minimum uptake by a plant associated with a specific yield, usually near maximum growth (Fox, 1981). It is also defined as the critical concentration for optimal crop growth or yield i.e. the nutrient concentration in plants sufficient to produce a certain proportion, e.g. 90%, of maximum dry matter yield (Föhse et al., 1988, Godwin and Blair, 1991). Therefore, plants growing under limited P conditions with a low internal P requirement may have a low external P requirement or may be inefficient acquiring P, but they must be efficient in using the P taken up to produce dry matter.

The external P requirement of plants is the P concentration in soil solution associated with adequate nutrition or growth (Fox, 1981) and thus very close related to the plant P efficiency. Concentration of P in soil solution, which is in the order of 0.32 – 19.37 μmol P L^{-1} (Wild, 1988; Kamprath and Watson, 1980), can be depleted rapidly by growing roots in soil. As solution Pi falls below its equilibrium concentration, it is replenished by labile Pi desorbed from clay mineral surfaces adjacent to the roots (Fox, 1981). Consequently, Pi moves from the adsorbed forms into solution and along a concentration gradient to the root where the concentration is low. However, in P-limited tropical soils, the quantity of labile P may be insufficient to maintain Pi solution concentration against depletion by plant root. Thus this specific soil condition influences the movement of P toward the root surface because gradient is the driving force of diffusive P flux. On the other hand, P inflow depends on the concentration at the root surface, for that P depletion may imply severe restriction of P inflow into plants

It has been reported that the P concentration in soil solution (external P requirement) necessary to achieve maximum growth differs widely among crops. Using flowing solution cultures, Asher and Loneragan (1967) showed a 25-fold difference in external P requirements among eight plant species and Asher (1978) reported a 200-fold difference for 18 species ranging from Stylosanthes guianensis to cassava. Fox, (1981) showed that the external P

requirements of a range of crops and vegetables estimated in the field on Hawaiian Oxisols using adsorption isotherms were equally variable. Kamprath and Watson (1980) summarized the external P requirements for several temperate and tropical crop species varies in the range of 2 to 22 μmol L^{-1}.

Hence, at a low P concentration in soil solution, P efficient plants may be either those with a low external P requirement or those which are able to achieve their external requirement by developing of morphological and/or physiological root mechanisms (PAE), always in connection with the P use efficiency.

Since different concepts of nutrient efficiency have been developed, some giving emphasis to productivity and other to internal nutrient requirement (Gourley et, al., 1994), it is important to clarify the definition of P efficiency for this research. Buso and Bliss (1988) defined that efficiency with regard to a specific mineral nutrient, is the ability of a species or cultivar to produce a high yield in a soil limiting in this particular nutrient element compared to a standard species or cultivar. Considering this definition, in this study **P efficiency is defined** as the genotype's ability to produce shoot biomass under low P availability in soil in comparison with other genotypes.

Plant adaptation to P limited tropical soils can be partially attributed to inherent genotypic differences in P use efficiency (PUE). Phosphorus use efficiency describes the amount of P that is needed to build one unit of shoot biomass. Efficient use of P is dependent on a number of plant attributes (Clark, 1990; Caradus, 1990) including: 1) high dry-matter yield per unit of P acquired; 2) growth duration and plant type; 3) partitioning of P between different pools and its translocation within the plant; 4) leaf death rate; and 5) partitioning of a greater proportion of biomass to harvestable yield. The ability of crop plants to remobilize P from vegetative to reproductive organs, and forage plants from senescing to growing points may form an important mechanism that allows plants to improve the use of acquired P (Caradus, 1990). Several researchers found that species (like white clover) adapted to low P soils generate a lower proportion of dead leaf to total leaf material when under P stress than species from high P soils (Beadle, 1966; Specht and Groves, 1966; Grime and Hunt, 1975).
A number of tropical crop and forage species can grow normally with low tissue P concentrations due to efficient use of P among the major biochemical fractions (soluble-

P, lipid-P, and residue-P). Lotus, which maintained relatively low tissue Pi concentrations, was found to be more tolerant to low P conditions than white clover, which exhibited high Pi concentrations in the tissues (Hart and Jessop, 1983). Pigeon pea is more tolerant to low P conditions compared to soybean because it maintains relatively low tissue concentration of Pi due to the efficient incorporation of the external Pi NMR (nuclear magnetic resonance) indicate that, under Pi deficiency, the vacuole acts as a Pi reservoir to maintain a constant cytoplasmic Pi concentration (Rao, 1996; Foyer and Spencer, 1986; Ratcliffe, 1994).

Phosphorus efficiency ratio(PER)

The phosphorus efficiency ratio (PER) reported for plants grown in field under dry land conditions range form about 250-565 kg grain kg^{-1} P in shoots. Plants grown under controlled environmental conditions have Phosphorus efficiency ratios ranging from as low as 40 to as high as 715g grains per mg of P in shoots. (Batten 1986a: Liprect 1964). Higher PE ratios are usually obtained at lower rates of applied P (Jones *et al.* 1989), but within a phosphorus rate treatment the PE ratio is correlated with the ratio of grain to total shoot dry weight the harvest index (Batten, 1986a). The uptake and utilization of P in the field are influenced by genotype and factors that affect water use efficiency (WUE) (French and Schultz 1984) including soil temperature (Barrow, 1974). Ogata *et al* (1988) showed that dry weights in plant parts increased with increasing P application rates.

Clark and Brown (1996) experimented with two corn inbreds for Phosphorus use efficiency for the two inbreds was tested by inducing P stresses. The more efficient inbreed Pa36 took up and accumulated higher amounts of P. When P concentration increased in nutrient solution, the P concentration in tops of pa 36 increased over 6 fold and P concentration in the tops of WH increased 4 fold. The P concentration in the roots of both inbreds increased from approximate 0.2 to 0.9%. Root and top yields reached a maximum for both inbreds at 1 mg P/litre.

Snapp *et. al* (1995) showed that low P treatment reduces biomass accumulation in shoot, pod and root tissues compared to high P treatment. In comparison to high P plants, low P

plants had significantly reduced root length in the main root system. In contrast, low P plants maintained fine root length comparable to high P plants. Root biomass investment in high P plants was reduced to 54% and P investment by 43% for low P plants compared to high P. P uptake from the P patch was 3 fold higher in high P plants compared to low P plants.

Many authors observed higher root to shoot ratio on P deficiency plant associated with a high production of carbohydrates being partitioned to roots and higher sugar concentration in roots (Cakmak *et al.*, 1994; Paul and Stitt, 1993). They observed effect of P starvation on leaf area per plant is consistent with the results of other authors who have reported a rapid and severe effect of P deficiency on leaf growth (Rao and Terry, 1989). In thier experiments leaf area accounts for major part of reduced total yield leaf biomass per plant. The PUE was not a strongly affected during first half of the experiment suggesting that photosynthesis per unit leaf area was not affected.

Root to shoot ratio was higher on P deprived plants, which indicates that shoot growth was more severely reduced than root growth. Brevedan *et al.* (2002) observed low P treatment reduced shoot growth significantly and main effect was a reduction on expansion of leaves (70%) root growth was less affected by low P.

Plant factors for P acquisition efficiency (PAE)

The ability of plants to adapt their morphological and physiological root characteristics to variable nutrient availability is genetically determined (Batten, 1992; Egle et al., 1999; Fransen et al., 1999; Horst et al., 1993). The low mobility of P in soil makes P acquisition by the plant very dependent on soil exploration in time and space (Nye and Tinker, 1977; Barber, 1984; Koide, 1991; Marschner, 1991). Efficiency of P acquisition depends markedly on rooting density and root distribution in the soil profile, and these, in turn, on plant genotype, soil chemical and physical properties, and cropping system (e.g. rotation). The rooting depth of annual crops increases as the growing season progresses, but in comparison, perennial forage species, particularly grasses, develop more vigorous root systems as an adaptive feature to low P availability in tropical soils (Friesen et al., 1997; Rao et al. 1996). Field and greenhouse studies (Otani and Ae, 1996) indicated that P uptake by crops is

strongly related to root length in soils where P availability is high, but not in soils with low P availability or where soil volume is limited.

Since the uptake of nutrients occurs on the root surface, root diameter and root length defines the maximum volume of soil which can be exploited with a given amount of photosynthate. Root diameter varies between species and cultivars and changes with plant age (Welbank et al., 1973; Atkinson, 1985). Dicotyledonous species have greater root diameter than monocotyledonous (Atkinson, 1990; Fitter, 1991).

As the mobility of P in soil is low, the root cylinder may not be enough to feed the plant. Therefore, plants grow root hairs, which are tubular shaped growing cells arising from root epidermal cells known as trichoblasts (Ridge, 1996). Jungk and Claassen (1989) found the influx of P per unit root length greatly enhanced by root hairs. This can be explained by the enlargement of the root surface area and because root hairs penetrate the soil perpendicular to the root axis, giving access to a larger volume of soil per unit root length. Consequently, P depletion profiles are found to differ in their radial extension depending on root hair length

(Hendriks et al., 1981). Assuming a frequency of 100 mm^{-1}, a radius of 0.005 mm and a dry matter content of 5%, Clarkson (1991) calculated a threefold increase in surface area could be achieved at an expense of less than 2% of root dry matter. Föhse et al. (1991) found that spinach had highest root hair density and length followed by bean, wheat and rape. Eticha and Schenk (2001) found that differences in P efficiency between two cabbages varieties could partly attributed to differences in root hair length.

Arbuscular mycorrhizae fungi (AMF) colonize the roots of most plants and serve as an extended link between plant roots and soil (Marschner and Dell, 1994). When root exploration of the soil is restricted by low P supply, up to 80% of the plant P can be delivered to the host plant by the external arbuscular-mycorrhizal (AM) hyphae, which explore soil to a distance of more than 10 cm from the root surface (Li et al., 1991). The mycorrhizal efficiency of P acquisition probably varies markedly among crop species. Cassava has a higher AM dependence than Stylosanthes guianensis, cowpea, beans, Andropogon gayanus, maize, or rice (Howeler et al., 1987). Soil and crop management practices (crop sequence,

tillage, fertilisers, and pesticides) can influence the total quantity of AMF development (Miller et al., 1994). Therefore, crop systems improving soil environment for high population of AMF species, could enhance P supply to plants, especially from strongly sorbed P pools in the soil, which the plant is unable to take up only through the enlargement of the root system (Dodd et al., 1987).

Phosphorus uptake by plants is also influenced by the uptake kinetics. These parameters include maximum net influx per centimetre of root length (I_{max}), Michaelis- constant (K_m) and minimum soil solution concentration (C_{Lmin}) (Nielsen, 1979). The absorption kinetics of the plant is controlled by these parameters. Under conditions in which the rate-determining step in P uptake is located in the root, P uptake will increase if root length per unit plant weight and I_{max} increase, and K_m and C_{Lmin} decrease (Nielsen, 1979). These parameters vary with P concentration in the soil solution. Fontes et al. (1986) reported that I_{max} values decrease with increase in P supply to the tomato plants. An average increase of 173 per cent in I_{max} was observed in plants grown at insufficient P supply. Buhse (1992) reported that the I_{max} of rape and sugar beet increases remarkable not only when the P supply decrease from 50 to 0.2 μM P but also when the root temperature increase from 10 to 25°C. On the other hand, K_m and C_{Lmin} values were not affected by P supply to the tomato plants. The assessment of the kinetic parameters (I_{max}, K_m and C_{Lmin}) characterizing the uptake system of a genotype is complicated by at least 3 factors (Sattelmacher et al., 1994): (i) the plasticity of the system in response to the P status of the plant (Jungk and Claassen, 1989; Abdou, 1989); (ii) the differences in P uptake along roots (Kuhlmann and Barraclough, 1987; Henriksen et al., 1992); and (iii) the dependence of P uptake on plant growth rate (Engels, 1993). Thus there is general agreement that the efficiency of the uptake system is of minor importance for P acquisition from soils because transport of P to the root surface rather than the uptake is the limiting step (Barber, 1984). Therefore it is less likely that selection for efficient P uptake kinetics will contribute to more efficient P acquisition from low-P soils.

The root release of organic acids (especially malic acid, citric acid and perhaps oxalic acid) is another key component in P acquisition. Organic acids differ markedly in their capacity to complex Fe and Al and thus solubilize the respective P compounds in soil bound by these

ions. Different cultivars produce a specific acid that complexes a specific mineral (Fe, Al, and Ca), for example, pigeon pea releases piscidic acid that complexes Fe but not Ca (Ae et al., 1990). Ae et al. (1996) proposed that cell walls of plant roots are involved in P-solubility activity. Root exudation of acid phosphatases (ectoenzymes) is common in plants and is usually enhanced under P deficiency (Ozawa et al. 1995). Acid phosphatases deplete organic P in the rhizosphere of lupin roots within about 2.5 mm of the root surface (Li et al., 1997). Secretion of phytase was highest in Brachiaria decumbens CIAT 606, Stylosanthes guianensis CIAT 184 and tomato. It is speculated that the secretion of phytase could provide an efficient mechanism for wide adaptation of the tropical forage grass B. decumbens CIAT 606 (planted on over 40 million ha) to the low P supplying tropical soils of Latin America.

Adaptation of plants to low P availability

Under low available P conditions an array of plant traits are known to contribute to high P uptake efficiencies, among them are acidification of rhizosphere (i.e. excretion of protons from roots) and exudation of organic anions by roots (Randall, 1995).

Plants respond to low levels of bioavailable P by increased root growth, higher expression of P-transporters (Muchhal et al., 1996; Leggewie et al., 1997;Liu et al., 1998; Burleigh and Harrison, 1999), and by alterations in metabolism including the induction of RNAses (Beriola et al., 1994). In addition, secretion of acid phosphatases from roots (APases; EC 3.1.3.2) is a notable consequence of P deficiency (Goldstein et al., 1988; Duff et al., 1991; Li and Tadano, 1996). The levels of induction of APase production and secretion in roots can be dramatic. Major increases of APase released from P-starved roots were emonstrated for various plant species (Ascencio, 1997). For example under P-deficient conditions, the secretion of APase from lupine roots increased up to 20 times compared with the P-sufficient conditions (Tadano and Sakai 1991), with large amounts of APase detected in soil
surrounding the roots (Li et al., 1997).

Capacity of plants to access P under limiting conditions depends on important adaptive traits, including organic acid excretion, alteration of pH of rhizosphere and increased ability to explore different layers of soil. (Schachtmnay *et al.*, 1998, Lopez-

Bucio *et al*, 2000, Corroll *et.al* (2003). Plants evolved a diverse array of strategies to obtain adequate P under limiting conditions including modifications to root architecture, carbon metabolism and membrane integrity changes, exudation of Low molecular weight (LMW) organic acids, protons and enzymes and enhanced expression of numerous genes involved in low P adaptation.

Acid phosphatase activity

Phosphatases (P-ases), classified either as acid or alkaline, constitute an enzyme group which is presumed to catalyze the hydrolysis of several organic phosphate monoesters, liberating available Pi, and occurring scattered in all tissue cells of plant organs (Juma & Tabatabai, 1988). Root-secreted phosphatase activity, named extracellular, is related to plant ability to make soil P available for absorption. The intracellular acid phosphatases, present in the cytosol, plastids and vacuoles, are responsible for the P-hydrolysis from organic compounds, favoring P mobilization and translocation from senescent tissues (Duff et al., 1994). Therefore, this enzyme activity is a physiological characteristic related to plant efficiency in relation to P acquisition and utilization, and is genetically variable (Tadano et al., 1993). Plants usually secrete root acid Pases when P availability is low; however, plant species differ in secretion ability and enzyme activity (Yan et al., 2001).

Acid phosphatases (EC. 3.1-3.2 Acid phosphatases) are ubiquitous in animals, plants and microorganisms and are involved in the metabolism and transport of phosphorus, which is essential to a large number of metabolic reactions.

Plant roots secrete Acid phosphatases, which are considered being important response to phosphorus deficiency. Acid phosphatases consist of group of enzymes, which catalyze the hydrolysis of phosphate monoesters. The acid phosphatase secreted by roots hydrolyses organic phosphate compounds and liberate orthophosphate in the rhizosphere so as to avoid phosphorus stress (Tarafdar and Claakssen 1988). Wool house (1969) suggested that increased P uptake or better adaptation of plants to P stress may be a result of increased phosphatase activity of plant roots

Increased secretion of acid phosphatases (APases) has been reported in many plant species, includ-ing maize, under P-deficient conditions (Elliott and Läuchli, 1986; Tadano and Sakai, 1991; Trull et al., 1997). Using a leaf disc APase assay for diagnosis of P starvation, Elliot and Läuchli (1986) found that APase activity per unit area increased 2–3 times in leaves of P-deficient maize plants compared with P- sufficient leaves. Similarly, intracellular APase activity increased in wheat shoots under P-deficient conditions (McLachlan et al., 1987). Remarkable difference in levels of APase secretion from roots under P- deficient conditions has been observed in many plant species (Tadano and Sakai, 1991). Secretion of APases increases in response to P starvation in cell suspension cultures of maize and other plant species (Goldstein et al., 1988b; Lefebvre et al., 1990; Miernyk, 1992). In cell suspension systems, both the synthesis and secre-tion of APase are altered under P deficiency (Ueki and Sato, 1977)

Phosphatases play a specific role in hydrolysis of organic P compounds (Tabatabai, 1988), they are present in soils but their activities in rhizosphere soil may increase due to the direct release of extracellular phosphatases from the roots of growing plants and due to the release by the growing microbial biomass utilizing the root exudates (Barber and Lynch, 1976). The rate-limiting step in the process of organic P mineralization might then be the availability of P-esters for hydrolysis and the activity of phosphatase in soil solution.

Plants contain many other kinds of Acid phosphatase isoenyzme in their tissues such as axis, cotyledon (Kaneko *et al* 1990) and roots (Panara *et al* 1990). Some of them play a role in the reutilization of bound phosphorus in the cytoplasm (Szabonasy *et al* 1987) and others are secreted form roots into the rhizosphere to enable organic phosphorus to become available to plants (Hirata *et al* 1982). The amount of Acid phosphatases secreted form roots was very larger in some plant species when plants grown under P-deficiency conditions. The secreted acid phosphatase could hydrolyse organic P in soils, such as nucleotide, Phospholipids and sugar phosphates into inorganic phosphate, which plants, are able to absorb.

Tadano *et al.* (1993) demonstrated differential interspecific genetic variability for root secretion and acid Pase activity for nine plant species, among them, rice, wheat,

tomato and lupin, and observed also different magnitudes in root enzyme activity increases in response to P deficiency. Mc Lachlan (1980a; 1980b) found variations in intact root enzyme activity among cultivated wheat species and their wild progenies. The cultivated species presented lower enzyme activities as compared to the wild, suggesting that selection for wheat plants more adapted to low P conditions might have occurred unconsciously

Farouq *et al*(1995) investigated the extent of variation among barley genotypes *(Hordeum vulgare* L. cv.) in their ability: i) to induce activity of soluble extracellular phosphatase in rhizosphere soil. ii) to withdraw bicarbonate extractable organic phosphorus (NaHCO3 –PO4). All the genotypes induced 3-4 times higher phosphatase activities in rhizosphere soil as compared to bulk soil. A high correlation (r = 0.79) was found between the activity of soluble extracellular phosphatase and the quantity of NaHCO3-Po withdrawn from the rhizosphere soil by the
barley genotypes.

Recently it has been postulated that the function of roots in the secretion of Acid phosphatase is a widespread adaptive mechanism of plants that enables them to grow in phosphorus deficient soils (Tadano and Sakai 1991). The acid phosphatases in soil, whether derived from plant roots or from microorganisms, are all capable of catalyzing the mineralization of organic P (Spear and Ross, 1978; Adams and Pats 1992).

Tarafdar and Jungk (1987) showed that acid phosphatase activity at root surface of rape was up to 8 times higher than that in bulk soil and found appreciable depletion of organic P in soil very close to the root surface. Clark and Brown (1974) proved that the phosphatase activity of roots of maize inbred was higher than those others. Phosphatase activities of roots of both inbred lines increased as P stress was induced on the plants by decreased addition of P.

Minggans hi *et al* (1997) observed a high acid phosphatase activity within root compartment of P deficient plants. The highest activity was 0.73 μg^{-1}. The Acid phosphatase activity decreased with the increase of the distance form the root

compartment. He showed that a relationship between Acid phosphatase activity and depletion of organic P in the rhizosphere. He observed a significant positive correlation (r=0.958**) between activity of Acid phosphatase and depletion of organic P in the soil.

Helal (1990) presented the results of a comparative study on P utilization form Ionositol hexa Phosphate (IP_6) by various bean varieties in relation to the activity and characteristics of their root phosphatases. All varieties tested were able to take up sufficient P for adequate growth from the initial nutrient solutions.

Xialong Yan *et al* (2001) compared Acid phosphatase activity in leaves of common bean (*Phaseous vulyaris*) of P efficient genotype (DOR 364), and P inefficient genotype (G 19833) and in their recombinant inbred lines (RILS). P deficiency increased leaf Acid phosphatases activity but that was much higher and more responsive to availability in DOR 364 than in G 19833. Leaf Acid phosphatases segregated in RILS with two discrete groups having either high (mean 1.71 µmol/mg protein/min) or low (0.36 µmol/mg protein/min) activity. Their results do not support a major role for leaf Acid phosphatases induction in regulating plant adaptation to P deficiency. However Xialong yong *et. al*(2001) had indicated that there were varietal differences both in P-uptake by young roots and their phosphatase activity. He shows further that root phosphatase activity is pH dependant. Varietal differences are especially evident in the more acid range (pH 4-6) and diminish at higher pH (6) the root phosphatase activity at pH 5 shows an inverse relation to the P content of plant. This dependence on root phosphatase activity for the P supply of plants has been already discussed by Helal and Sauerbeck (1987b). There is the positive relation is evident between the utilization of IP_6-P by various varieties and the phosphatase activity of their roots at pH 5.

A pronounced pH dependency of root phosphatase activity is indicative of the significance of rhizosphere pH not only for the availability of inorganic phosphorus but also for the activity of root enzymes and the related turnover of organically bound nutrients. Secretory acid phosphatases plays significant role in the utilization of the organic phosphate compounds (Li *et al*, 1997). It was reported that dry matter production

of tomato and beet increased when secertory Acid phosphatases from lupin roots was added to rhizosphere of these plants (Tadano and Komatsu 1994)

Kenji Ozawa *et al* (1995) reported that P treatment resulted in the enhancement of activity of secreted Acid phosphatase due to P deficiency, while activity in + P treatment was nearly constant on a dry weight basis. The activity of Acid phosphatase on a root dry weight basis in the P treatment become approximately 20 times as high as that in the + P treatment at 15 days after the treatment. These results indicated that lupin plants quickly responded to P deficiency and secreted Acid phosphatase before their growth or metabolism was markedly distributed which supported the concept of a biochemical early warming system proposed by Soldstein *et al* (1988).

Silberburh *et al* (1981) reported that activity of Acid phosphatase in plant tissues increased under P deficient conditions. Hirata *et. al*(1982) has been reported that the activity of Acid phosphatases secreted by rice roots under P deficiency increased about 2 fold compared with that of control roots. It has also been reported that the activity of Acid phosphatases, which was secreted by tomato roots and suspension, cultured cells, increased under P deficiency (Goldstein *et al* 1988). The increase of activity of Acid phosphatases in the rhizosphere of wheat and clover was considered to induce the depletion of organic phospatase (Tarafdar and Jungk 1987).

Tashiaki Tadano and Hiroshi sakal(1991) investigated the activities of acid phosphatase secreted by roots for 24 hr. Nine crop species grown under P deficient and P sufficient conditions. The activity of Acid phosphatases secreted by roots under P deficient conditions was remarkably high than in lupin and tomato, high in cabbage and radish. The increased rates of activity of Acid phosphatases secreted under P deficient conditions when compared with those under P-sufficient conditions ranged from 1.5 times in azuki bean to 19.9 times in lupin. The activities of Acid phosphatases in lupin were much higher than those in rice. Both activities of Acid phosphatases increased with the decrease of P concentration in the nutrient solution, and were substantially high at the initial growth stage.A combination of phosphatase activity and enhanced Pi uptake may help plants acquire required amounts of Pi from the rhizosphere (Raghothama, 2001).

It is interesting that during P starvation increased activity of phosphate and phytase was found throughout plant tissues and in the rhizosphere as reported by Hubel and Beck, (1996). Similarly, An increase in concentration of phosphatase, phytase and RNases was observed in the absence of exogenous supply of Pi (Bosse and Kocls 1998).Tomasche, *et al.* (2004) demonstrated that root associated acid phosphatases pool is increased in Arabidopsis when Pi is limiting and documented five acid phosphatases isoforms secreted from roots. Similarly Lim *et al.* (2003) investigated tissue and isoform specific responses of acid phosphatases to P deficiency in three rice genotypes in all genotypes acid phosphatase activity increased in P deficient plants.

MATERIAL AND METHODS

III. MATERIALS AND METHODS

Experiments were conducted to determine the genetic variability for P uptake and utilization from inorganic and organic fixed forms of P (Al-P, Fe P, Ca p.KH_2PO_4. and phytate) by pigeon pea genotypes and to investigate the mechanisms adopted by contrasting genotypes for high and low P uptake and accumulation of P in shoots was investigated. The materials used and procedures adopted are given in this chapter.

3.1 Screening of pigeon pea genotypes for high P uptake and utilization

Experimental details

3.1.1 Plant material

Thirty -six pigeonpea genotypes of medium duration group (165-175 days) were selected from the core collection of 250 Accessions received/supplied by ICRISAT Patenchuru, Hyderabad. AICRP (Pigeon pea), GKVK. Bangalore, and ZARC, Gulbarga were used. List of genotypes used for the study are presented in the (Table .1).

3.1.2 Vermiculite Properties

Vermiculite supplied by KELTECH industries was used as media for the experiment. The physical and chemical properties of vermiculite are given in appendix.3

3.1.4 Estimation of available P in vermiculte

Available phosphorus was estimated by following Brays No. 1 method (Bray and Kurtz, 1970). 1g of **vermiculte** was taken in 100 ml beaker containing 50 ml of Brays exractant. It was shaken for 30 min on end-to-end shaker, and filtered through Whatman No. 42 filter paper. Known amount of aliquot was taken and available P was estimated by ascorbic acid method. The list of reagents required is given in Appendix.

Ascorbic acid method: Reagent A & B was prepared fresh and added to 5 ml of aliquot taken in a 25 ml volumetric flask. The volume was made upto to 25ml. Colour intensity was read at 660nm.

Table 1: Genotype selected for experiment

Sl No	Genotype	Sl No	Genotype
1	ICP 40	19	BSMR 736
2	ICP218	20	BRG-2
3	ICP1029	21	BDN 2010
4	ICP 2933	22	LCV 10
5	ICP 3226	23	LCV 40
6	ICP 4557	24	H 3C
7	ICP 5466	25	TTB 7
8	ICP 7118	26	JKM 189
9	ICP 8477	27	WRP I
10	ICP 8863	28	JS 5
11	ICP 9306	29	ICP7035
12	ICP 12764	30	JSA 59
13	ICP 14064	31	JSA 81
14	ICP 14252	32	GT 101
15	ICP14352	33	GRG 207
16	WRGE 38	34	BRG 1
17	JKM 205	35	ICP96047
18	JKM207	36	SEL20129

3.1.5.1 Preparation of standard curve

Standard curve was prepared by pipetting out 0.5, 1, 2, 3, 4 and 5 ml of standard stock solution (0.02195 g of $KHPO_4$ dissolved in one lit of distilled water, this gives 5 ppm of P in the solution) to 25 ml volumetric flasks, reagents added to develop colour and absorbance was measured at 660nm.

3.1.6 Evaluation of 36 genotypes for P uptake and utilization

The selected 36 genotypes were grown in the Black polythene bags 3kg capacity. The vermiculite mixture prepared was filled into these bags and Pre-germinated seeds of pigeon pea genotypes were sown and allowed to grow under normal condition for one week.

All recommended nutrients were provided with modified Hoagland solution for normal growth and development for 15days.once the seedlings attained good growth phosphorous stress was imposed by using different forms of fixed P and control with following treatments.

Treatments

T_1 - organically bound P as fixed P. 50ml of 3mM phytic acid supplied to each polythene bag. (20mM of P)

T2 - Inorganically bound forms of P-AlPO4, CaHPO4, and FePO4 supplied to each polythene bag (270mg of P, ie 90mg/bag) which will contain (20 mM of P)

T3 - Normal P (KH2PO4) control (Full strength Hoagland's solutions

(20mM of P)

T4 - Deficient P (No P)

Replication – Four

Treatment was imposed after 10 DAS by giving the half strength hoaglands solution that was prepared using required amounts of nutrients and 100 ml solution was fed to each bag after 5 days of sowing.

3.1.6.2 Aftercare

To check the rodent attack the ploythene bags were covered with mesh which helped in protecting the seedlings, Prophylactic plant protection measures were taken up as per recommended package.

The plants were allowed to grow up to 45 days by providing different sources and concentration of P with hoaglands solution for every three days, After experimental period the following plant growth parameters were recorded.

3.1.7 Growth parameters

3.1.7.1 Shoot parameters

The plant height was measured from collar region to tip of the plant and expressed in cm. Leaves were harvested by plucking and total leaf area per plant was recorded using leaf are meter (Delta T devices) and expressed as cm^2. The shoot portion was harvested at the end of 45 days after sowing by cutting the stems at collar region and dry weight of shoot including leaf and stem was recorded and expressed as g per plant.

Extraction of roots

The intact roots were extracted after cutting the bags on one side and the vermiculite was separated in plastic trays by removing the vermiculite particles adhering to the root and roots were cut with sesior and separated from shoot and the separated root, were wrapped with aluminium foil and put in the liquid nitrogen and these root samples were used for enzyme extraction.

3.1.7.2 Root parameters

Root length was taken after removing the roots from shoot using graduated scale and expressed in cm. Root volume was measured by immersing the roots in known volume of water taken in measuring jar and calculating the raise in the volume of water after deduction from the earlier volume. The root volume was expressed in cm^3 per plant. Then the roots were dried in a hot air oven to get root dry weight. Dry weight of plant samples were recorded after drying at 80^0C for 2 days in hot air oven and weight expressed as gram per plant. Root dry weight was recorded as gram per plant. Total biomass was calculated by adding shoot dry weight and root dry weight and it was expressed in grams per plant.

3.1.7.3 Root to shoot dry weight ratio

The ratio of root to shoot dry weight was calculated by dividing Root dry weight with shoot dry weight. Expressed as ratio.

3.1.8 Estimation of Phosphorus content in plant samples

The P content in leaf and root samples was estimated separately by following Vanado-molybdate method (Jackson, 1973). The composition of Vanado-molybate reagent is given in appendix. The oven-dried Root and leaf samples were grounded to fine powder and then used for the estimation of P content. The powder was taken in 250ml conical flask and triacid digestion was done.

Acid digestion

Known amount of (0.250g) grounded powder of plant samples was taken in 250ml conical flask, the 5 ml of single acid (concentrated HNO_3) was added to flask and kept overnight. Then 5 ml of di-acid (prepared by using conc. HNO_3 and Hyphochloric acid in 10:4) was added. The conical flask was kept on sand bath without shaking and allowed to digest until the contents were clear and white precipitate appeared.

Then volume was made to 25 ml using distilled water. From that, 5 ml of aliquot was taken for P estimation.

3.1.8.1 Preparation of standard curve for estimation of P in plant samples

0.2195g of KH_2PO_4 dissolved in 400ml of distilled H_2O. Then 25 ml of 7 N H_2SO_4 was added and volume was made up to 1 litre with distilled H_2O.

The calculated quantities of 50 ppm P stock solution was pipetted out to a series 25 ml volumetric flasks and 5 ml of molybdate reagent is added to each flask and volume was made up. The intensity of colour was read at 430 nm after 30 min.

To prepare plant samples for estimation of P, 5 ml aliquot of digested samples (single/diacid) was taken in 25 ml volumetric flasks and 5 ml of molybdate reagent was added, volume is made up. The colour intensity was read at 430 nm. Compare the unknown sample reading with standard curve to get required P concentration and percent P was calculated in the plant sample using the following formula.

$$\% P = \frac{\text{Graph ppm} \times \text{vol. of Digested sample} \times \text{vol. madeup}}{10^6 \times \text{weight of sample} \times \text{Aliquot taken}} \times 100$$

3.1.8.2. Phosphorus content in root and leaves

The P content in leaf and root was estimated according to procedure described above and expressed as percent. The total shoot and root P was worked out by multiplying P (%) with corresponding dry weight and expressed as mg/plant. The root P content was also calculated as mentioned above. The total P content in plants was calculated by adding the P contents of shoot and P content of roots and the results were expressed in mg/plant. Total leaf P (mg/plant) accumulation was taken as contrasting character with different root weight was considered as criteria for selection with root dry weight as constant.

3.1.8.3 Phosphorus uptake efficiency

The P uptake efficiency of genotypes was calculated as the ratio of shoot P per unit root dry weight. It expressed as mg total P/g root dry weight.

3.1.8.4 Phosphorus utilization efficiency

The P utilization efficiency calculated as the ratio of gm total dry matter produced per mg of shoot P accumulated. It was expressed as g TDM per mg of shoot P

3.2 Selection of contrasting genotypes for high and low P content

From the first set of accessions six genotypes were selected using Z-distribution and correlation studies that was carried out for all 36 genotypes with respect to their leaf P% and with high enzyme activity. These six contrasting genotype the Z-distribution were selected by picking four genotypes from four quadrants and two from of middle of distribution for further studies. These selected genotypes were again grown in separate set of experiment with four treatments imposed and screened for conformation of the physiological parameter like p% and enzyme activity.

3.3.8 Acid phosphatase activity

Acid phosphatase activity was determined using the substrate P nitro phenyl-1-phosphate (Sigma St Louis,) the reaction stopped after 15 min by adding – NaOH to the reaction mixture. The formation of yellow P-nitro phenol was quantified at 410 nm in spectrophotometer. The acid phosphatase activity was estimated in root samples using the following procedure.

Fresh samples collected at the end of experimental period of 45 days were assayed for acid phosphatase activity in root using modified universal buffer with pH value 4.5. The P- nitrophenol phosphate disodium salt was used as the substrate and yellow color developed due to hydrolysis of ester bonds was measured against a blank for each samples. The samples were incubated at 37^0C for 15 min. The calibration curve was prepared using P-nitrophenol and results expressed µg P-Nitrphenol formed/mg protein/min.

Procedure

Sodium Hydrixide 0.085N

Dissolve 0.85 sodium hydroxide in 250ml water.

Subtrate solution; Dissolve 1.49g EDTA, 0.84g citric acid and 0.03g PNP in 100ml water and adjust PH 5.3

Standard; 69.75mg of PNP was weighed and dissolved in 5ml of distilled water (100mm)

Enzyme extraction; Homogenise 1gm fresh tissue in 10ml of ice cold 50mm citrate buffer(PH.5.3) in a prechilled pestel and orter filter through four layers of cheese cloth centrifuge the filtrate at 10,000rpm for 10 minutes use the supernantant as enzyme source.

1. Incubate 3ml of the substrate solution at 37c for 5 minutes.
2. Add 0.5ml enzyme extract and mix well
3. Remove immediately 0.5ml of the solution and mix it with 9.5ml of sodium hydroxide 0.085N. this corresponds to the zero time assay
4. Incubate the remaining solution (substrateand enzyme) for 15 minutes at 37c
5. Draw 0.5ml sample and mix it with 9.5ml sodium hydroxide solution.
6. Measure the absorbance of the blank and incubate tubes at 405nm.
7. Take 0.2 to 1.0ml (4 to 20mm) of the standard dilutes to 10ml with NAOH solution read the colour and draw the standard curve.

Protein quantification by CBB method;

CBB G-250(25 mg) was dissolved in 12.5ml of 95% methonal to this solution 25ml of orthophosphoric acid was added and the volume of the resultairy solution was made up to 250ml with distilled water the freshly prepared reagent was used every time.

A known volume of the cell free extract of the protein was added to 3ml Brodford reagent and mixed thoroughly the absorbance of the solution was read at 595nm after three minutes and within 30 minutes using a spectrometer. The protein content was calculated by comparing the absorbance of the samples with standard curve developed using BSA. The standard graph was developed between 5g and 40mg of BSA the protein content was expressed in mg/g fresh weight.

EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

Experiments were conducted to identify genetic variability among pigeon pea genotypes for phosphorus uptake and its utilization from organic P and inorganic P and to examine the biochemical mechanisms involved in higher P uptake under P deficient conditions. Thirty -six medium duration pigeon pea genotypes were selected from germplasm lines and these selected lines were grown in polythene bags containing vermiculite provided with fixed forms of inorganic and organic P for 45 days under green house conditions. Root and shoot growth traits, acid phosphatase activity and plant P contents of these genotypes were determined. Based on leaf P content and root acid phosphatase activity, six contrasting genotypes were selected for the further physiological and biochemical studies. These six genotypes were grown in second experiment with same conditions with objective to study root traits and to examine the activity of root acid phosphates.

The results of these above experiments are presented here.

Variability in growth attributes and P uptake amongst pigeon pea genotypes

4.1 Experiment – I

To study the genotypic variation in P uptake, 36 pigeon pea genotypes of medium duration (160-180 days) were grown in polythene bags under green house conditions with four P treatments. Plants were maintained at 100 per cent FC for 45 days through frequent watering and providing all nutrients through Hoagland's solution. An observation on growth parameters, root acid phosphatase activity and plant phosphorus contents were made at the end of 45 days.

4.1.1. Growth parameters

Plant growth among pigeon pea genotypes grown in +P and fixed forms of P showed significant differential response. The growth of pigeon pea genotypes in +P was

good compared to the plants grown in fixed forms of P. Data of these parameters are given in Table 2 to 6 and mean and range values of these growth traits is given in Table 19.

4.1.1.1 Root volume.

Significant variation in root volume amongst genotypes and also between the treatments was noticed. Under P deficient conditions, the root volume ranged from 0. 8 to 2.05 cm^3/pl (Table.2) with a mean of 1.59 cm^3/plant. Under –P conditions Genotypes ICP3226 and ICP 12764 recorded higher root volume of 2.05, 1.87cm^3/pl (Table.2). Under P sufficient conditions, genotypes ICP12764 and ICP 3226 had the higher root volume of 1.2 cm^3 per plant, 1.1cm^3 per plant. Under organic P conditions root volume varied from 0.2 to 2.0 cm^3/plant. Highest rot volume was recorded by ICP 3226(2.0cm^3/plant). Plants grown under organic p sourse showed higher root volume compare to inorganic P conditions. Data indicates increase in the root volume under P deficient conditions compare to other treatments (Fig.1, Plate 1)

4.1.1.2 Root length.

There was a considerable variation in root length among pigeon pea genotypes under different P source (Table.3). Root length was recorded high (28.8) with range of 21.25 to 35.5cm under P deficiency compare to other treatments. The genotypes like, ICP 12764 and ICP 8477 produced longer root lengths of 35, 34.75cm respectively. Under P sufficient conditions root length is comparatively low (17.14cm) and ranged from 12.75 to 20.5cm (Table.3). Under organic P root length was more than inorganic condition (Fig2 Plate 1)

Table 2 : Root Volume of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	1.025	0.525	0.675	1.500
2	ICP218	1.000	0.525	0.725	1.575
3	ICP1029	1.050	0.550	0.675	1.550
4	ICP 2933	1.425	0.575	0.700	0.825
5	ICP 3226	2.000	0.575	1.200	2.050
6	ICP 4557	0.750	0.500	0.500	1.650
7	ICP 5466	1.000	0.550	1.000	1.450
8	ICP 7118	1.000	0.525	0.900	1.700
9	ICP 8477	1.000	0.525	0.890	0.800
10	ICP 8863	1.225	1.000	1.100	1.550
11	ICP 9306	0.525	0.475	0.598	1.550
12	ICP 12764	1.175	0.550	1.100	1.870
13	ICP 14064	0.500	0.425	1.000	1.575
14	ICP 14252	0.525	0.550	0.930	1.525
15	ICP14352	1.000	0.525	1.000	1.225
16	WRGE 38	1.300	1.000	1.000	1.650
17	JKM 205	1.250	1.000	0.820	1.650
18	JKM207	0.475	0.375	0.525	1.375
19	BSMR 736	0.425	0.525	0.681	1.425
20	BRG-2	1.000	0.525	1.000	1.625
21	BDN 2010	1.000	0.525	1.000	1.200
22	LCV 10	0.350	0.500	1.000	1.525
23	LCV 40	1.000	1.000	0.920	1.650
24	H 3C	0.475	0.475	1.000	1.725
25	TTB 7	1.000	1.000	1.025	1.950
26	JKM 189	1.325	1.000	1.000	1.925
27	WRP 1	0.450	1.000	1.050	1.850
28	JS 5	0.450	0.425	0.600	1.375
29	ICP7035	1.775	1.000	1.025	2.000
30	JSA 59	0.275	0.500	0.674	2.000
31	JSA 81	0.375	0.500	0.500	1.625
32	GT 101	0.400	0.300	0.525	1.475
33	GRG 207	0.550	1.050	0.824	1.725
34	BRG 1	1.000	1.000	1.100	1.550
35	ICP96047	0.275	0.375	1.000	1.875
36	SEL20129	0.375	0.500	0.475	1.825
	MEAN	**0.853**	**0.638**	**0.892**	**1.594**
	Range	**0.275-2**	**0.3-1.05**	**0.475-1.1**	**0.8-2.05**
	CD	**0.066**			
	CV	**9.34%**			

Table 3: Root Length of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	27.000	25.000	19.000	30.000
2	ICP218	20.000	19.000	15.000	25.000
3	ICP1029	18.500	20.500	14.750	21.250
4	ICP 2933	21.250	20.500	14.500	25.500
5	ICP 3226	31.250	25.000	14.750	24.250
6	ICP 4557	23.000	22.250	18.250	27.500
7	ICP 5466	21.500	20.500	14.750	25.000
8	ICP 7118	23.250	20.500	17.250	24.000
9	ICP 8477	30.750	27.500	20.250	35.000
10	ICP 8863	24.500	20.250	18.500	27.750
11	ICP 9306	30.250	27.250	20.000	31.250
12	ICP 12764	22.500	26.750	20.500	35.500
13	ICP 14064	21.500	19.000	15.500	27.250
14	ICP 14252	25.500	19.500	17.000	30.500
15	ICP14352	18.250	17.000	12.750	23.000
16	WRGE 38	20.500	18.000	14.500	26.000
17	JKM 205	26.750	19.000	14.500	31.750
18	JKM207	29.750	27.500	20.000	32.000
19	BSMR 736	29.500	25.000	18.500	34.000
20	BRG-2	25.500	20.500	14.500	30.250
21	BDN 2010	20.750	20.500	14.750	30.500
22	LCV 10	25.000	20.750	15.250	30.750
23	LCV 40	25.500	20.500	14.750	30.000
24	H 3C	24.250	21.250	18.250	29.000
25	TTB 7	25.500	20.500	18.000	29.000
26	JKM 189	28.500	21.000	20.000	29.500
27	WRP 1	29.750	27.250	20.250	32.250
28	JS 5	25.750	20.500	18.750	30.500
29	ICP7035	24.500	20.500	19.750	28.750
30	JSA 59	25.500	20.750	18.250	30.500
31	JSA 81	19.000	18.500	15.500	25.250
32	GT 101	24.250	20.500	18.000	30.250
33	GRG 207	25.000	20.750	18.500	29.750
34	BRG 1	23.750	21.000	18.000	31.000
35	ICP96047	24.250	20.500	15.250	28.500
36	SEL20129	25.500	23.250	19.250	26.750
	MEAN	**24.660**	**21.625**	**17.146**	**28.882**
	Range	**18.25-31.25**	**17-27.5**	**12.75-20.5**	**21.25-35.5**
	CD	**0.567**			
	CV	**3.54%**			

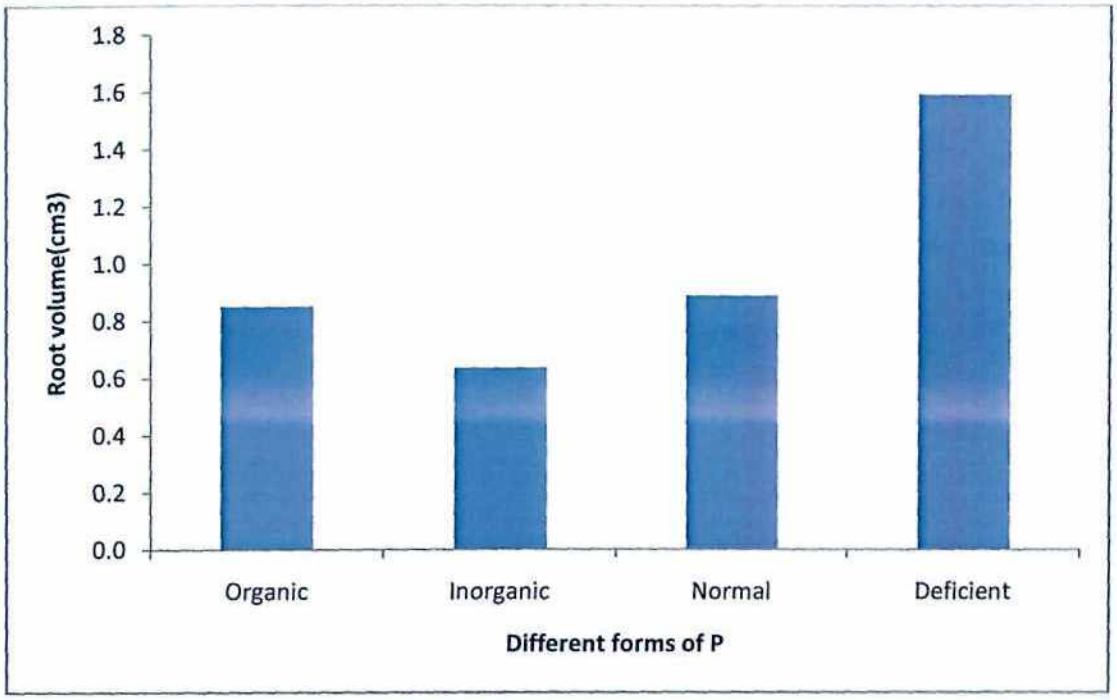


Fig. 1 Effect of different sources of P on root volume of 36 pigeon pea genotypes grown for 45 days

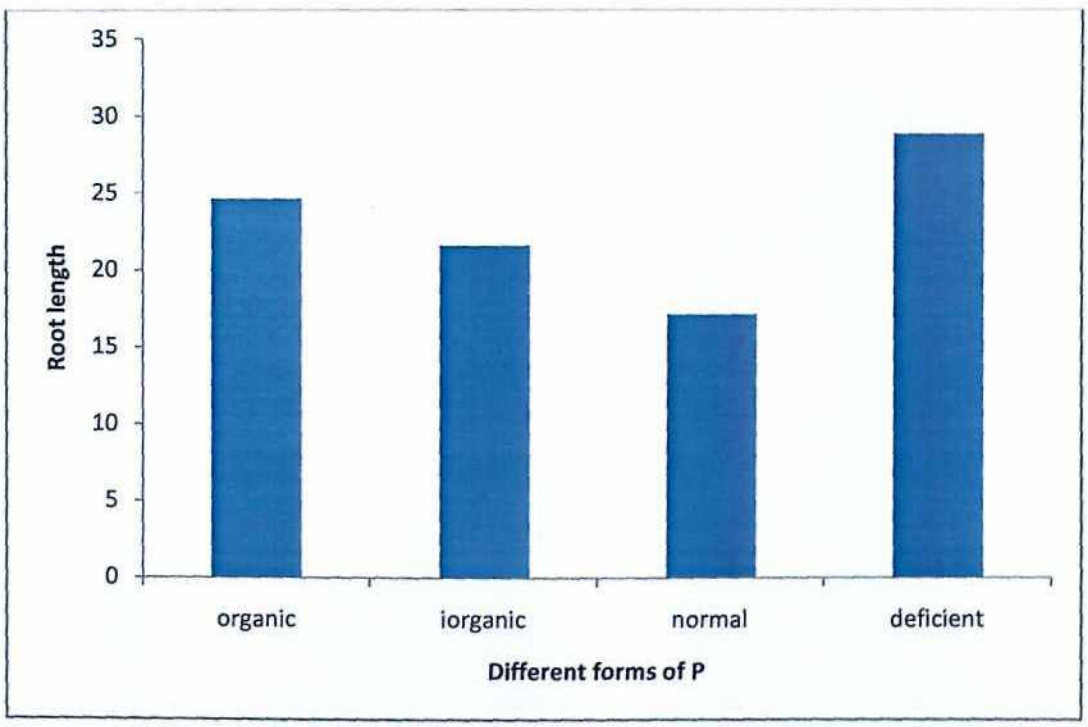


Fig. 2 Effect of different sources of P on root lenght of 36 pigeon pea genotypes grown for 45 days

4.1.2 Plant dry weight

4.1.2.1 Root dry weight

There was a good variation in root dry weight between the genotypes under different sources of P level (table 4). Genotypes like ICP 3226, ICP 8477, accumulate more root dry weight under organic P where as genotypes like ICP 7035,ICP 96047 and lcv-10 accumulated low root weight. Under inorganic P condition genotypes like ICP3226, ICP12764 and ICP8477 accumulated high root weight compare to normal condition. Under normal P the genotypes root weight ranged from 0.432-0.638. Genotypes like ICP2933, JKM 207 and BSMR-736 showed high root weight. Under p deficient condition the genotypes like ICP12764, ICP3226 and ICP 8477 showed high root weight like 1.018,0.940, gm/pt. Among all treatments root weight was more under deficient P condition followed by organic P condition (Fig.3) this could be due to the fact that under P deficient condition the plant is spending more energy and allocating more resources to the root. Hence root is growing at the cost of shoot.

4.1.2.2 Shoot dry weight.

There was a good variation noticed in shoot dry weight amongst the 36 genotypes grown under different P sources (Table.5). Under organic condition the shoot dry weight ranges from 0.683-2.288 gm. There was a good variation seen between genotypes the genotypes like ICP-12764, ICP 3226, and ICP8477, recorded highest shoot weight like 2.288, 1.965 and 1.647 under organic condition. Under inorganic condition the range is less compared to organic form it ranges from 0.67-1.875, genotypes like ICP-12764 ICP1029 and JKM 205 showed highest shoot weight. The shoot weight was low under deficient condition which ranged from0.690-1.233. ICP-3226 ICP12764 and ICP 8477 recorded highest shoot weight where as genotypes like Sel 20129 BRG-207 and ICP 218 recorded lowest shoot weight. This shows that P is the crucial nutrient required for the plant growth and development. Variation in shoot biomass indicates that even under P deficient condition, some of the genotypes are efficiently translocating the metabolites to shoots (fig .3).

Table 4: Root Wt of 36 Genotypes Grown under Different Source of Phosphorus

	Organic	Inorganic	Normal	Deficient
ICP 40	0.545	0.367	0.470	0.620
ICP218	0.560	0.335	0.458	0.642
ICP1029	0.525	0.457	0.432	0.642
ICP 2933	0.638	0.440	0.638	0.722
ICP 3226	0.968	0.592	0.538	0.940
ICP 4557	0.255	0.252	0.545	0.892
ICP 5466	0.537	0.435	0.505	0.752
ICP 7118	0.542	0.420	0.550	0.850
ICP 8477	0.770	0.541	0.545	0.917
ICP 8863	0.257	0.530	0.543	0.820
ICP 9306	0.438	0.472	0.540	0.723
ICP 12764	0.665	0.582	0.527	1.018
ICP 14064	0.438	0.340	0.563	0.770
ICP 14252	0.348	0.382	0.543	0.785
ICP14352	0.543	0.475	0.545	0.682
WRGE 38	0.640	0.555	0.570	0.775
JKM 205	0.642	0.532	0.557	0.763
JKM207	0.343	0.260	0.470	0.693
BSMR 736	0.343	0.475	0.552	0.620
BRG-2	0.528	0.462	0.537	0.688
BDN 2010	0.545	0.367	0.565	0.643
LCV 10	0.257	0.372	0.542	0.643
LCV 40	0.552	0.555	0.570	0.793
H 3C	0.442	0.352	0.550	0.770
TTB 7	0.552	0.537	0.530	0.865
JKM 189	0.632	0.535	0.555	0.892
WRP 1	0.350	0.527	0.543	0.845
JS 5	0.345	0.247	0.435	0.668
ICP7035	0.242	0.242	0.543	1.018
JSA 59	0.565	0.445	0.522	0.897
JSA 81	0.320	0.420	0.442	0.763
GT 101	0.348	0.445	0.438	0.673
GRG 207	0.440	0.510	0.522	0.822
BRG 1	0.525	0.527	0.530	0.750
ICP96047	0.255	0.465	0.532	0.870
SEL20129	0.355	0.440	0.460	0.800
Mean	**0.503**	**0.439**	**0.525**	**0.769**
Range	**0.242-0.968**	**0.242-0.592**	**0.432-0.638**	**0.62-1.018**
CD	**0.022**			
CV	**6.810%**			

Table 5 ; Shoot Wt of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	1.262	1.333	1.410	0.835
2	ICP218	1.320	1.243	1.353	0.856
3	ICP1029	1.453	1.560	1.637	0.787
4	ICP 2933	1.370	1.233	1.348	0.775
5	ICP 3226	1.965	1.175	1.637	1.233
6	ICP 4557	1.243	1.238	1.380	0.690
7	ICP 5466	1.308	1.023	1.228	0.815
8	ICP 7118	1.030	0.860	1.002	0.930
9	ICP 8477	1.647	1.173	1.543	0.978
10	ICP 8863	1.262	0.973	1.348	0.675
11	ICP 9306	1.245	0.957	1.208	0.800
12	ICP 12764	2.288	1.875	1.565	1.130
13	ICP 14064	0.983	0.688	0.975	0.837
14	ICP 14252	0.775	0.775	0.867	0.892
15	ICP14352	1.145	1.265	1.353	0.920
16	WRGE 38	0.973	0.780	0.970	0.860
17	JKM 205	0.960	0.968	1.245	0.725
18	JKM207	1.255	1.192	0.960	0.730
19	BSMR 736	0.978	0.773	1.220	0.780
20	BRG-2	1.267	1.167	1.175	0.835
21	BDN 2010	0.877	0.772	1.245	0.765
22	LCV 10	1.257	0.970	1.240	0.800
23	LCV 40	1.192	0.670	1.330	0.810
24	H 3C	0.957	0.875	1.230	0.745
25	TTB 7	1.250	1.238	1.353	0.770
26	JKM 189	1.662	1.580	1.243	0.847
27	WRP 1	0.965	1.263	1.325	0.850
28	JS 5	1.245	1.155	1.157	0.782
29	ICP7035	1.350	1.177	1.562	0.713
30	JSA 59	0.985	0.790	1.245	0.767
31	JSA 81	0.863	0.775	1.245	0.843
32	GT 101	0.978	0.865	1.250	0.755
33	GRG 207	0.858	0.757	1.255	0.675
34	BRG 1	0.860	0.802	1.257	0.775
35	ICP96047	0.865	0.770	1.348	0.805
36	SEL20129	0.683	0.838	0.970	0.690
Mean		**1.183**	**1.043**	**1.252**	**0.838**
Range		**0.683-2.288**	**0.67-1.875**	**0.867-1.637**	**0.690-1.233**
CD		**0.049**			
CV		**6.240%**			

4.1.2.3 Root to shoot dry weight ratio.

High root to shoot ratio is a desirable trait under deficient conditions. It also helps in acquiring more nutrients through increased surface area between roots. Root to shoot ratio varied significantly among 36-pigeonpea genotypes. High root to shoot dry weight ratio was observed under P deficient condition which ranged from 0.505-1.225 with a mean of 0.941 (Table.5a) under inorganic condition it ranged from0.215-0.827 with a mean of 0.448. Mean root/shoot ratio with and without P was shown in Table.5.over all root to shoot ratio was high in deficient condition followed by inorganic and organic condition. It was less in normal condition compared to other treatments. This indicates some genotypes are producing more root biomass than shoot biomass under –P condition. (Fig6)

4.1.2.4 Total dry matter

There was a significant variation observed between genotypes in total dry matter (table.6). Under organic it ranges from 1.04-2.95 amongst the genotypes ICP 12764, ICP3226, and ICP8477.Showed good TDM accumulation of 2.95, 2.93and 2.29.gm/pt with mean of 1.68. Under inorganic form of P. under inorganic form it ranges from 1.02-2.32 genotypes like ICP12764, ICP 8477 and ICP 3226. Showed good TDM accumulation as 2.32, 2.11, and 2.01 respectively. The TDM ranges were increased in normal condition it ranges form 1.41-2.11 with a mean of 1.77 ICP3226. ICP8477 and ICP 12764 accumulated high TDM like 2.11 2.09 and 2.07gm/pt. In deficient condition the genotypes showed low TDM compared to other form of P which ranges from1.22-2.14 with a mean of 1.569. Genotypes like ICP 7035, (2.148), ICP 8863(1.870) and ICP12764 (1.852) showed high TDM and genotypes like ICP14352 (1.220) and ICP218 (1.355) and BDN2010 showed low TDM under deficient condition. The TDM accumulation was more under normal condition compared to other forms of P it was less in inorganic condition because of fixed forms of P which is bounded to other nutrients which will not be available (fig.3).

Table 5a : R/S Ratio of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	0.432	0.275	0.335	0.505
2	ICP218	0.425	0.268	0.340	0.905
3	ICP1029	0.363	0.293	0.265	0.820
4	ICP 2933	0.465	0.358	0.475	0.935
5	ICP 3226	0.672	0.660	0.625	1.138
6	ICP 4557	0.568	0.475	0.395	0.925
7	ICP 5466	0.417	0.430	0.413	0.938
8	ICP 7118	0.530	0.490	0.550	0.920
9	ICP 8477	0.610	0.678	0.587	0.775
10	ICP 8863	0.535	0.545	0.402	0.760
11	ICP 9306	0.352	0.495	0.448	0.910
12	ICP 12764	0.623	0.620	0.577	1.225
13	ICP 14064	0.450	0.497	0.422	0.922
14	ICP 14252	0.447	0.498	0.400	0.892
15	ICP14352	0.472	0.375	0.403	0.765
16	WRGE 38	0.660	0.715	0.440	0.908
17	JKM 205	0.290	0.555	0.450	1.055
18	JKM207	0.273	0.223	0.490	0.963
19	BSMR 736	0.353	0.238	0.453	0.803
20	BRG-2	0.415	0.397	0.458	0.828
21	BDN 2010	0.623	0.478	0.455	0.843
22	LCV 10	0.205	0.385	0.438	0.813
23	LCV 40	0.465	0.827	0.428	1.000
24	H 3C	0.462	0.402	0.447	1.047
25	TTB 7	0.442	0.437	0.392	1.170
26	JKM 189	0.380	0.340	0.363	1.088
27	WRP 1	0.363	0.420	0.407	1.002
28	JS 5	0.278	0.215	0.377	0.867
29	ICP7035	0.572	0.503	0.348	0.915
30	JSA 59	0.245	0.567	0.417	1.183
31	JSA 81	0.373	0.543	0.355	0.940
32	GT 101	0.358	0.283	0.350	0.917
33	GRG 207	0.512	0.400	0.415	0.970
34	BRG 1	0.345	0.377	0.420	0.975
35	ICP96047	0.292	0.328	0.395	1.083
36	SEL20129	0.520	0.525	0.477	1.190
	MEAN	**0.435**	**0.448**	**0.428**	**0.941**
	Range	**0.205-0.672**	**0.215-0.827**	**0.265-0.625**	**0.505-1.225**
	CD	**0.538**			
	CV	**14%**			

Table 6: Total Dry Matter of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	1.807	1.700	1.880	1.542
2	ICP218	1.880	1.578	1.810	1.355
3	ICP1029	1.978	1.620	2.070	1.430
4	ICP 2933	2.007	1.673	1.985	1.498
5	ICP 3226	2.933	2.017	2.105	1.852
6	ICP 4557	1.947	1.820	1.925	1.870
7	ICP 5466	1.845	1.457	1.732	1.568
8	ICP 7118	1.572	1.280	1.552	1.780
9	ICP 8477	2.295	2.115	2.090	1.852
10	ICP 8863	1.938	1.502	1.890	1.907
11	ICP 9306	1.683	1.430	1.748	1.523
12	ICP 12764	2.952	2.320	2.070	2.148
13	ICP 14064	1.420	1.027	1.537	1.607
14	ICP 14252	1.123	1.157	1.410	1.678
15	ICP14352	1.688	1.740	1.897	1.220
16	WRGE 38	1.613	1.335	1.540	1.635
17	JKM 205	1.603	1.500	1.803	1.488
18	JKM207	1.597	1.452	1.430	1.423
19	BSMR 736	1.320	1.248	1.772	1.400
20	BRG-2	1.795	1.630	1.712	1.522
21	BDN 2010	1.423	1.140	1.810	1.407
22	LCV 10	1.515	1.343	1.782	1.442
23	LCV 40	1.745	1.225	1.900	1.603
24	H 3C	1.400	1.228	1.780	1.515
25	TTB 7	1.802	1.775	1.882	1.635
26	JKM 189	2.213	1.642	1.787	1.765
27	WRP 1	1.315	1.790	1.868	1.695
28	JS 5	1.590	1.403	1.593	1.450
29	ICP7035	2.120	1.770	1.887	1.775
30	JSA 59	1.228	1.235	1.767	1.665
31	JSA 81	1.183	1.195	1.688	1.605
32	GT 101	1.325	1.108	1.688	1.428
33	GRG 207	1.298	1.268	1.778	1.498
34	BRG 1	1.385	1.330	1.787	1.525
35	ICP96047	1.120	1.022	1.880	1.675
36	SEL20129	1.037	1.278	1.430	1.490
Mean		**1.686**	**1.482**	**1.777**	**1.569**
Range		**1.04-2.95**	**1.02-2.32**	**1.41-2.11**	**1.22-2.148**
CD		**0.049**			
CV		**9.820%**			

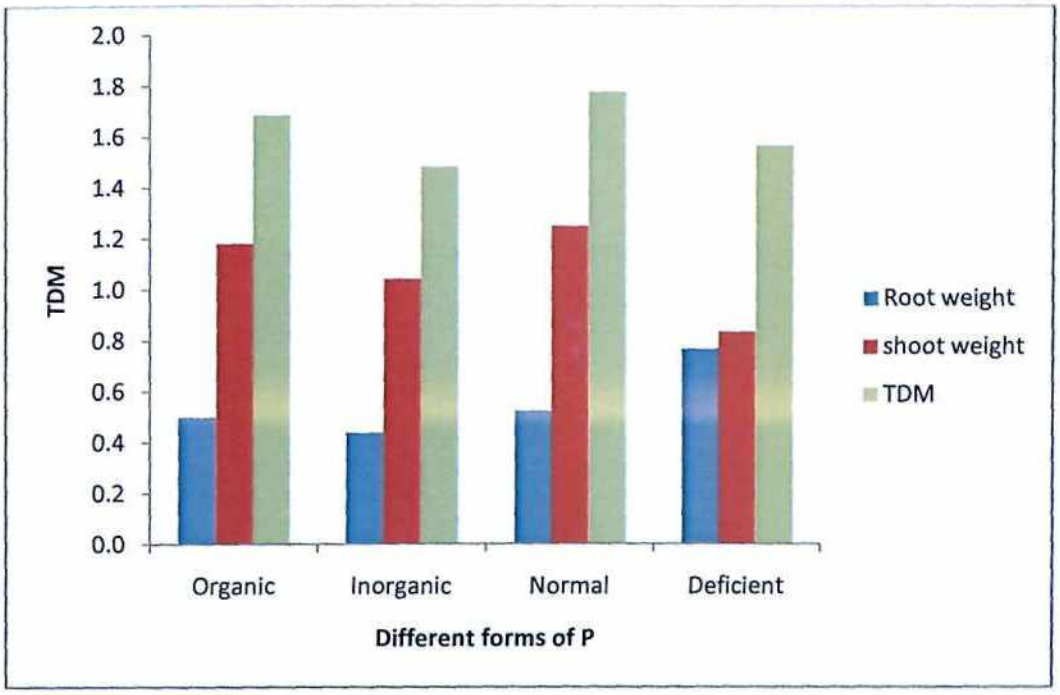


Fig 3 Effect of different sources of P on biomass of 36 pigeon pea genotypes grown for 45 days

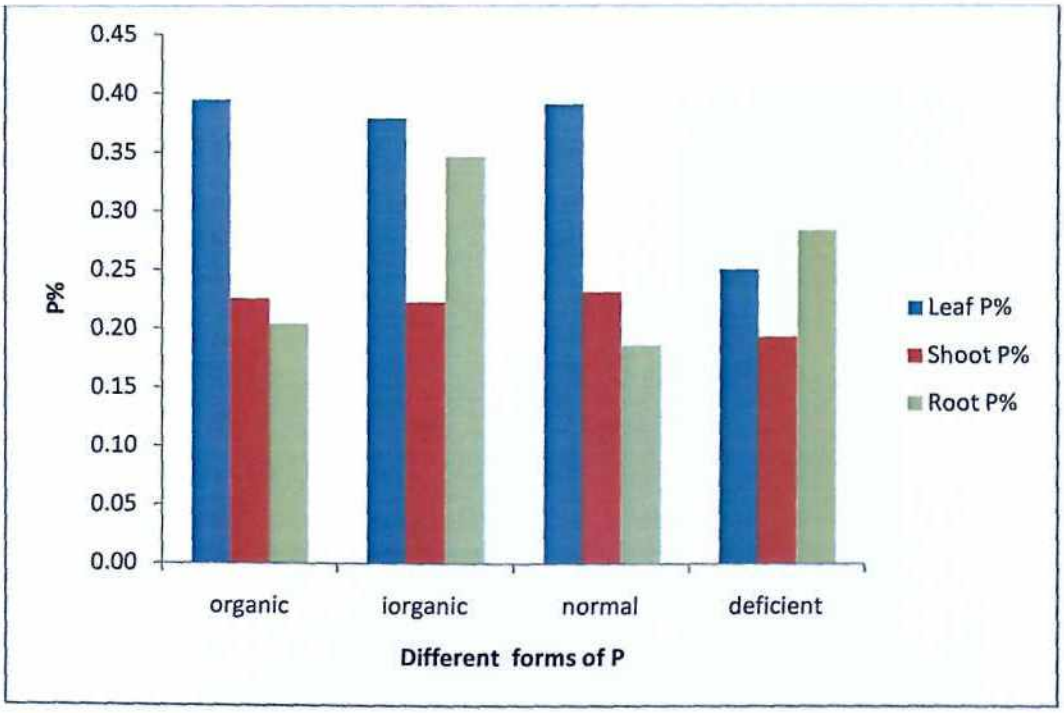


Fig. 4 Effect of different sources of P on leaf, root and shoot P% of 36 pigeon pea genotypes grown for 45 days

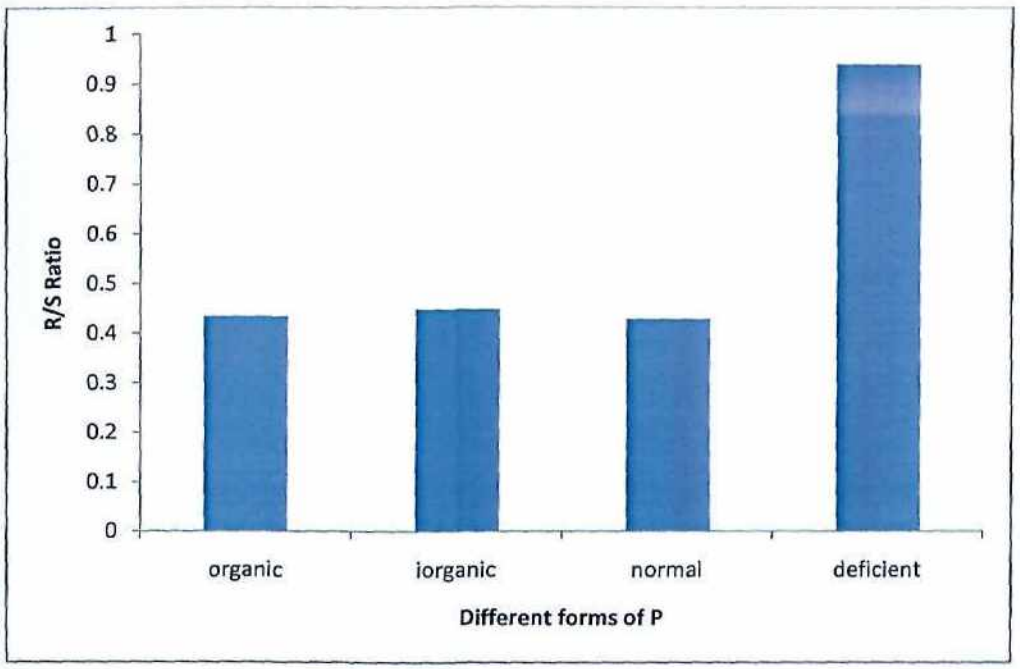


Fig. 5 Effect of different sources of P on R/S Ratio of 36 pigeon pea genotypes grown for 45 days

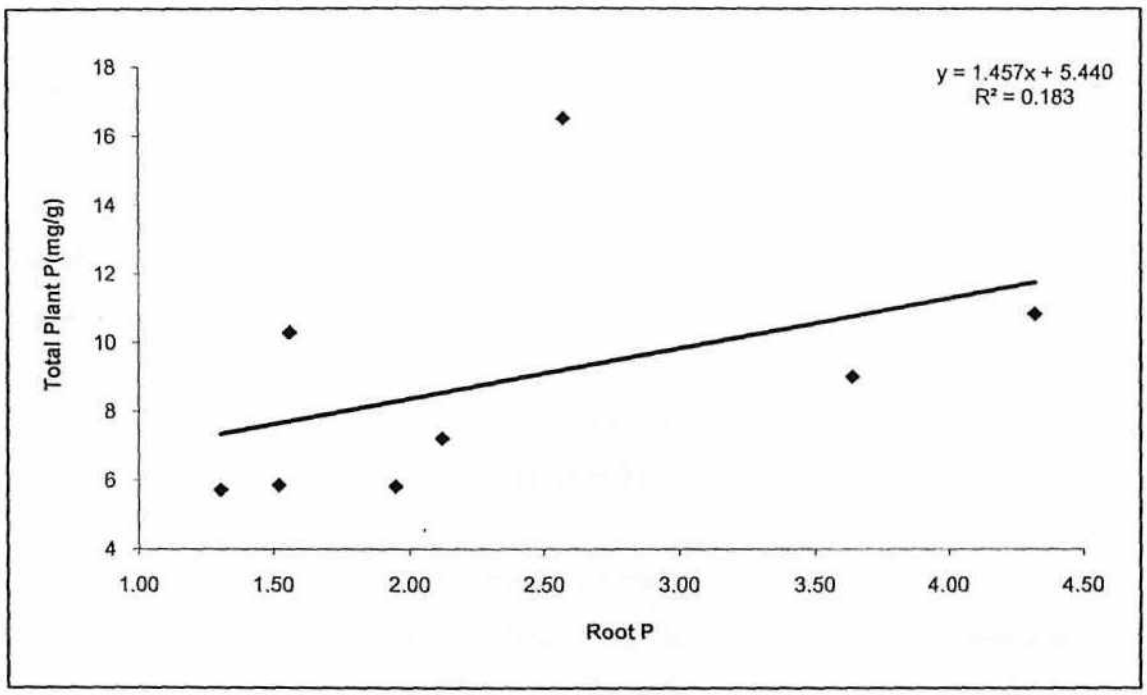


Fig 5a. Relationship between TOTAL PLANT p and ROOT P of high and low P uptake genotypes grown for 45 days under different P sources

4.1.3 Plant P contents of pigeon pea genotypes grown under different forms of phosphorus conditions

The P content of root and leaf was estimated by **Vanado-molybdate method** (Jackson, 1973) and the values expressed in percentage and also in mg/pl. are given in Table. 7, 8, 9, Total mean values and range are shown in Table

4.1.3.1 Root P

The first plant organ, which comes in contact with mineral nutrients to acquire and absorb, are roots. Root P was expressed as percent and calculated for total root biomass to get total root P in terms of mg $Plant^{-1}$. Both under sufficient and insufficient P conditions the genotypes varied significantly in the total root P content (Table.9). The genotypes grown in P sufficient condition showed more P concentration in the root than the genotypes grown under deficiency of P (Fig.7).

Roots are the major plant part with comes in contact with the soil to absorb nutrients and translocated to shoot it was expressed in % first and then calculated for total root biomass to get the total root P in terms of mg/pt. Under deficient P condition root P accumulation is lowest compare to other treatments. Under organic condition the root P ranged from 0.125-0.58% with a mean of 0.204. Genotypes like 1CP3226 (0.580) ICP8477 (0.575) and ICP 12764 (0.308) accumulated high root P% and genotypes like ICP8863 (0.125) sel-20129(0,150) and icp4557 (0.163) showed low root P%. Under inorganic condition the root p% ranged from 0.122-0.468 with a mean of 0.346. Genotypes like ICP 3226(0.456) and ICP8447 (0.364) and ICP 12764(0.250) ranged high root P content and ICP 14252(0.122%) and ICP8863 (0.123) and BDN 2010(0.133) showed low root P content. Where as in normal condition the root P % ranged from 0.128-0.267 with a mean of 0.186. Here the genotypes like SEL20129 (0.128) ICP218 (0.130) and TTB7 (0.132) showed low root P% where as ICP12764, (0.267) ICP3226 (0.245) and ICP8477 (0.257) showed high root P%. In P deficient condition root p% ranges from 0.105-1.278 with a mean of 0.284. Here genotypes like ICP8447 (1.278) ICP3226 (0.173) and ICP12764 (0.178) showed high root p% compared to other. Under

Table 7: Root P % of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	0.218	0.143	0.137	1.053
2	ICP218	0.235	0.232	0.130	0.885
3	ICP1029	0.132	0.160	0.140	1.035
4	ICP 2933	0.305	0.177	0.150	0.105
5	ICP 3226	0.580	0.456	0.245	0.173
6	ICP 4557	0.163	0.158	0.205	0.112
7	ICP 5466	0.255	0.190	0.158	0.155
8	ICP 7118	0.165	0.205	0.220	1.023
9	ICP 8477	0.575	0.364	0.128	1.278
10	ICP 8863	0.125	0.123	0.160	1.040
11	ICP 9306	0.227	0.157	0.180	0.112
12	ICP 12764	0.308	0.250	0.267	0.178
13	ICP 14064	0.128	0.143	0.223	0.112
14	ICP 14252	0.230	0.237	0.245	0.105
15	ICP14352	0.133	0.122	0.160	0.180
16	WRGE 38	0.160	0.140	0.143	0.130
17	JKM 205	0.168	0.162	0.163	0.112
18	JKM207	0.133	0.148	0.178	0.105
19	BSMR 736	0.133	0.185	0.255	0.170
20	BRG-2	0.163	0.165	0.223	0.140
21	BDN 2010	0.160	0.133	0.183	0.115
22	LCV 10	0.147	0.133	0.175	0.127
23	LCV 40	0.580	0.133	0.160	0.143
24	H 3C	0.153	0.163	0.158	0.110
25	TTB 7	0.168	0.150	0.132	0.107
26	JKM 189	0.170	0.147	0.155	0.127
27	WRP 1	0.145	0.143	0.237	0.110
28	JS 5	0.145	0.163	0.160	0.175
29	ICP7035	0.163	0.125	0.223	0.133
30	JSA 59	0.170	0.188	0.183	0.150
31	JSA 81	0.155	0.145	0.158	0.107
32	GT 101	0.232	0.157	0.155	0.107
33	GRG 207	0.172	0.140	0.185	0.128
34	BRG 1	0.255	0.227	0.240	0.138
35	ICP96047	0.185	0.165	0.237	0.110
36	SEL20129	0.150	0.160	0.257	0.117
Mean		**0.204**	**0.346**	**0.186**	**0.284**
Range		**0.125-0.58**	**0.122-0.468**	**0.128-0.267**	**0.105-1.278**
CD		**0.748**			
CV		**14.28%**			

deficient condition the P% was low compared to other treatment because of non-availability of the phosphorus. This indicates under –P condition, plants have to struggle to acquire the needed P through physiological and biochemical adaptations under P stress. The pigeon pea genotypes significantly varied in taking up P indicating the scope for identifying high uptake types.

4.1.3.2. Shoot P.

Shoot P was one of the important parameter considered in this experiment. It was estimated by vanado-molybdate method. And it was expressed in percent. There was a significant variation amongst the genotypes was observed between the four treatments. Under organic condition the shoot P% ranged from 0.137-0.432% with a mean of 0.226. The genotypes like ICP 8477(0.432) ICP 3226(0.0.310)and ICP 12764(0.258) showed high shoot P% and genotypes like ICP 5466(0.137) ICP 7035(0.145) and ICP 8863(0.182) showed low shoot P % where as in inorganic condition the shoot P% ranges from 0.137-0.427% with a mean of 0.0.223%. In this treatment the genotypes like ICP8477 (0.427) ICP 12764(0.270) and ICP 3226(0.328) showed high accumulation of shoot P % and the genotypes like ICP 4557(0.137) ICP 7035(0.193%) and ICP 8863(0.257) showed low shoot P %. In normal condition the shoot P % ranges from 0.16-0.32% with a mean 0.232. Here ICP 8477(0.322) ICP3226 (0.265) and ICP 12764(0.253).These genotypes showed high shoot P%. And JSA 59 (0.16) and ICP 8863(0.225) where as in deficient condition shoot Prangs from 0.11-1.175. Here the shoot P% was very much low compared to other three treatments. With a mean of 0.194,

4.1.3.2 Leaf P.

Leaf P was one of the important parameter considered in this experiment. It was estimated by vanado-molybdate method. And it was expressed in percent. There was a significant variation amongst the genotypes was observed between the four treatments. Under organic condition the leaf P% ranged from 0.232-0.668% with a mean of 0.395. The genotypes like ICP 8477(0.668) ICP 3226(0.595)and ICP 12764(0.582) showed high leaf P% and genotypes like ICP 4557(0.232) ICP 7035(0.255) and ICP 8863(0.241)

Table 8: Shoot P % of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	0.253	0.222	0.220	0.112
2	ICP218	0.233	0.262	0.225	0.112
3	ICP1029	0.227	0.238	0.183	0.220
4	ICP 2933	0.183	0.165	0.175	0.133
5	ICP 3226	0.310	0.328	0.265	0.388
6	ICP 4557	0.225	0.252	0.212	0.170
7	ICP 5466	0.137	0.232	0.245	1.175
8	ICP 7118	0.245	0.215	0.232	0.175
9	ICP 8477	0.432	0.427	0.322	0.285
10	ICP 8863	0.182	0.257	0.225	0.172
11	ICP 9306	0.247	0.242	0.233	0.163
12	ICP 12764	0.258	0.210	0.253	0.195
13	ICP 14064	0.225	0.197	0.217	0.188
14	ICP 14252	0.223	0.237	0.265	0.158
15	ICP14352	0.223	0.210	0.240	0.120
16	WRGE 38	0.215	0.270	0.232	0.163
17	JKM 205	0.230	0.220	0.238	0.122
18	JKM207	0.257	0.240	0.240	0.160
19	BSMR 736	0.218	0.262	0.255	0.178
20	BRG-2	0.237	0.137	0.273	0.135
21	BDN 2010	0.233	0.210	0.225	0.148
22	LCV 10	0.195	0.170	0.255	0.138
23	LCV 40	0.250	0.240	0.253	0.110
24	H 3C	0.247	0.233	0.250	0.140
25	TTB 7	0.257	0.183	0.245	0.190
26	JKM 189	0.267	0.223	0.225	0.150
27	WRP 1	0.145	0.232	0.247	0.170
28	JS 5	0.253	0.165	0.265	0.133
29	ICP7035	0.180	0.193	0.163	0.223
30	JSA 59	0.153	0.183	0.160	0.135
31	JSA 81	0.235	0.235	0.242	0.142
32	GT 101	0.180	0.207	0.188	0.162
33	GRG 207	0.178	0.230	0.227	0.123
34	BRG 1	0.255	0.183	0.227	0.115
35	ICP96047	0.202	0.140	0.220	0.185
36	SEL20129	0.143	0.183	0.220	0.212
Mean		**0.226**	**0.223**	**0.232**	**0.194**
Range		**0.137-0.432**	**0.137-0.427**	**0.16-0.322**	**0.11-1.175**
CD		**0.038**			
V		**25.17%**			

Table 9: Leaf P % of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	0.448	0.355	0.383	0.218
2	ICP218	0.367	0.410	0.367	0.185
3	ICP1029	0.360	0.363	0.375	0.248
4	ICP 2933	0.335	0.348	0.365	0.183
5	ICP 3226	0.595	0.447	0.547	0.330
6	ICP 4557	0.232	0.262	0.257	0.173
7	ICP 5466	0.450	0.372	0.430	0.268
8	ICP 7118	0.425	0.450	0.435	0.280
9	ICP 8477	0.668	0.615	0.660	0.435
10	ICP 8863	0.241	0.270	0.280	0.237
11	ICP 9306	0.425	0.332	0.425	0.313
12	ICP 12764	0.582	0.535	0.522	0.298
13	ICP 14064	0.480	0.355	0.447	0.298
14	ICP 14252	0.427	0.430	0.460	0.293
15	ICP14352	0.355	0.430	0.407	0.185
16	WRGE 38	0.350	0.417	0.355	0.250
17	JKM 205	0.232	0.328	0.362	0.175
18	JKM207	0.267	0.348	0.318	0.210
19	BSMR 736	0.447	0.455	0.418	0.285
20	BRG-2	0.525	0.427	0.500	0.250
21	BDN 2010	0.525	0.427	0.520	0.315
22	LCV 10	0.325	0.392	0.348	0.223
23	LCV 40	0.500	0.435	0.430	0.330
24	H 3C	0.330	0.252	0.278	0.147
25	TTB 7	0.373	0.343	0.350	0.225
26	JKM 189	0.348	0.325	0.328	0.220
27	WRP 1	0.355	0.360	0.340	0.262
28	JS 5	0.357	0.330	0.362	0.225
29	ICP7035	0.255	0.352	0.265	0.260
30	JSA 59	0.333	0.360	0.358	0.245
31	JSA 81	0.330	0.328	0.308	0.213
32	GT 101	0.355	0.337	0.330	0.245
33	GRG 207	0.335	0.370	0.427	0.303
34	BRG 1	0.422	0.415	0.415	0.310
35	ICP96047	0.422	0.335	0.345	0.232
36	SEL20129	0.330	0.335	0.360	0.187
Mean		**0.395**	**0.379**	**0.391**	**0.251**
Range		**0.232-0668**	**0252-0.615**	**0.257-0.66**	**0.147-0.435**
CD		**0.022**			
CV		**9.82%**			

showed low leaf P % where as in inorganic condition the leaf P% ranges from 0.252-0.615% with a mean of 0.379%. In this treatment the genotypes like ICP8477 (0.615) ICP 12764(0.535) and ICP 3226(0.447) showed high accumulation of leaf P % and the genotypes like ICP 4557(0.262) ICP 7035(0.252%) and ICP 8863(0.270) showed low leaf P %. In normal condition the leaf P % ranges from 0.257-0.66% with a mean 0.391. Here ICP 8477(0.66) ICP3226 (0.547) and ICP 12764(0.522).These genotypes showed high leap P%. And ICP 4557(0.257) and ICP 8863(0.280) where as in deficient condition the genotypes showed good responses in leaf P%. Here the leaf P% was very much low compared to other three treatments. It ranges from 0.147-0.435 with a mean of 0.251, here also the some genotypes like ICP 8447(0.435) ICP 3226(0.330) and ICP12764 (0.305) showed low leaf P %.(Fig7)

4.1.3.3 Root P.

Root P (mg/g) was calculated and expressed in mg/g and given in Table.10 and fig. Under organic condition it ranges form 2.2-13.31,2.20-10, 2.6-8.2 and 1.1-2.9 under inorganic normal and deficient condition with a mean of 2.03, 1.67,1.86 2.83.root P(mg/pt) was also calculated and mean of these treatments are 0.73,0.98,2.08 and ranges from 0.38-3.7,0.38-1.2,0.59-1.4,0.7-8.5 under organic inorganic, deficient and normal condition(table10a)

Frequency distribution in fig.6 indicates under organic P condition about 50 per cent of genotypes having root P (mg/g) in the range of 1.1-2.0 mg/g whereas under inorganic P condition about 60% of genotypes have the root P content in the range of 3.0-4.0 mg/g. Under normal conditions, some genotypes are able to accumulate the root P to an extent of 1.0-3.0mg/g. Under P deficient condition all genotypes have less root P accumulation in the range of 1.1-2.0 mg/g. (Fig13)

4.1.3.4 Shoot P.

The shoot P % is calculated using shoot dry weight and leaf p % Shoot P translocated from root has greater significance in producing more biomass and there by

more productivity. the shoot p (mg/g)(Table 11,11a) was calculated and the mean ranges form 3.9,3.7,3.9,2.5 and ranges from 2.32-6.6,2.5-6.1,2.5-6.6,and 4-4.3 under organic, inorganic, deficient, and normal condition. Hence total shoot P per plant is calculated using shoot dry weight and leaf P (%) and expressed as mg/pl (Table.10). Under P deficiency genotypes ICP 8477,ICP3226 and ICP12764 showed high shoot P content (mg/plant) of 3.78,2.578 and 2.890 in the shoot and genotypes ICP 4557, ICP8863 and ICP7035 showed low shoot P (mg/plant) content of 1.69,1.84 and 1.86.Under +P conditions, genotypes ICP3226 and ICP 8477 had showed high shoot P content of 7.3,8.2, and 6.47 and genotypes ICP 7035,8863 and 4557 showed low shoot P content (mg/plant) of 3.49,3.2 and 3.5.under organic and inorganic condition ICP3226,ICP12764,and ICP8477 showed high total shoot p content and Pigeon pea genotypes had accumulated more shoot P under sufficient P conditions . This indicates plants that are provided with sufficient P accumulate more shoot P without expending of extra energy.

Frequency distribution in fig.5 indicates under organic P condition about 50 per cent of genotypes having shoot P (mg/g) in the range of 2.1-3.0 mg/g whereas under inorganic P condition about 60% of genotypes have the shoot P content in the range of 3.0-5.0 mg/g. Under normal conditions, some genotypes are able to accumulate the shoot P to an extent of 7.0mg/g. Under P deficient maximum number of genotypes has less shoot P accumulation in the range of 2-3.0 mg/g

4.1.3.5 Total Plant P.

Total plant P is the addition of root P and shoot P and it was expressed in mg/plant and mg/g the P content ranged from 2.78-14.7,2.7-11.11,3.5-8.8,1.9-12.35and mean from 5.85,4,69,5.8,4.19 under organic inorganic normal and deficient condition(table. The genotypes ICP12764, ICP8477 and ICP3226 which showed high total plant P (mg per plant) of 10.78, 9.7 and 9.1 under organic conditions. And genotypes ICP4557, ICP8863 and ICP7035m showed low total P content of 3.98, 4.7 and 4.1mg/plant (Table.12, 12a). Under inorganic condition genotypes ICP8477, ICP12764 and ICP3226 showed high total P content of 8.3, 7.8 and 6.9mg/plant and genotypes

Table 10 : Total Root P Mg/gm of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	3.075	1.425	1.382	1.525
2	ICP218	2.350	2.313	1.300	8.850
3	ICP1029	1.313	1.600	1.400	1.350
4	ICP 2933	2.450	1.770	1.495	5.250
5	ICP 3226	5.750	2.440	2.450	1.725
6	ICP 4557	1.612	1.575	2.050	1.125
7	ICP 5466	2.550	1.900	1.575	1.550
8	ICP 7118	1.650	2.050	2.200	1.225
9	ICP 8477	3.050	2.225	1.275	1.775
10	ICP 8863	1.250	1.225	1.600	1.400
11	ICP 9306	2.275	1.575	1.800	1.125
12	ICP 12764	2.175	2.500	2.675	1.775
13	ICP 14064	1.275	1.425	2.225	1.125
14	ICP 14252	2.300	2.375	2.450	1.050
15	ICP14352	1.325	1.225	1.600	1.800
16	WRGE 38	1.600	1.400	1.425	1.300
17	JKM 205	1.675	1.625	1.625	1.125
18	JKM207	1.325	1.475	1.770	1.050
19	BSMR 736	1.325	1.850	2.550	1.700
20	BRG-2	1.625	1.650	2.225	1.400
21	BDN 2010	1.600	1.325	1.825	1.150
22	LCV 10	1.468	1.325	1.750	1.275
23	LCV 40	5.787	1.325	1.600	1.425
24	H 3C	1.525	1.625	1.563	1.100
25	TTB 7	1.667	1.500	1.307	1.075
26	JKM 189	1.697	1.475	1.550	1.275
27	WRP 1	1.450	1.425	2.375	1.100
28	JS 5	1.450	1.620	1.600	1.750
29	ICP7035	1.615	1.255	2.225	1.325
30	JSA 59	1.700	1.875	1.825	1.500
31	JSA 81	1.550	1.450	1.575	1.075
32	GT 101	2.307	1.578	1.540	1.075
33	GRG 207	1.710	1.400	1.840	1.275
34	BRG 1	2.550	2.275	2.405	1.375
35	ICP96047	1.850	1.650	2.375	1.100
36	SEL20129	1.488	1.567	2.563	1.175
	MEAN	**2.038**	**1.675**	**1.861**	**2.835**
	Range	**1.25-5.787**	**1.225-2.5**	**1.275-2.675**	**1.05-12.775**
	CD	**0.654**			
	CV	**16.68%**			

Table11 : Total Shoot P mg/g of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	4.438	3.530	3.810	2.175
2	ICP218	3.640	4.080	3.673	1.850
3	ICP1029	3.580	3.593	3.723	2.475
4	ICP 2933	3.350	3.475	3.650	1.825
5	ICP 3226	5.950	4.475	5.475	3.000
6	ICP 4557	2.325	2.625	2.575	1.725
7	ICP 5466	4.500	3.725	4.288	2.675
8	ICP 7118	4.250	4.500	4.350	2.800
9	ICP 8477	6.675	6.150	6.600	4.350
10	ICP 8863	3.475	3.475	3.175	2.375
11	ICP 9306	4.250	3.325	4.250	3.125
12	ICP 12764	5.815	5.332	4.980	2.975
13	ICP 14064	4.800	3.550	4.475	3.050
14	ICP 14252	4.275	4.300	4.600	2.925
15	ICP14352	3.550	4.300	4.085	1.850
16	WRGE 38	3.500	4.175	3.550	2.500
17	JKM 205	2.325	3.275	2.650	1.737
18	JKM207	2.675	2.700	2.800	2.100
19	BSMR 736	4.475	4.550	4.173	2.850
20	BRG-2	5.250	4.270	5.225	2.500
21	BDN 2010	5.250	4.275	5.200	3.150
22	LCV 10	3.250	3.925	3.475	2.225
23	LCV 40	5.000	4.350	4.295	3.300
24	H 3C	3.300	2.525	2.775	1.475
25	TTB 7	3.725	3.425	3.500	2.250
26	JKM 189	3.475	3.250	3.275	2.200
27	WRP 1	3.550	3.600	3.400	2.625
28	JS 5	3.575	3.300	3.625	2.250
29	ICP7035	2.550	3.505	3.625	2.600
30	JSA 59	3.325	3.600	3.575	2.450
31	JSA 81	3.300	3.275	3.075	2.125
32	GT 101	3.550	3.375	3.300	2.450
33	GRG 207	3.350	3.700	4.275	3.025
34	BRG 1	4.225	4.150	4.150	3.100
35	ICP96047	4.225	3.350	3.450	2.325
36	SEL20129	3.300	3.350	3.600	1.875
MEAN		3.946	3.788	3.908	2.508
Range		2.325-6.675	2.525-6.15	2.575-6.6	1.475-4.35
CD		0.595			
CV		0.407			

Table 11a : Total Shoot P mg/pt of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	5.605	4.705	5.370	2.680
2	ICP218	4.803	5.055	4.965	1.308
3	ICP1029	5.200	5.600	6.095	1.955
4	ICP 2933	4.593	4.285	4.923	1.407
5	ICP 3226	11.695	5.257	7.392	2.578
6	ICP 4557	2.890	3.252	3.555	1.693
7	ICP 5466	5.880	3.820	5.262	2.185
8	ICP 7118	4.395	3.867	4.350	2.602
9	ICP 8477	11.003	7.207	8.200	3.780
10	ICP 8863	4.388	3.383	3.295	1.840
11	ICP 9306	5.283	3.185	5.140	2.520
12	ICP 12764	13.315	10.000	6.477	2.890
13	ICP 14064	4.702	2.440	4.358	2.557
14	ICP 14252	3.303	3.322	3.983	2.657
15	ICP14352	4.062	5.442	5.525	1.698
16	WRGE 38	3.392	3.245	3.445	2.160
17	JKM 205	2.222	3.160	4.270	1.258
18	JKM207	3.355	3.220	2.690	1.543
19	BSMR 736	4.365	3.505	5.123	2.215
20	BRG-2	6.655	4.977	6.143	2.028
21	BDN 2010	4.593	3.290	6.205	2.405
22	LCV 10	4.088	3.808	4.305	1.790
23	LCV 40	5.955	2.915	5.710	2.680
24	H 3C	3.160	2.205	3.413	1.122
25	TTB 7	4.658	4.237	4.732	1.733
26	JKM 189	5.775	5.138	5.065	1.865
27	WRP 1	3.428	4.548	4.505	2.242
28	JS 5	4.455	3.810	4.195	1.787
29	ICP7035	3.442	4.125	3.493	1.860
30	JSA 59	3.265	2.843	4.452	1.870
31	JSA 81	2.843	2.528	3.830	1.810
32	GT 101	3.460	2.915	4.127	1.855
33	GRG 207	2.875	2.805	5.370	2.037
34	BRG 1	3.632	3.320	5.220	2.400
35	ICP96047	3.655	2.570	4.635	1.867
36	SEL20129	2.255	2.803	5.667	1.310
Mean		**4.796**	**3.966**	**4.875**	**2.051**
Range		**2.222-13.315**	**2.205-10**	**2.69-8.2**	**1.122-2.98**
CD		**0.309**			
CV		**11.32%**			

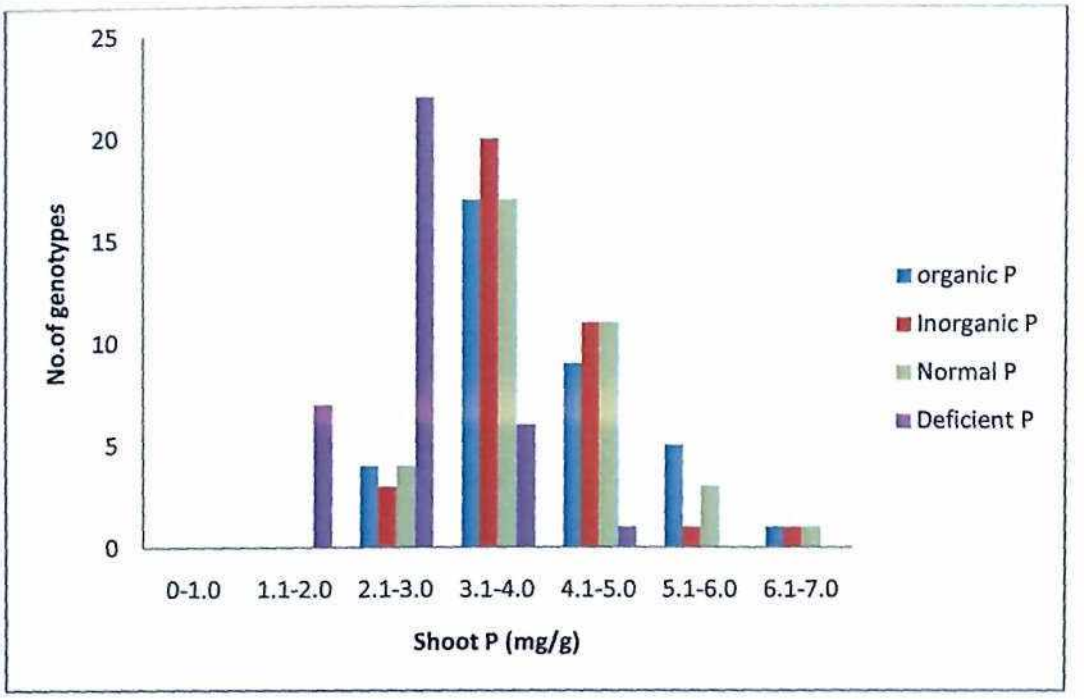


Fig 6. Frequency distribution of 36 pigeonpea genotypes for shoot mg/g P under different sources of P

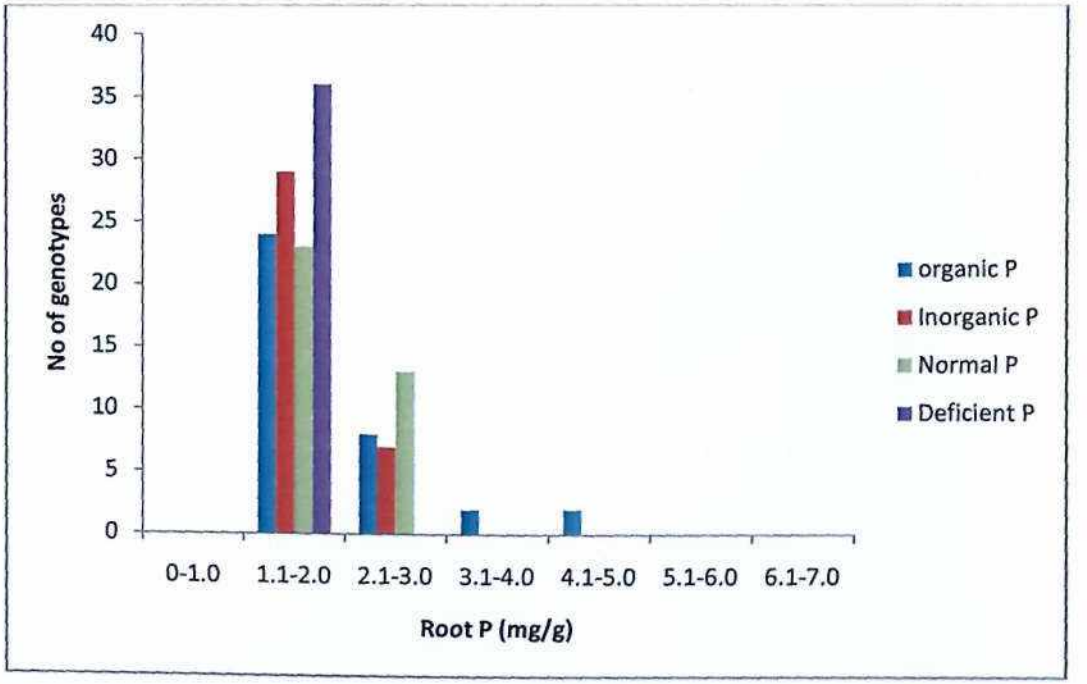


Fig 7. Frequency distribution of 36 pigeonpea genotypes for root Pmg/g P% under different sources of P

Table12 : Total P mg/g of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	7.283	5.227	6.025	9.212
2	ICP218	6.120	5.830	5.563	6.935
3	ICP1029	5.890	6.325	6.702	8.642
4	ICP 2933	8.290	5.067	5.875	2.168
5	ICP 3226	14.065	6.342	8.710	4.105
6	ICP 4557	4.028	4.170	4.670	2.685
7	ICP 5466	7.250	4.645	6.055	3.360
8	ICP 7118	5.287	4.727	5.560	11.172
9	ICP 8477	12.722	8.250	8.892	12.358
10	ICP 8863	5.230	4.033	5.147	11.172
11	ICP 9306	6.275	3.930	6.113	3.330
12	ICP 12764	14.765	11.110	7.612	4.490
13	ICP 14064	5.260	2.925	5.607	3.420
14	ICP 14252	4.102	4.232	5.317	3.482
15	ICP14352	4.782	6.025	6.398	2.923
16	WRGE 38	4.418	4.020	4.260	3.180
17	JKM 205	3.298	4.027	4.205	2.113
18	JKM207	3.810	3.600	3.522	2.270
19	BSMR 736	4.815	4.377	6.520	3.272
20	BRG-2	7.512	5.742	7.340	2.993
21	BDN 2010	5.462	3.783	7.508	3.138
22	LCV 10	4.468	4.295	5.255	2.605
23	LCV 40	9.058	3.655	6.622	3.838
24	H 3C	3.842	2.778	4.273	1.965
25	TTB 7	5.580	5.040	5.428	2.667
26	JKM 189	6.850	5.928	5.925	3.035
27	WRP 1	3.932	5.300	5.793	3.170
28	JS 5	4.955	4.207	4.890	2.958
29	ICP7035	4.685	4.865	6.875	4.278
30	JSA 59	3.675	3.678	5.405	3.215
31	JSA 81	3.340	3.138	4.527	2.625
32	GT 101	4.260	3.303	4.797	2.588
33	GRG 207	3.627	3.517	6.335	3.095
34	BRG 1	4.975	4.520	6.495	3.440
35	ICP96047	4.125	2.985	5.897	2.823
36	SEL20129	2.780	3.487	4.670	2.245
	MEAN	**5.856**	**4.697**	**5.855**	**4.193**
	Range	**2.78-14.765**	**2.778-11.11**	**3.522-8.892**	**1.965-12.358**
	CD	**0.470**			
	CV	**13.15%**			

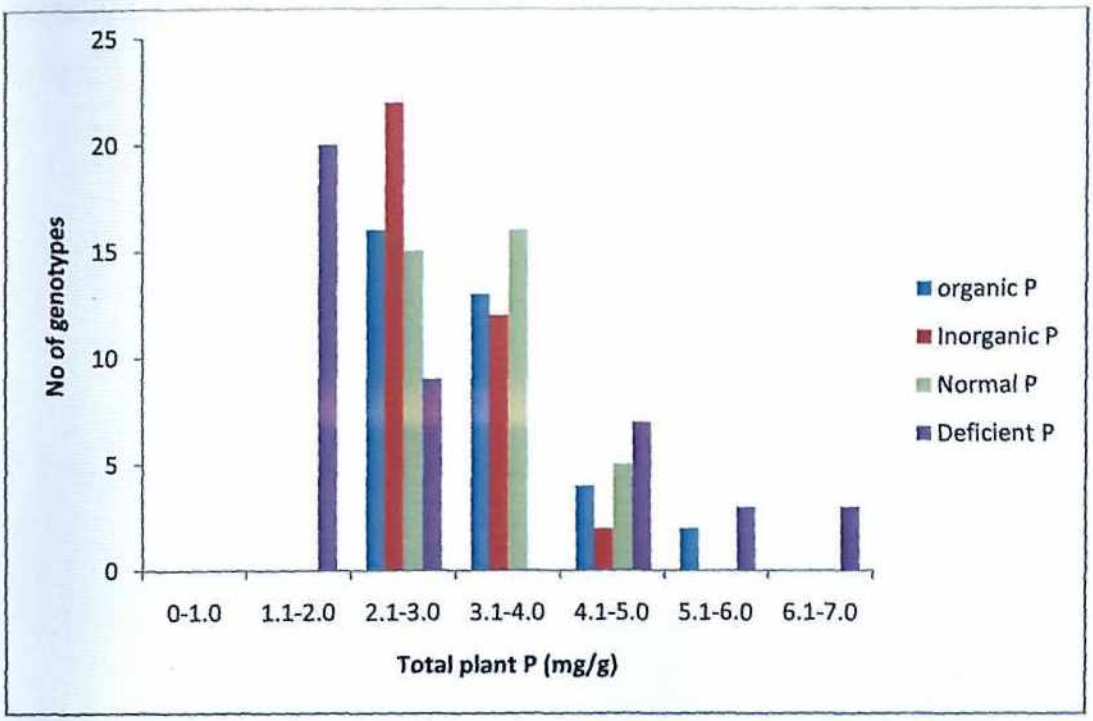


Fig 8. Frequency distribution of 36 pigeonpea genotypes for total P mg/g under different sources of P

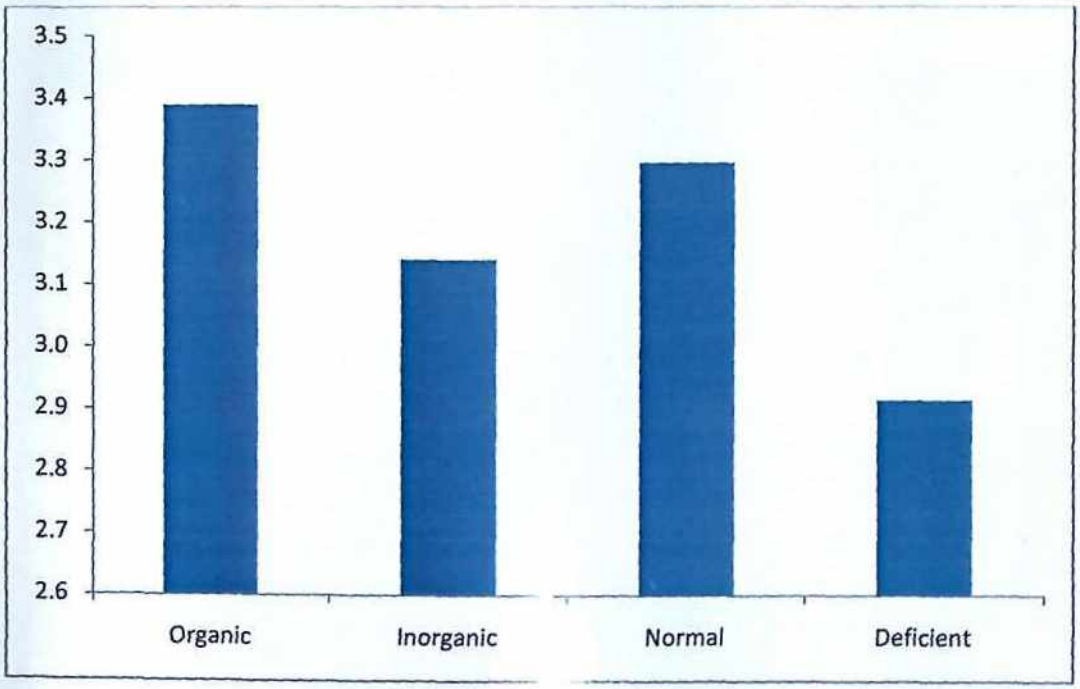


Fig 9. Effect of different sources of P on PAE 36 pigeon pea genotypes grown for 45 days

Table 12a : Total Root P mg/pt of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	1.678	0.525	0.653	6.533
2	ICP218	1.318	0.775	0.595	5.630
3	ICP1029	0.688	0.725	0.602	6.687
4	ICP 2933	2.360	0.782	0.953	0.760
5	ICP 3226	3.768	1.087	1.317	6.195
6	ICP 4557	1.140	0.920	1.120	1.003
7	ICP 5466	1.370	0.830	0.795	1.175
8	ICP 7118	0.895	0.860	1.210	8.570
9	ICP 8477	1.722	1.042	0.692	8.577
10	ICP 8863	0.843	0.652	0.867	8.595
11	ICP 9306	0.995	0.742	0.975	0.810
12	ICP 12764	3.117	1.110	1.410	1.597
13	ICP 14064	0.560	0.485	1.255	0.862
14	ICP 14252	0.802	0.913	1.333	0.825
15	ICP14352	0.718	0.582	0.872	1.225
16	WRGE 38	1.025	0.773	0.813	1.020
17	JKM 205	1.072	0.865	0.902	0.855
18	JKM207	0.452	0.380	0.830	0.728
19	BSMR 736	0.452	0.875	1.400	1.057
20	BRG-2	0.858	0.763	1.197	0.965
21	BDN 2010	0.873	0.487	1.030	0.733
22	LCV 10	0.383	0.487	0.948	0.815
23	LCV 40	1.440	0.735	0.912	1.158
24	H 3C	0.680	0.573	0.860	0.843
25	TTB 7	0.920	0.802	0.692	0.935
26	JKM 189	1.070	0.790	0.863	1.170
27	WRP 1	0.508	0.755	1.293	0.930
28	JS 5	0.500	0.400	0.697	1.170
29	ICP7035	1.242	0.740	1.210	1.352
30	JSA 59	0.410	0.835	0.955	1.345
31	JSA 81	0.495	0.610	0.698	0.815
32	GT 101	0.797	0.383	0.672	0.733
33	GRG 207	0.752	0.710	0.965	1.058
34	BRG 1	1.342	1.202	1.275	1.040
35	ICP96047	0.470	0.418	1.265	0.955
36	SEL20129	0.528	0.690	1.180	0.935
	MEAN	**1.061**	**0.731**	**0.981**	**2.086**
	Range	**0.383-3.7**	**0.38-1.202**	**0.595-1.41**	**0.728-8.595**
	CD	**0.470**			
	CV	**13.15%**			

I3C, ICP4557 and JKM205 showed low total P content of 4.1, 4.2, and 4.7 mg/plant espectively. And in normal condition ICP12764, ICP8477 and ICP3226 showed high otal p 7.9, 7.8 and 7.6and genotypes like ICP8863, ICP7035, and ICP4557 showed low otal p.and in deficient condition ICP3226 ICP8477and ICP 12764 showed high total P ;ontent of 13.02, 17.12, and 12.8.

1.1.3.6 Phosphorus acquisition efficiency PAE (mg P/g RW).

In this parameter the genotypes accumulated P acquisition which ranges from 2.063-5.75 with a ean of 3.389 in organic P source condition the genotypes like ICP 8477(5.750) ICP 3226(4.798) and ICP12764(4.997) showed more acquisition efficiency where as genotypes like ICP 7035 (2.063) ICP4557(2.067) and ICP8863(2.700) showed low acquisitions efficiency but under inorganic condition it ranges from 2.262-5.023 with a mean of 3.140 genotypes like ICP 8477(5.023) ICP 12764(2.748) and ICP3226(3.915) showed high acquisition efficiency where as H3c(2.262) JSA 81(2.630) ICP 4557(2.292) showed very low PAE and in normal condition it ranges from 2.335-4.977. With a mean of 3.297 where in genotypes ICP 8477(4.977) ICP3226 (4.610) and ICCP 12764(4.295) recorded high. Where in under deficient condition the PAE ranges from (1.292-3.448)with a mean of 2.365 here the genotypes like 8447(8.010) ICP3226(6.135 and ICP12764(6.005) showed high PAE and genotypes like ICP7035(1.292) ICP4557(1.435) and ICP8863(1.422)showed low PAE overall under organic condition PAE was more compared to other treatment.(Fig8,9,10)

4.1.3.7 Phosphorus utilization efficiency (PUE)-g TDM/ mg shoot P;

Here the pue of the plants were shown significant differences among genotypes and between the treatments also the PUE range form 0.173-0.485 under organic condition with a mean of 0.316 genotypes like ICP4557 (0.485) ICP 7035(0.453) and ICP 8863(0.483) showed high PUE where as genotypes like ICP8477 (0.173) ICP12764 (2.00) and ICP 3226(0.210) showed low PUE under this treatment. Where as in inorganic condition genotypes like ICP4557 (0.438) ICP8863 (0.375) and ICP 7035(0.447) showed high PUE where as ICP 8477(0.200) ICP3226 (0.255) and ICP12764 (0,210) showed low pue where in normal condition ICP7035 (4.17) JKM207 (0.415) and ICP 4557(0.412)

Table 13: PAE of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	4.025	3.072	4.968	3.202
2	ICP218	3.257	3.702	5.107	3.072
3	ICP1029	2.978	3.137	2.407	3.238
4	ICP 2933	4.125	3.030	1.447	2.958
5	ICP 3226	4.798	3.915	6.135	4.610
6	ICP 4557	2.067	2.292	1.435	2.425
7	ICP 5466	3.933	3.180	2.138	3.498
8	ICP 7118	3.352	3.695	2.317	3.582
9	ICP 8477	5.750	5.023	8.010	4.977
10	ICP 8863	2.700	2.680	1.422	2.725
11	ICP 9306	3.735	2.748	2.183	3.490
12	ICP 12764	4.997	4.788	6.005	4.295
13	ICP 14064	3.715	2.847	2.125	3.650
14	ICP 14252	3.663	3.668	2.055	3.775
15	ICP14352	2.835	3.457	3.448	3.373
16	WRGE 38	2.742	3.018	1.935	2.762
17	JKM 205	2.063	2.688	5.800	2.335
18	JKM207	2.387	2.475	1.590	2.460
19	BSMR 736	3.655	3.523	2.337	3.665
20	BRG-2	4.185	3.522	1.990	4.287
21	BDN 2010	3.848	3.322	2.235	4.148
22	LCV 10	2.947	3.200	1.805	2.953
23	LCV 40	5.305	2.983	2.377	3.487
24	H 3C	2.740	2.262	1.292	2.400
25	TTB 7	3.095	2.842	1.625	2.880
26	JKM 189	2.982	2.803	1.723	2.817
27	WRP 1	2.990	2.960	1.870	3.100
28	JS 5	3.115	3.002	1.292	3.070
29	ICP7035	2.213	2.752	1.990	3.263
30	JSA 59	3.002	2.980	1.930	3.055
31	JSA 81	2.827	2.630	1.625	2.682
32	GT 101	3.220	2.983	1.817	2.845
33	GRG 207	2.795	2.777	2.065	3.558
34	BRG 1	3.595	3.405	2.257	3.632
35	ICP96047	3.688	2.930	1.692	3.145
36	SEL20129	2.680	2.735	1.505	3.268
	MEAN	**3.389**	**3.140**	**2.365**	**3.297**
	Range	**2.063-5.75**	**2.262-5.023**	**1.292-3.448**	**2.335-4.977**
	CD	**0.310**			
	CV	**14.04%**			

Table 14: PUE of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	0.250	0.322	0.205	0.315
2	ICP218	0.308	0.275	0.208	0.325
3	ICP1029	0.335	0.320	0.170	0.310
4	ICP 2933	0.275	0.333	0.692	0.340
5	ICP 3226	0.210	0.255	0.440	0.220
6	ICP 4557	0.485	0.438	0.702	0.412
7	ICP 5466	0.255	0.315	0.473	0.288
8	ICP 7118	0.300	0.272	0.158	0.280
9	ICP 8477	0.173	0.200	0.127	0.203
10	ICP 8863	0.370	0.375	0.175	0.368
11	ICP 9306	0.270	0.367	0.467	0.285
12	ICP 12764	0.200	0.210	0.422	0.235
13	ICP 14064	0.267	0.350	0.477	0.273
14	ICP 14252	0.275	0.273	0.502	0.263
15	ICP14352	0.352	0.290	0.077	0.295
16	WRGE 38	0.365	0.333	0.530	0.365
17	JKM 205	0.483	0.375	0.707	0.430
18	JKM207	0.422	0.405	0.630	0.415
19	BSMR 736	0.275	0.288	0.430	0.283
20	BRG-2	0.242	0.282	0.600	0.232
21	BDN 2010	0.260	0.305	0.450	0.240
22	LCV 10	0.340	0.318	0.558	0.340
23	LCV 40	0.210	0.335	0.422	0.288
24	H 3C	0.368	0.447	0.787	0.417
25	TTB 7	0.323	0.355	0.617	0.350
26	JKM 189	0.335	0.360	0.585	0.358
27	WRP 1	0.340	0.340	0.543	0.322
28	JS 5	0.327	0.338	0.495	0.328
29	ICP7035	0.453	0.367	0.512	0.315
30	JSA 59	0.338	0.335	0.520	0.328
31	JSA 81	0.355	0.383	0.622	0.373
32	GT 101	0.318	0.338	0.552	0.355
33	GRG 207	0.360	0.365	0.485	0.282
34	BRG 1	0.280	0.295	0.443	0.275
35	ICP96047	0.275	0.347	0.593	0.322
36	SEL20129	0.375	0.365	0.670	0.310
	MEAN	**0.316**	**0.330**	**0.474**	**0.315**
	Range	**0.173-0.485**	**0.2-0.447**	**0.077-0.787**	**0.203-0.431**
	CD	**0.031**			
	CV	**13.50%**			

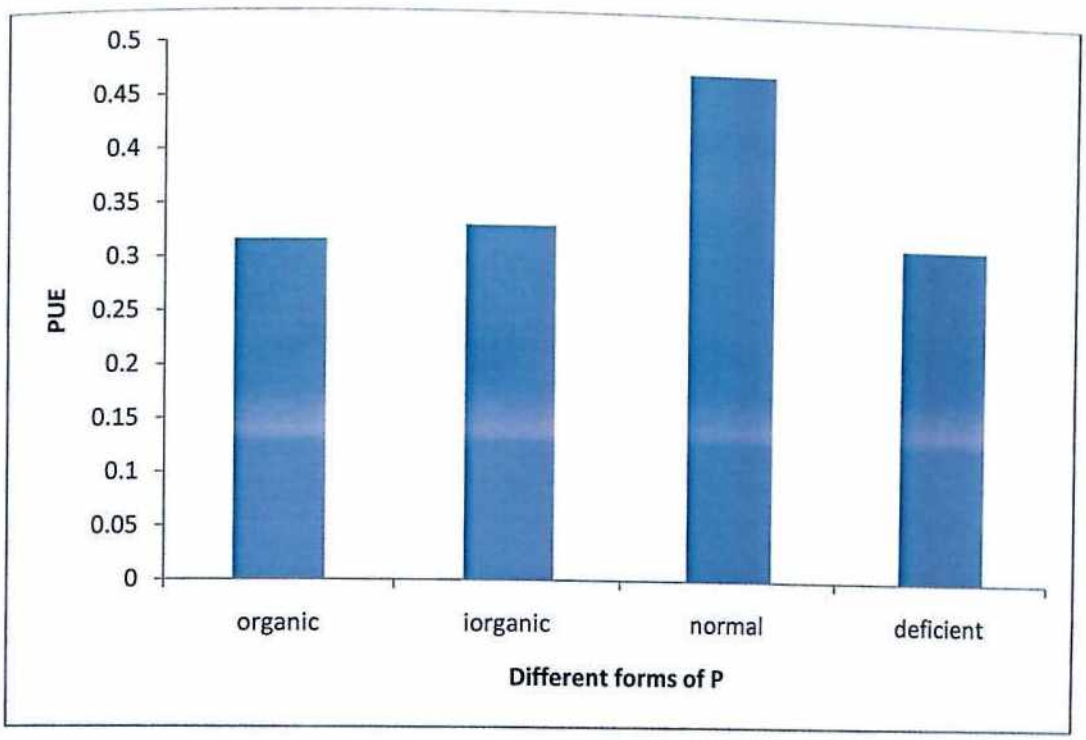


Fig 10. Effect of different sources of P on PUE 36 pigeon pea genotypes grown for 45 days

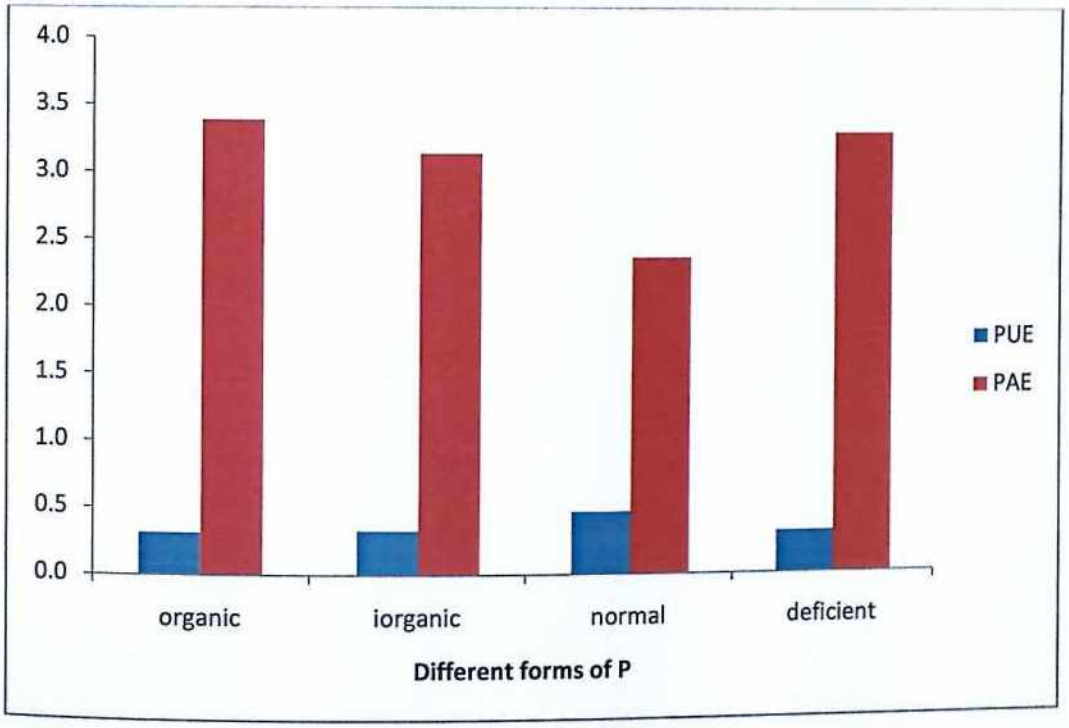


Fig11. Effect of different sources of P on PUE and PAE of 36 pigeon pea genotypes grown for 45 days

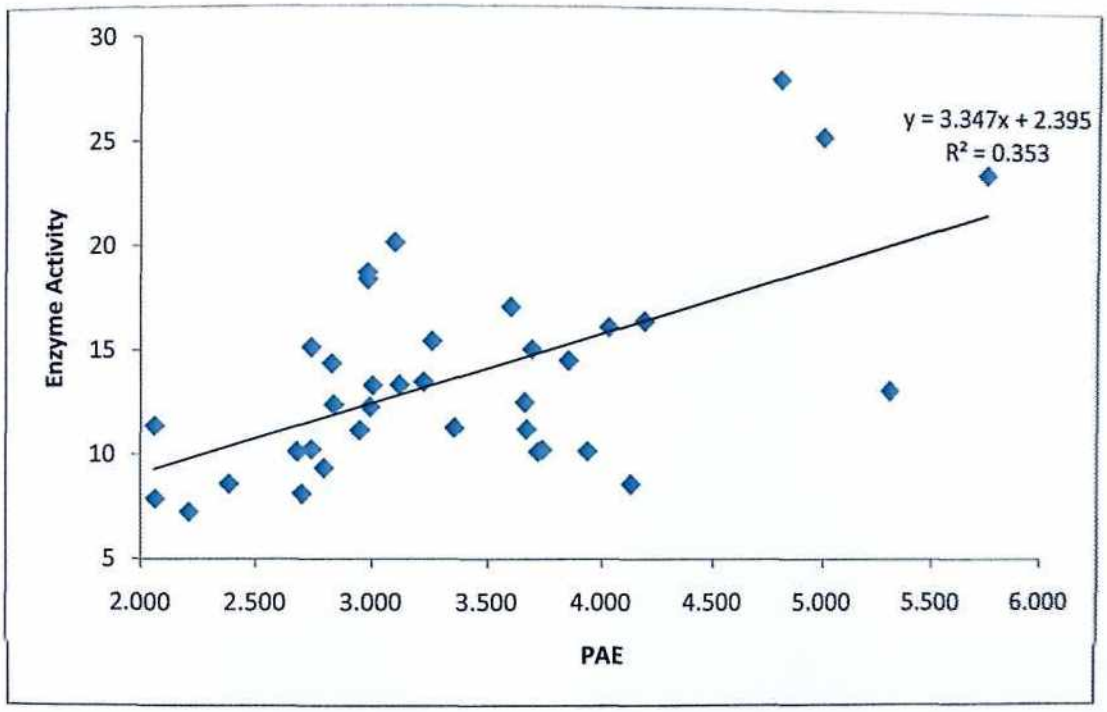


Fig12. Relationship between PAE and Enzyme activity in 36 genotypes grown for 45 days under organic condition

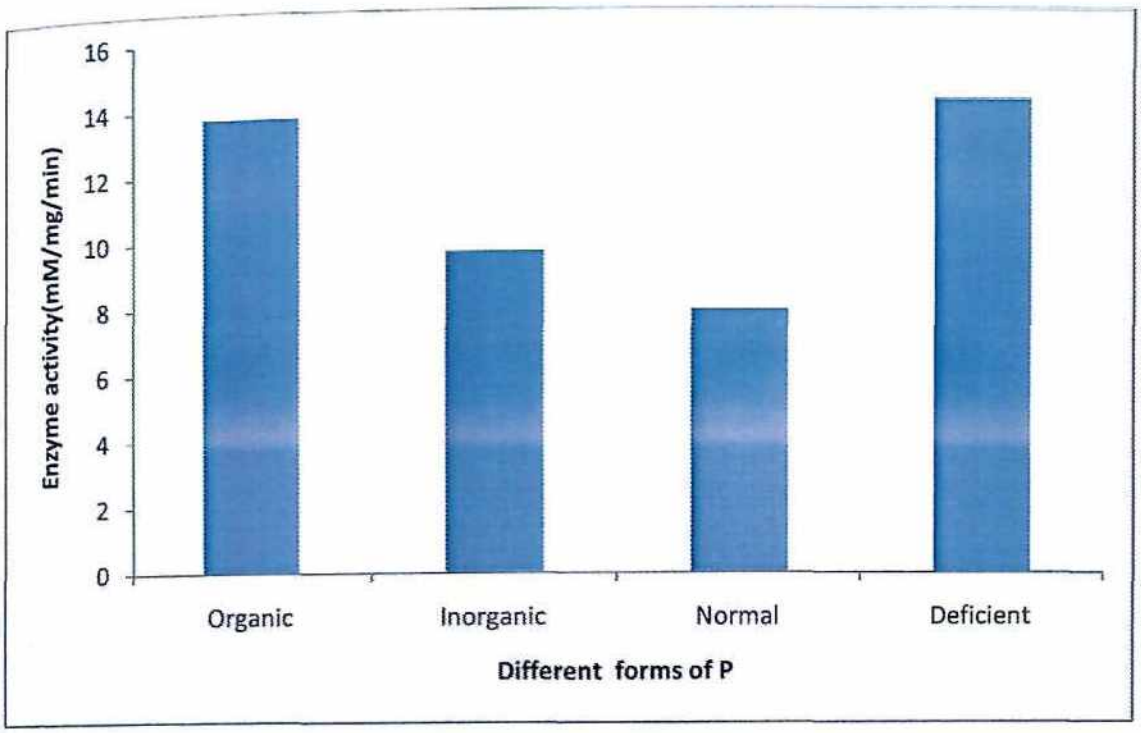


Fig. 13 Effect of different sources of P on Enzyme activity of 36 pigeon pea genotypes grown for 45 days

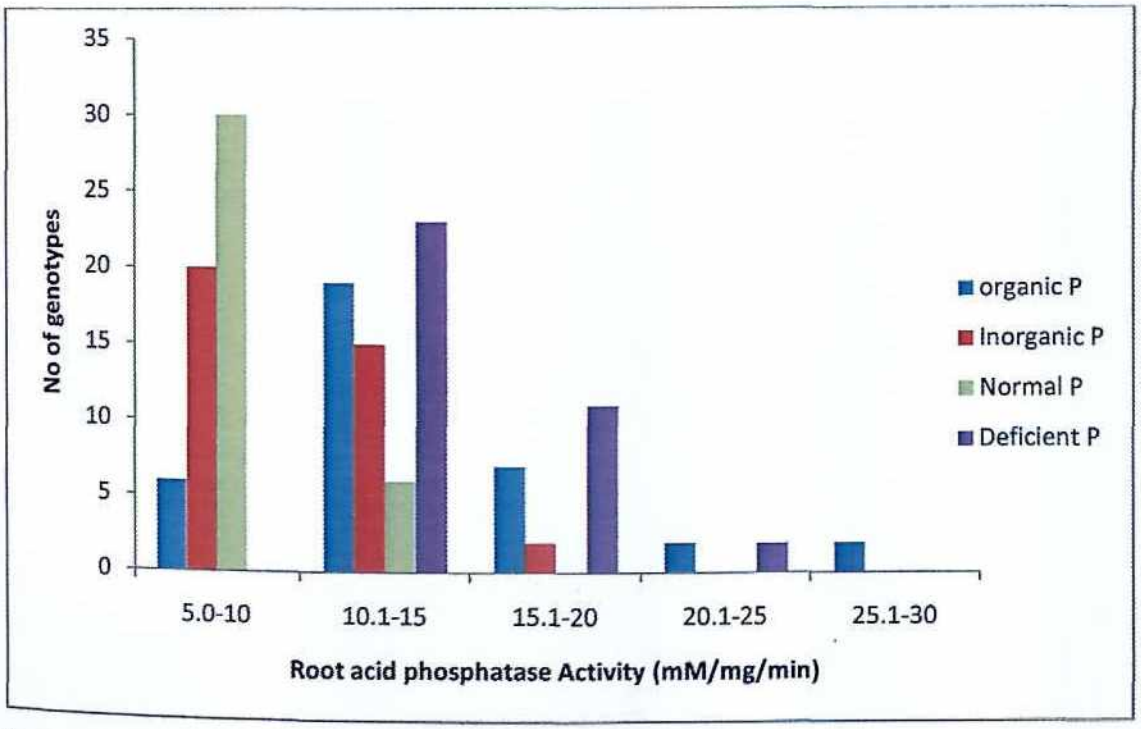


Fig 14. Frequency distribution of 36 Pigeonpea genotypes for enzyme activity under different sources of P

showed high PUE where as in genotypes like.ICP3226, ICP8477, and ICP12764 showed low acquisition both under normal and deficient condition.

4.1.3.8 Root acid phosphatase activity. (mM/mg/min)

Here the genotypes showed good response between the genotypes and treatments under deficient condition the genotypes like ICP 3226(24.452) ICP 8477(21.321) and ICP12764(18.273) showed high enzyme activity and genotypes like ICP4557(10.375) ICP8863(11.175)and ICP 7035(11.25)showed low enzyme activity where as in organic condition ICP 3226(28.072) ICP12764(25.332) and ICP 8477(23.593) showed high enzyme activity where in ICP7035(7.250) ICP8863(7.875) ICP4557(7.875) with a range of 7.25-28.072 and mean 13.744 In inorganic condition ICP3226(15.680) ICP8477(14.580) and ICP 12764(15.453) showed high enzyme activity where as genotypes like ICP 4557((5.750) ICP8863(6.075) and ICP 7035(6.320) showed low enzyme activity where as in normal condition the enzyme was low compared to other treatments here ICP12764(12.295) ICP3226(10.165) and ICP 8477(10.265) showed high enzyme activity ICP4557(5.325) ICP8863(5.750) ICP7035(5.350) showed low enzyme activity.

It is very well documented that activity of these enzymes increased under P deficient conditions to solubilise more P and help plant to acquire and utilize bound P. The acid phosphates activity increases in roots under –P condition. However, our results indicate a significant increase in roots under P deficiency in all the genotypes but the increase was higher in efficient genotypes.

Frequency distribution in fig.12 indicates under organic P condition all the genotypes having enzyme activity in the range of 10-30 mM/mg/min whereas under inorganic P condition about 60% of genotypes have the enzyme activity in the range of 5-18mM/mg/min. Under normal conditions, 80% genotypes show enzyme activity in the range of 5-14mM/mg/min. Under P deficient maximum number of genotypes have the enzyme activity from 10-28mM/mg/min.

Table 15 : Enzyme Activity of 36 Genotypes Grown under Different Source of Phosphorus

Sl No		Organic	Inorganic	Normal	Deficient
1	ICP 40	8.125	12.370	9.393	15.305
2	ICP218	15.433	5.750	5.750	14.193
3	ICP1029	18.705	7.325	10.300	12.000
4	ICP 2933	8.607	10.233	6.303	11.813
5	ICP 3226	28.072	15.680	10.165	24.452
6	ICP 4557	7.875	5.750	5.325	10.375
7	ICP 5466	10.190	9.072	6.197	16.367
8	ICP 7118	11.295	8.542	7.215	13.305
9	ICP 8477	23.593	14.580	10.265	21.321
10	ICP 8863	8.607	6.075	5.750	11.175
11	ICP 9306	10.252	9.320	8.250	13.285
12	ICP 12764	25.332	15.453	12.295	18.237
13	ICP 14064	10.162	8.560	6.235	13.308
14	ICP 14252	11.233	7.520	8.473	15.497
15	ICP14352	12.393	10.117	8.277	16.285
16	WRGE 38	10.258	8.467	7.440	13.548
17	JKM 205	11.375	7.425	5.288	14.290
18	JKM207	8.585	6.470	5.437	12.443
19	BSMR 736	12.513	8.445	6.380	12.588
20	BRG-2	16.420	10.320	8.332	18.420
21	BDN 2010	14.510	10.363	8.280	16.382
22	LCV 10	11.168	8.248	8.423	13.317
23	LCV 40	13.142	8.235	6.240	12.385
24	H 3C	15.133	10.295	8.328	12.112
25	TTB 7	20.140	12.002	10.210	15.233
26	JKM 189	18.382	10.155	8.293	13.483
27	WRP 1	12.285	10.185	7.680	12.440
28	JS 5	13.332	10.160	8.175	13.257
29	ICP7035	7.250	6.320	5.350	11.250
30	JSA 59	13.308	10.283	7.165	14.438
31	JSA 81	14.358	10.310	8.468	14.440
32	GT 101	13.483	9.287	7.270	15.307
33	GRG 207	9.350	7.805	8.080	13.210
34	BRG 1	17.082	12.408	10.185	15.377
35	ICP96047	15.055	10.260	8.210	16.307
36	SEL20129	10.133	8.138	7.795	13.217
	MEAN	13.744	9.674	7.928	14.247
	Range	7.25-28.072	5.75-15.68	5.288-12.295	10.335-24.452
	CD	0.494			
	CV	6.24%			

Table 16 : Mean and range of different growth parameters of 36 pigeon pea genotypes grown with different forms of phosphorus

Sl No	Growth Parameters	Range				Mean				CD@ P=0.01
		Organic	Inorganic	Normal	Deficient	Organic	Inorganic	Normal	Deficient	
1	Shoot length (cm)	15.750-8.500	15.25021.750	20.25031.250	12.75019.5	20.57	18.75	25.181	15.465	0.510
3	Shoot drt wt(gm)	0.683-2.288	0.67-1.875	0.867-1.637	0.690-1.233	1.183	1.043	1.252	0.838	0.049
4	Root volume(cm3)	0.275-2	0.3-1.05	0.475-1.1	0.8-2.05	0.853	0.638	0.892	1.595	0.066
5	Root dry wt(g)	0.242-0.968	0.242-0.592	0.432-0.638	0.62-1.018	0.503	0.439	0.525	0.769	0.022
6	Root length(cm)	18.25-31.25	17-27.5	12.75-20.05	21.25-35.5	24.660	21.625	17.146	28.882	0.567
7	R/S ratio	0.205-0.672	0.215-0.827	0.265-0.625	0.505-1.225	0.435	0.448	0.428	0.941	0.538
8	TDM (g)	1.04-2.95	1.02-2.32	1.41-2.11	1.22-2.148	1.686	1.482	1.777	1.569	0.049
9	Leaf P (%)	0.232-0668	0252-0.615	0.257-0.66	0.147-0.435	0.395	0.379	0.391	0.251	0.022
10	Root P (%)	0.125-0.58	0.122-0.468	0.128-0.267	0.105-1.278	0.204	0.346	0.186	0.284	0.748
11	Root P (mg/pl)	0.383-3.7	0.38-1.2	0.59-1.4	0.72-8.59	1.061	0.731	0.981	2.086	0.470
12	Shoot P (mg/pl)	2.22-13.31	2.20-10	2.69-8.2	1.122-3.78	4.79	3.966	4.875	2.108	0.309
13	Total P (mg/pl)	3.937-10.788	4.15-8.375	4.275-7.925	2.57517.125	5.984	5.463	5.769	5.343	0.654
14	PUE (mg P/gRW)	0.173-0.48	0.2-0.44	0.2-0.43	0.170-0.787	0.316	0.330	0.474	0.315	0.031
15	PAE(gTDM/mg shoot P)	2.063-5.75	2.262-5.023	2.335-4.977	1.29213.448	3.389	3.140	2.915	3.297	0.310
16	Enzyme activity	7.25-28.072	5.75-15.68	5.288-12.295	10.33524.452	13.744	9.674	7.928	14.247	0.494

Relationships.

There was a good correlation between TDM and shoot P content (Fig. 6a). It indicates the contribution of shoot P towards accumulation of biomass in pigeon pea genotypes under different P sources (r=0.185). Fig.9 indicates a good relationship between root dry weight and total plant P of 36 pigeon pea genotypes under different P sources. This signifies role of root dry weight in acquiring P and translocating to shoot under P deficiency (r^2=0.202).(Fig15,16,17,18) indicates a significant relationship between Leaf P% and Enzyme activity this shows the role of enzyme activity in leaf P% under different source of P.

4.1.4 Selection of contrasting genotypes with respect to leaf P (%) and root acid phosphatase activity

Based on the results obtained on leaf P (per cent), and enzyme activity under different P sources and the relationship observed between shoot P, TDM, root dry weight and total plant P (from the first experiment), standardized normal distribution plot was performed (Z-plot) between leaf P% and root acid phosphates activity .the contrasting genotypes were selected having low leaf P% and low enzyme activity and high leaf P% and high enzyme activity.

Since the shoot P content is a derived value from leaf P content. The leaf P (%) content was considered for selecting the genotypes. Hence based on leaf P (per cent) content and root acid phosphatase activity, three genotypes having high p content and high acid phosphatase activity and low p content and low acidphosphatae activity was selected and they are classified into following categories.

High leaf P (per cent) types and high acid phosphates activity.

1. ICP 3226
2. ICP 8477
3. ICP 2764

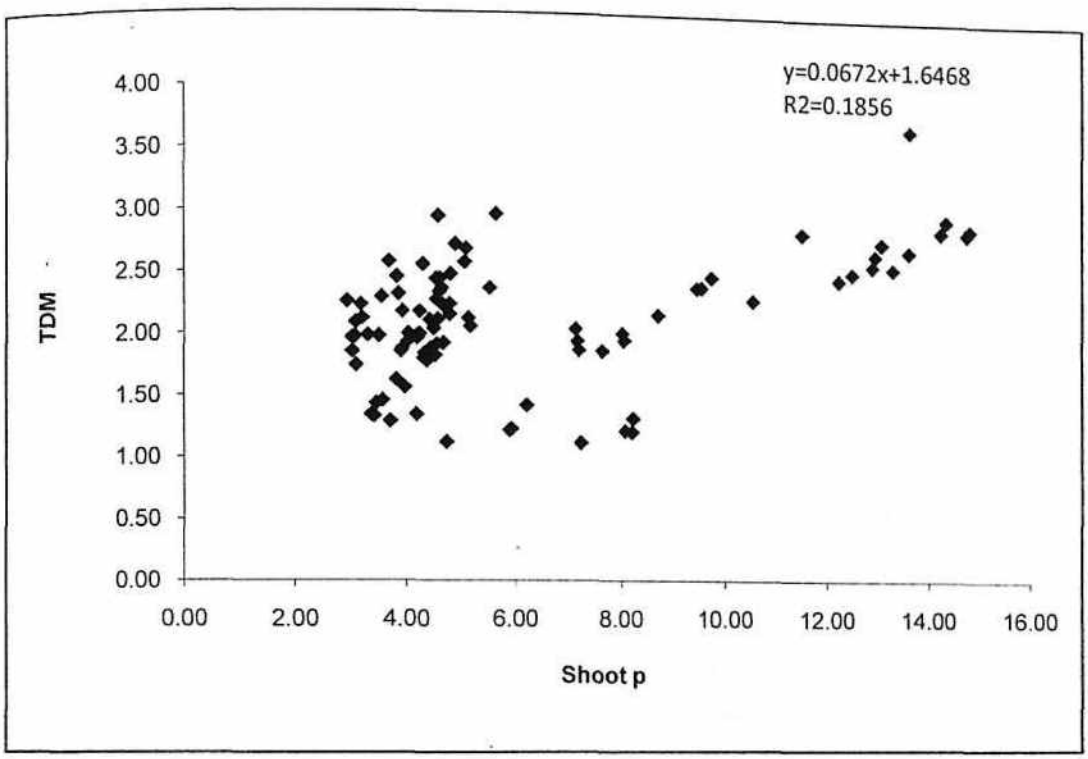


Fig 6a. Relationship between TDM and Shoot P content of 36 genotypes grown for 45days under different sources of P

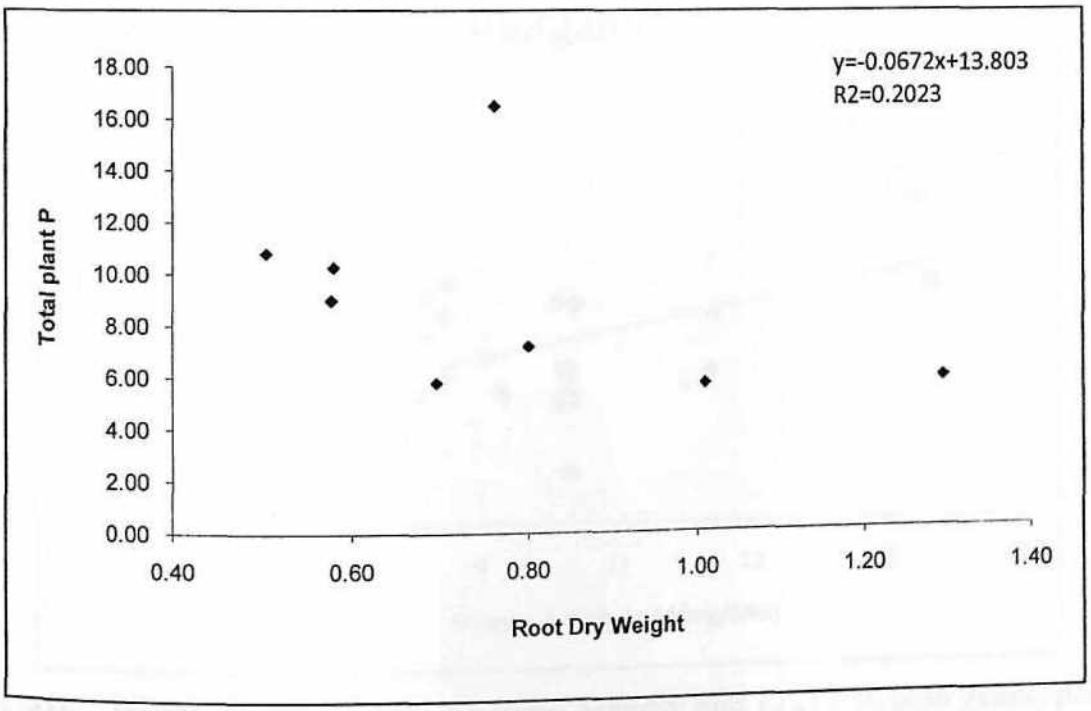


Fig 6b. Relationship between total plant P and root dry weight of36 genotypes grown for 45 days under different P sources

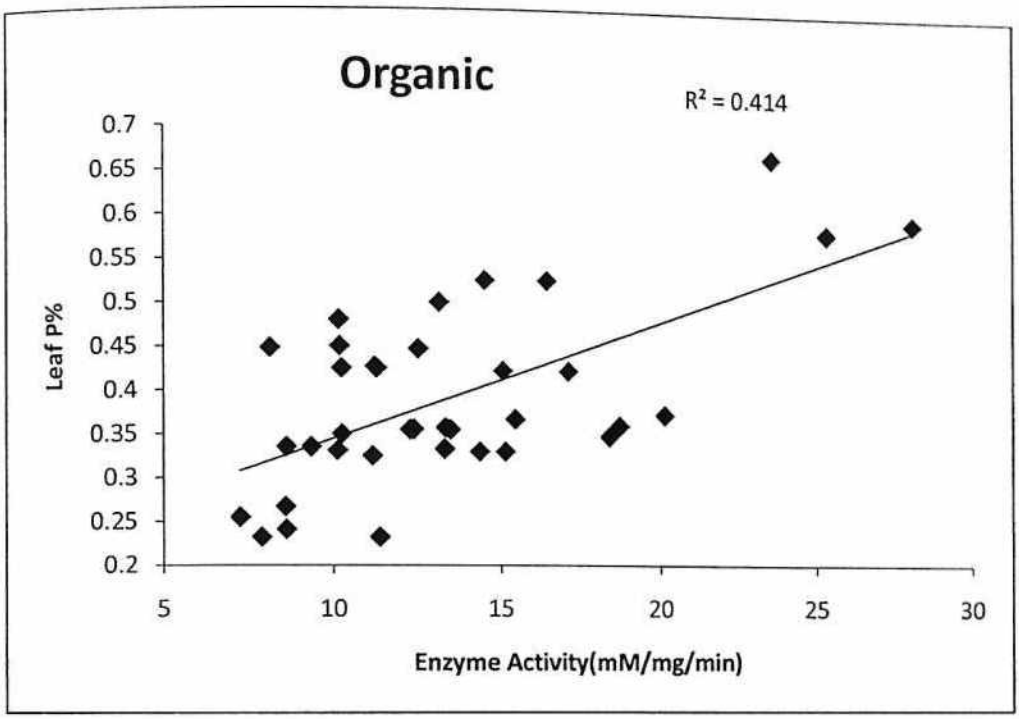


Fig15. Relationship between Enzyme Activity and Leaf P% of36 genotypes grown for 45 days under Organic P sources

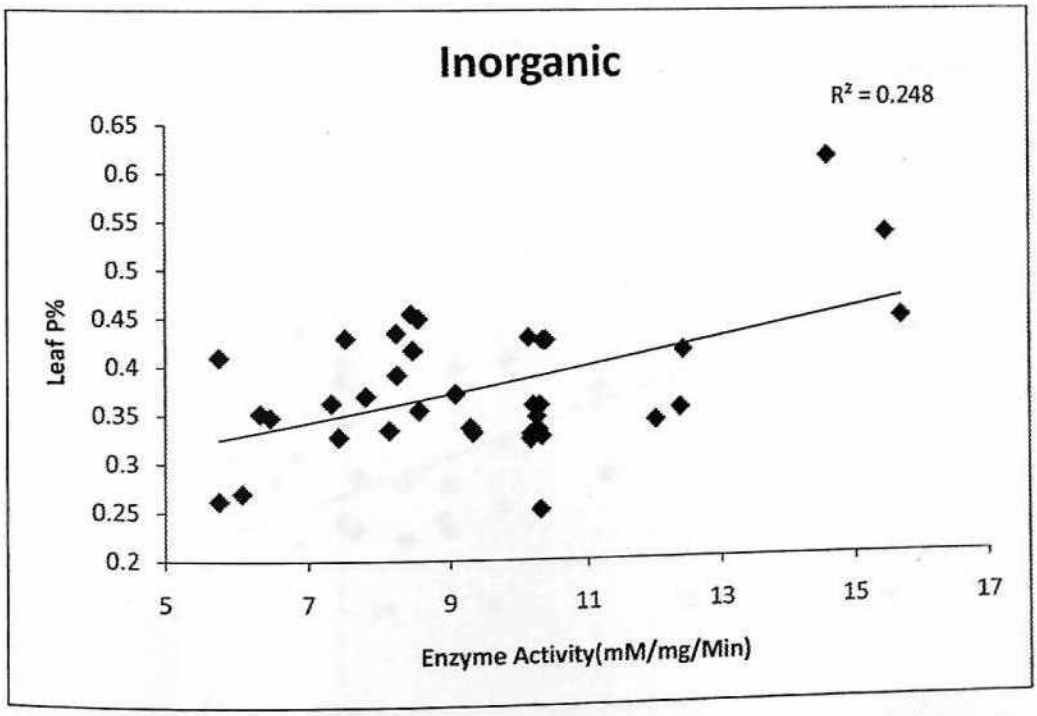


Fig16. Relationship between Enzyme Activity and Leaf P% of36 genotypes grown for 45 days under Inorganic P sources

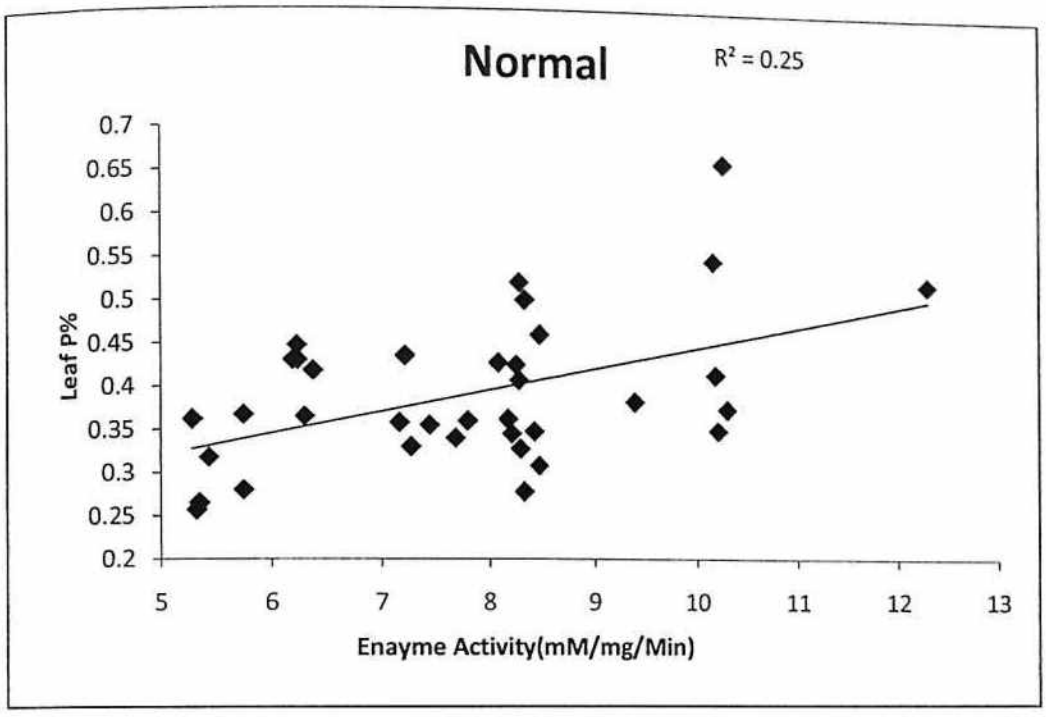


Fig17. Relationship between Enzyme Activity and Leaf P% of36 genotypes grown for 45 days under Normal P sources

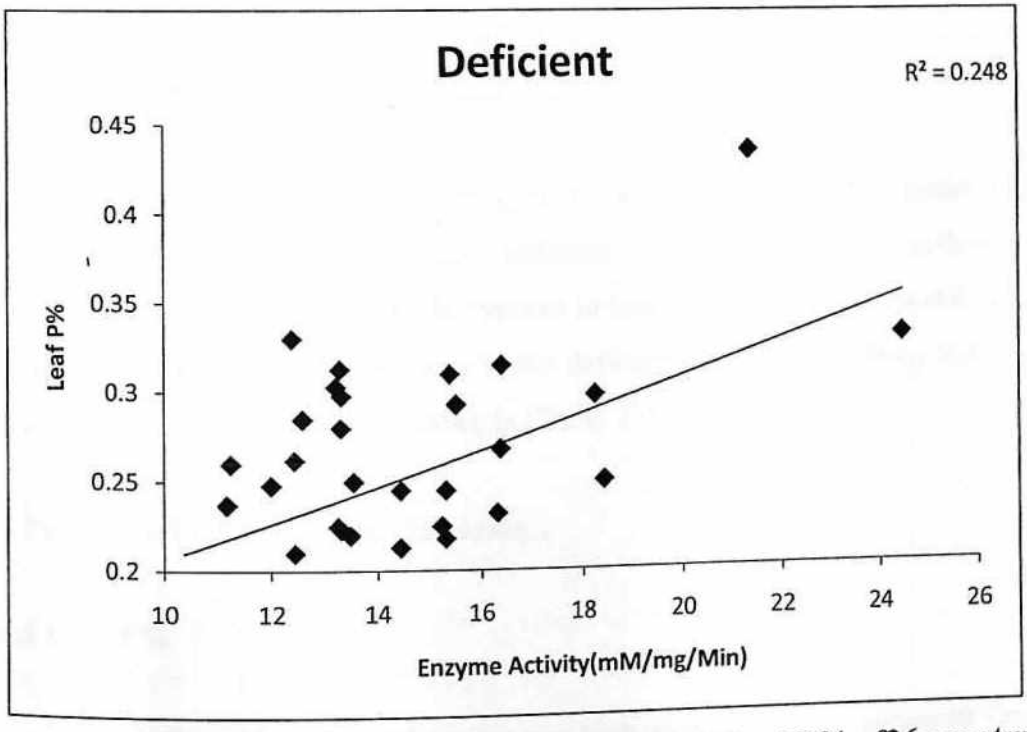


Fig18. Relationship between Enzyme Activity and Leaf P% of36 genotypes grown for 45 days under Deficient P sources

Low leaf P (per cent) types and low acid phosphates activity.

1. ICP 7025

2. ICP 4557

3. ICP 8863

These identified contrasting genotypes were used to examine the effect of P deficiency on root growth characteristics and biochemical mechanisms adapted by high and low P uptake types. For the clarity and reference, the growth characteristics and P related traits of selected contrasting genotypes have been provided in Tables in 19A, B and C.

High P uptake genotypes showed high TDM compared to low P uptake once under all treatments and TDM (2.694) was high under normal condition, and it was recorded low under deficient condition followed by organic and inorganic condition, in high P types. In low P types the TDM was high under normal condition compared to other treatments, the TDM was low in deficient condition followed by organic and inorganic condition. In table 20,21,22, and fig2.

And also high P uptake genotypes showed high leaf P% under organic condition(0.665%) and low under deficient condition(0.454%) followed by normal(0.578) and inorganic(0.542%) were as in low P uptake types showed high leaf P under normal condition (0.349)and low under deficient condition (0.249%) followed by organic(0.334%) and inorganic(0.306%), in (Table 23)

4.4.4 Plant P and its distribution in plants.

4.4.4.1 Root P%

The root P% of high P uptake types was high under organic condition (0.376) and low under deficient condition(0.254) followed by inorganic (0.268) and normal(0.318),among high P uptake types ICP3226 accumulated more leaf P%

compared to other two types and ICP 12764 accumulated low leaf p%. under low P types the root P was more under normal condition(0.423) and less under deficient Condit (0.267) followed by inorganic(0.282) and organic(0.305) amount these genotypes ICP4557 accumulated more leaf P% and ICP7025 accumulated less(0.217).(Table 25).

4.4.4.2 Leaf P.

The high P uptake genotypes showed high leaf P% under organic condition(0.665%) and low under deficient condition(0.454%) followed by normal(0.578) and inorganic(0.542%) were as in low P uptake types showed high leaf P under normal condition (0.349)and low under deficient condition (0.249%) followed by organic(0.334%) and inorganic(0.306%), in (Table 23)

Phosphorus acquisition efficiency was high in high P uptake types compared to low P uptake types it was high under organic condition (19.892) and recorded low under deficient condition (6.609). There was not much difference observed under low P types and it was less under deficient condition.

Phosphorus utilization efficiency was high in low P uptake types under inorganic condition (0.342) and under normal (0.324). It was low in deficient condition (0.265) but in high P uptake types it was low compared to low p types under normal condition (0.146) followed by organic and inorganic.

Enzyme activity was more under high P uptake types under organic condition (29.316) followed by deficient condition (22.183) it was low under control condition (11.573) here ICP3226 recorded high enzyme activity 30.892 under organic condition. Under low P uptake types the enzyme activity was less, in this condition the activity was more under organic condition (17.653) followed by deficient condition (17.226), it was less under inorganic condition.(Table34)

Shoot dry weight of the high P uptake types was more compared to low P uptake types. In high P uptake types there was good difference between different source of P the

17. **Root volume of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus**

H		Organic	Inorganic	Normal	Deficient	Mean
	ICP3226	3.050	2.475	2.125	3.525	2.794
	ICP8477	3.075	2.150	2.000	3.375	2.650
	ICP12764	3.075	2.025	2.100	3.225	2.606
	MEAN	**3.067**	**2.217**	**2.075**	**3.375**	
L	ICP4557	2.025	2.175	1.195	3.025	2.300
	ICP8863	2.025	2.025	1.525	3.200	2.195
	ICP7025	2.000	2.100	2.100	3.050	2.312
	MEAN	**2.017**	**2.100**	**1.867**	**3.092**	
	CV=4.75	CD@0.05 A=0.012 B=0.096 AB=0.1669				

18. **Shoot length (cm) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus**

H		Organic	Inorganic	Normal	Deficient	Mean
	ICP3226	30.500	25.250	32.500	20.500	27.188
	ICP8477	31.750	25.500	33.000	20.750	27.750
	ICP12764	34.000	24.500	31.750	25.000	28.813
	MEAN	**32.083**	**25.083**	**32.417**	**22.083**	**27.917**
L	ICP4557	21.750	22.500	25.250	23.250	23.188
	ICP8863	22.250	23.000	25.000	22.750	23.250
	ICP7025	21.500	18.500	24.250	20.750	21.250
	MEAN	**21.833**	**21.333**	**24.833**	**22.250**	**22.563**
	CV=4.75%	CD@0.05 A=0.254 B=0.1673 AB=1.686				

19. Root length (cm) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

H		Organic	Inorganic	Normal	Deficient	Mean
	ICP3226	36.750	26.000	25.250	32.750	30.188
	ICP8477	38.750	25.250	29.000	34.000	31.750
	ICP12764	36.250	25.000	25.750	33.250	30.063
	MEAN	**37.250**	**25.417**	**26.667**	**33.333**	**30.667**
L	ICP4557	26.000	23.250	22.500	27.500	24.813
	ICP8863	25.250	21.750	24.500	26.000	24.375
	ICP7025	24.000	20.500	24.250	26.500	23.813
	MEAN	**25.083**	**21.833**	**23.750**	**26.667**	**24.334**
	CV=3.36%	CD@0.05 A=0.0246 B=0.154 AB=1.304				

20: Shoot dry wt (g) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

H		Organic	Inorganic	Normal	Deficient	Mean
	ICP3226	2.005	1.737	1.218	0.880	1.460
	ICP8477	1.870	1.353	1.280	0.877	1.345
	ICP12764	2.245	1.728	1.248	1.248	1.617
	MEAN	**2.040**	**1.606**	**1.249**	**1.002**	**1.474**
L	ICP4557	1.318	1.250	1.325	1.085	1.244
	ICP8863	1.328	1.265	1.517	1.295	1.351
	ICP7025	1.530	1.288	1.355	1.352	1.381
	MEAN	**1.392**	**1.268**	**1.399**	**1.244**	**1.325**
	CV=7.87%	CD@0.05 A=0.165 B=0.124 AB=1.1545				

21: Root dry wt (g) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

H		Organic	Inorganic	Normal	Deficient	Mean
	ICP3226	0.957	0.572	0.503	1.163	0.799
	ICP8477	0.663	0.567	0.535	0.935	0.675
	ICP12764	0.658	0.593	0.502	0.928	0.670
	MEAN	**0.759**	**0.577**	**0.513**	**1.009**	**0.715**
L	ICP4557	0.932	0.663	0.630	1.203	0.857
	ICP8863	0.722	0.635	0.573	1.303	0.808
	ICP7025	0.885	0.788	0.525	1.377	0.894
	MEAN	**0.846**	**0.695**	**0.576**	**1.294**	**0.853**
	CV=16.36	CD@0.05 A=0.021 B=0.014 AB=0.1030				

22. Total biomass dry wt (g) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	2.963	2.310	2.530	1.387	2.260
	ICP8477	2.537	1.925	2.820	1.410	2.023
	ICP12764	2.903	2.325	2.733	1.750	2.289
	MEAN	**2.801**	**2.187**	**2.694**	**1.516**	**2.191**
L	ICP4557	2.252	1.917	2.380	1.715	2.104
	ICP8863	2.055	1.905	2.220	1.868	2.162
	ICP7025	2.415	2.077	2.180	1.882	2.277
	MEAN	**2.241**	**1.966**	**2.260**	**1.822**	**2.181**
	CV=8.05	CD@0.05 A=0.011 B=0.010 AB=0.143				

shoot dry weight was more under organic condition(2.040g) followed by inorganic (1.606g) and normal (1.249g) the shoot dry weight was low under deficient condition (1.002g) compared to other forms. In low P uptake types there was not much difference organic (1.392g), inorganic (1.268g), normal (1.399g) and deficient (1.244g). (Table20 Fig3b)

Root dry weight of the selected genotypes was more under deficient condition compared to other forms. The dry weight was more under deficient condition (1.009g) followed by organic (0.759g) inorganic (0.577g) and normal (0.513g). Under low P uptake types also showed the same response as high P types it was high in deficient condition (1.294g) followed by organic (0.846g) inorganic (0.695g) and normal (0.576g) (Table21 Fig4b)

Total dry matter (TDM) of high P uptake types was more under organic condition (2.801g) followed by normal (2.694g) inorganic (2.187g) and deficient condition (1.516g), under low P uptake types tdm was more under normal condition (2.260g) followed by organic (2.241g) inorganic (1.966g) the was less tdm under deficient condition (1.822g). (Table22 Fig5b)

Root volume of the selected genotypes was high under high P uptake types under deficient condition (3.375 cm3) and in organic condition it was (3.067) followed by inorganic and normal condition. Under low P uptake types root volume was more under deficient (3.092 cm3) it was least in normal condition (1.867cm3). (Table 17)

Root length of the selected genotypes was more in high P uptake types compared to low P uptake types root length was more under organic condition (37.250cm) followed by deficient condition (33.33cm) and it was observed less under inorganic condition (25.417), in low P uptake types root length was more under deficient condition (26.667cm), and in organic condition (25.083cm), over all ICP8477 (38.750) recorded more root weight and ICP7025 (20.5) recorded low root length.

23: Leaf P (%) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Defecient	Mean
H	ICP3226	0.668	0.558	0.653	0.512	0.598
	ICP8477	0.680	0.552	0.625	0.500	0.589
	ICP12764	0.647	0.517	0.455	0.350	0.492
	MEAN	**0.665**	**0.542**	**0.578**	**0.454**	**0.560**
L	ICP4557	0.370	0.320	0.358	0.277	0.331
	ICP8863	0.352	0.348	0.365	0.245	0.327
	ICP7025	0.280	0.250	0.325	0.225	0.271
	MEAN	**0.334**	**0.306**	**0.349**	**0.249**	**0.310**
	Grand Mean	0.500	0.424	0.402	0.414	0.435
	CV=5.22%	CD@0.05 A=0.021 B=0.010 AB=0.044				

24: Shoot P (%) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	0.568	0.510	0.465	0.325	0.467
	ICP8477	0.440	0.320	0.453	0.335	0.387
	ICP12764	0.453	0.330	0.270	0.303	0.339
	MEAN	**0.487**	**0.387**	**0.396**	**0.321**	**0.398**
L	ICP4557	0.325	0.242	0.330	0.250	0.287
	ICP8863	0.273	0.232	0.275	0.207	0.247
	ICP7025	0.392	0.255	0.247	0.247	0.286
	MEAN	**0.330**	**0.243**	**0.284**	**0.284**	**0.273**
	GRAND MEAN	**0.409**	**0.315**	**0.316**	**0.303**	**0.336**
	CV=6.98%	CD@0.05 A=0.0124 B=0.0121 AB=0.0446				

25. Root P (%) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	0.440	0.322	0.365	0.225	0.338
	ICP8477	0.340	0.240	0.318	0.250	0.287
	ICP12764	0.348	0.242	0.270	0.288	0.287
	MEAN	**0.376**	**0.268**	**0.318**	**0.254**	**0.304**
L	ICP4557	0.240	0.235	0.335	0.205	0.254
	ICP8863	0.322	0.337	0.420	0.232	0.328
	ICP7025	0.352	0.273	0.515	0.365	0.376
	MEAN	**0.305**	**0.282**	**0.423**	**0.267**	**0.319**
	CV=7.07%	CD@0.05 A=0.0112 B=0.0312 AB=0.0141				

26: Root P (mg/plant) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	4.113	2.145	4.375	1.425	3.014
	ICP8477	2.460	1.535	4.155	1.445	2.399
	ICP12764	3.065	1.925	3.717	1.502	2.553
	MEAN	**3.213**	**1.868**	**4.083**	**1.458**	
L	ICP4557	2.327	1.348	3.837	1.035	2.137
	ICP8863	2.158	1.923	3.933	1.250	2.316
	ICP7025	2.323	1.607	4.780	1.827	2.634
	MEAN	**2.269**	**1.626**	**4.183**	**1.371**	
	CV=14.42	CD@0.05 A=0.103 B=0.142 AB=0.510				

27: Shoot P (mg/plt) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient
H	ICP3226	13.315	9.703	7.930	4.512
	ICP8477	12.725	7.498	8.025	4.382
	ICP12764	14.540	8.968	5.680	4.373
	MEAN	**13.527**	**8.723**	**7.212**	**4.422**
L	ICP4557	4.873	4.015	3.665	3.878
	ICP8863	4.693	4.392	3.700	4.708
	ICP7025	4.308	3.197	3.045	4.425
	MEAN	**4.624**	**3.868**	**3.470**	**4.337**
	CD@0.05=0.8417				
	CV=9.51	CD@0.05 A=0.312 B=0.231 AB=0.8417			

28: Total plant P (mg/plt) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient
H	ICP3226	17.427	11.848	9.352	8.890
	ICP8477	15.190	9.033	9.467	8.537
	ICP12764	17.605	10.900	7.180	8.088
	MEAN	**16.741**	**10.593**	**8.667**	**8.505**
L	ICP4557	7.195	5.360	4.698	7.717
	ICP8863	6.850	6.313	4.953	8.642
	ICP7025	6.625	4.803	4.873	9.200
	MEAN	**6.890**	**5.492**	**4.841**	**8.520**
	CV=7.27	CD@0.05 A=0.213 B=0.324 AB=0.899			

29. **Root P (mg/g) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus**

		Organic	Inorganic	Normal	Deficient
H	ICP3226	4.403	3.235	3.348	2.040
	ICP8477	3.413	2.415	4.203	2.347
	ICP12764	3.458	2.442	5.155	3.650
	MEAN	3.758	2.698	3.174	2.537
L	ICP4557	2.417	2.357	3.637	2.255
	ICP8863	3.245	3.375	3.183	2.507
	ICP7025	3.525	2.710	2.702	2.850
	MEAN	3.062	2.814	4.235	2.679
		CD@0.05 A=0.0121 B=0.0140			
	CV=6.73	AB=0.295			

30. **Shoot P (mg/g) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus**

H	I	Organic	Inorganic	Normal	Deficient	Mean
	CP3226	6.648	5.590	6.520	5.133	5.973
	ICP8477	6.803	5.540	6.258	5.018	5.904
	ICP12764	6.485	5.178	4.550	3.500	4.928
	MEAN	**6.645**	**5.436**	**5.776**	**4.550**	
L	ICP4557	3.695	3.208	2.762	3.560	3.306
	ICP8863	3.525	3.467	2.438	3.635	3.266
	ICP7025	2.805	2.488	2.252	3.240	2.698
	MEAN	**3.342**	**3.054**	**2.486**	**3.478**	
	CV=5.04	CD@0.05 A=0.0141 B=0.0212 AB=0.309				

31.Total P (mg/g) of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

H		Organic	Inorganic	Normal	Deficient	Mean
	ICP3226	11.050	8.825	8.775	8.770	9.355
	ICP8477	10.215	7.955	8.765	8.200	8.784
	ICP12764	9.943	7.620	7.400	6.203	7.791
	MEAN	**10.403**	**8.133**	**8.313**	**7.724**	
L	ICP4557	6.112	5.565	4.803	6.907	5.847
	ICP8863	6.770	6.843	4.785	7.837	6.559
	ICP7025	6.330	5.198	5.908	8.395	6.457
	MEAN	**6.404**	**5.868**	**5.165**	**7.713**	
	CD@0.05=0.4438					
	CV=4.21	CD@0.05 A=0.101 B=0.121 AB=0.443				

32. P A E of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	18.660	17.868	.14.823	7.390	14.685
	ICP8477	21.120	14.205	16.950	6.555	14.708
	ICP12764	19.898	14.065	13.665	5.883	13.377
	MEAN	**19.892**	**15.379**	**15.146**	**6.609**	
L	ICP4557	8.402	9.485	9.428	6.875	8.548
	ICP8863	10.322	11.130	9.310	9.235	9.999
	ICP7025	10.033	8.102	9.995	9.935	9.516
	MEAN	**9.586**	**9.572**	**9.578**	**8.682**	
	CV=12.47	CD@0.05 A=0.210 B=0.421 AB=2.076				

33. P U E of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	0.168	0.202	0.130	0.238	0.184
	ICP8477	0.173	0.220	0.132	0.255	0.195
	ICP12764	0.178	0.235	0.175	0.327	0.229
	MEAN	**0.173**	**0.219**	**0.146**	**0.273**	
L	ICP4557	0.315	0.340	0.285	0.292	0.308
	ICP8863	0.290	0.292	0.307	0.255	0.286
	ICP7025	0.333	0.392	0.380	0.247	0.338
	MEAN	**0.313**	**0.342**	**0.324**	**0.265**	
	CD@0.05=0.044					
	CV=5.75	CD@0.05 A=0.003 B=0.001 AB=0.044				

34. Enzyme activity of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	30.892	15.358	10.502	28.370	21.281
	ICP8477	29.000	15.137	13.608	26.090	20.959
	ICP12764	28.057	14.358	10.610	12.088	16.278
	MEAN	**29.316**	**14.951**	**11.573**	**22.183**	**19.506**
L	ICP4557	19.385	11.672	14.280	17.582	15.730
	ICP8863	17.898	12.760	12.602	16.132	14.848
	ICP7025	15.675	11.352	10.977	17.963	13.992
	MEAN	**17.653**	**11.928**	**12.620**	**17.226**	**14.857**
	CV=3.80	CD@0.05 A=0.112 B=0.142 AB=0.532				

35: R/S Ratio of selected Pigeon pea genotypes grown for 45 days with different sources of phosphorus

		Organic	Inorganic	Normal	Deficient	Mean
H	ICP3226	0.477	0.333	0.950	0.572	0.583
	ICP8477	0.358	0.422	0.733	0.612	0.531
	ICP12764	0.293	0.350	0.750	0.405	0.449
	MEAN	**0.376**	**0.368**	**0.811**	**0.530**	
L	ICP4557	0.710	0.532	0.907	0.593	0.686
	ICP8863	0.542	0.502	0.860	0.443	0.587
	ICP7025	0.585	0.610	0.101	0.392	0.651
	MEAN	**0.613**	**0.548**	**0.927**	**0.476**	
	CD@0.05					
	CV=14.54%	CD@0.05 A=0.001 B=0.003 AB=0.0142				

Relationships

Fig.2b indicates a good relationship between root p and TDM under different P sources (r=0.606). Fig.2a indicates a good relationship between TDM and RDW different sources of P (r^2=0.441).Fig5a.indicates there was a good relationship between root P and total plant P (r^2=0.183).Fig3a.there was a good relationship between leaf p% and enzyme activity in different sources of P (r^2=202).

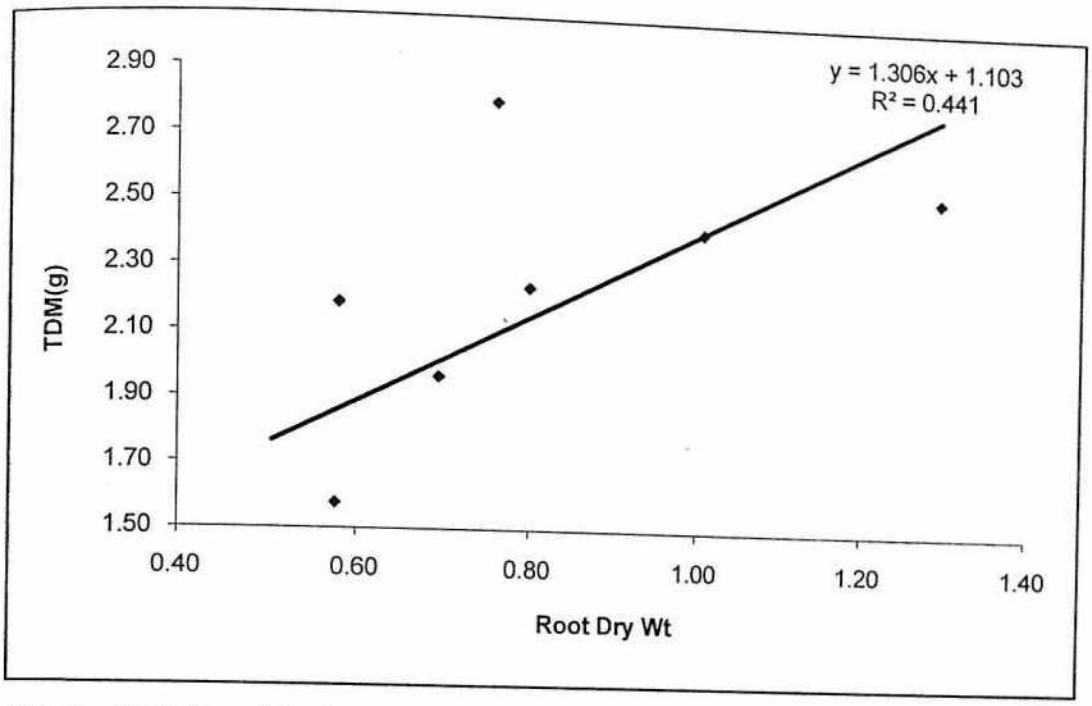


Fig 2a. Relationship between root dry weight and TDM of high and low P uptake genotypes grown for 45 days under different P sources.

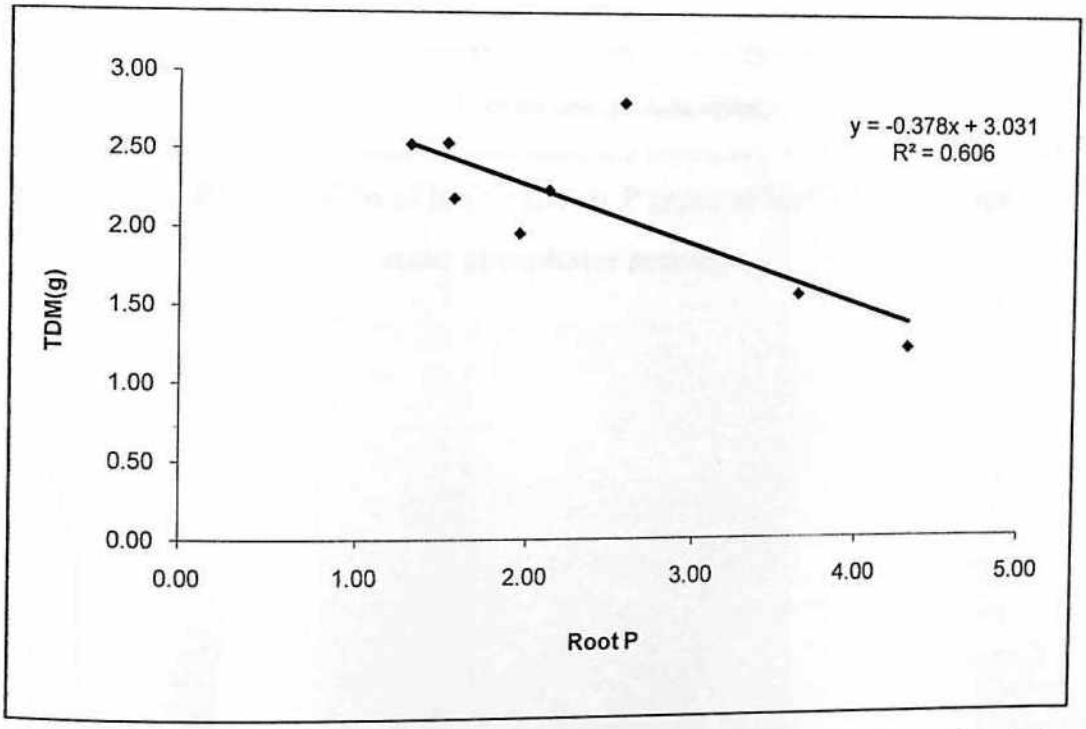


Fig 2b. Relationship between TDM and ROOT P of high and low P uptake genotypes grown for 45 days under different P sources.

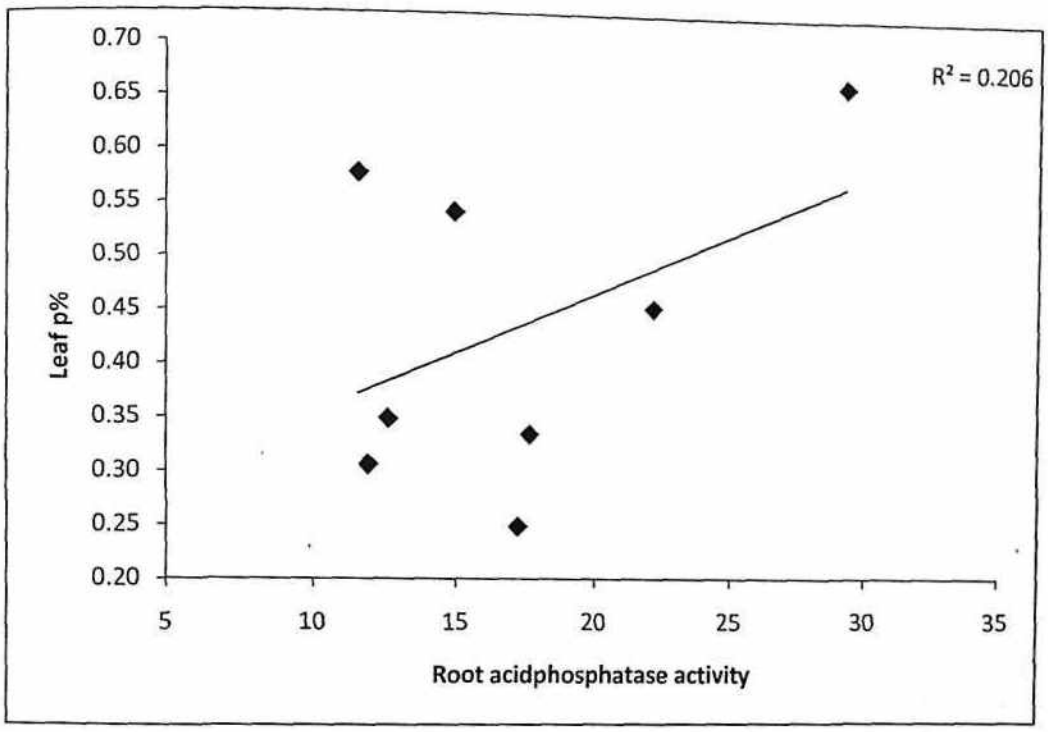
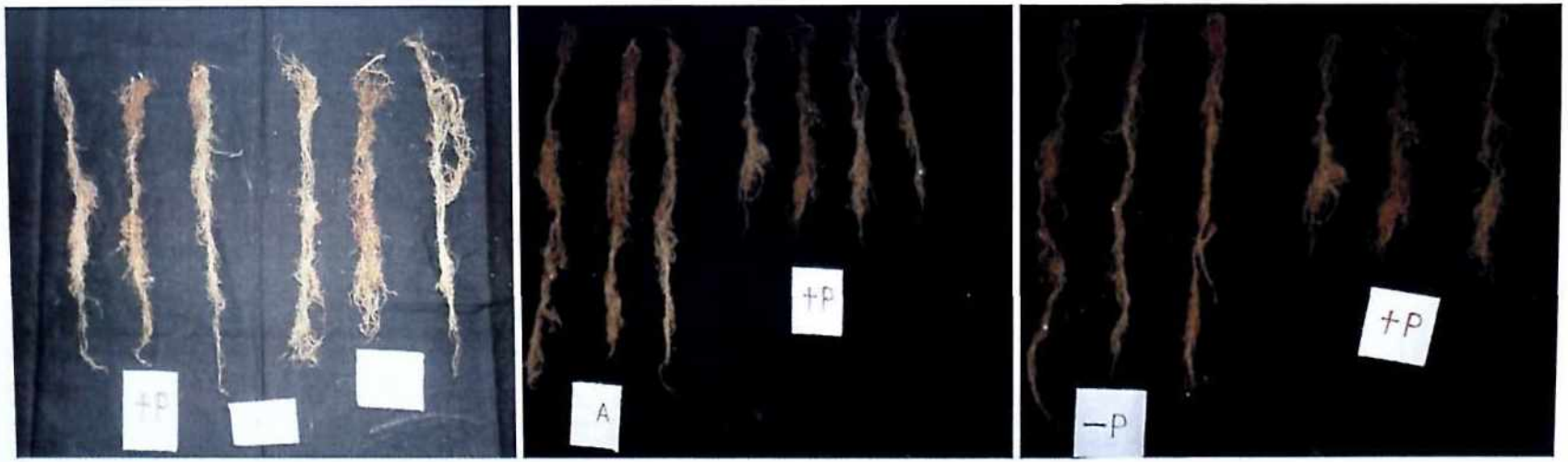


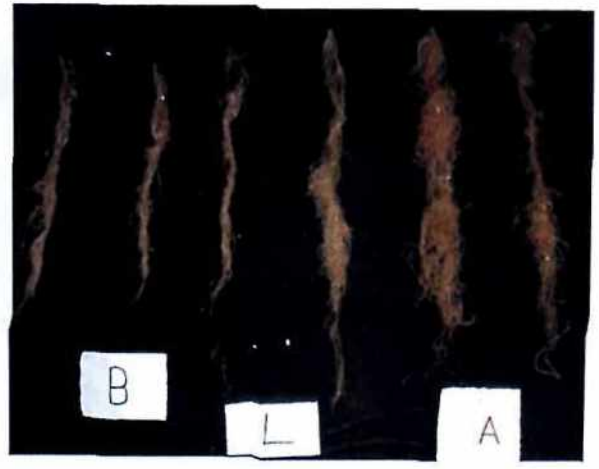
Fig. 19 Relationship of high and low P types of leaf P% and Root Acid phosphates activity



Normal P and Inorganic P | Organic and Normal P | Deficient and Normal P

Plate.1.Effect of root growth on different forms of Phosphorus High P Uptake Genotypes

Deficient and Normal P



Inorganic P and Organic p

Plate,2.Effect of root growth on different forms of Phosphorus Low P Uptake Genotypes

DISCUSSION

V. DISCUSSION

In soil, distribution of P is often stratified. Because of its reactivity, the total amount of P in soil may be high, but its availability for plant uptake is low (Holford, 1997). The capacity of plants to access P under limiting conditions depends on important adaptive traits including modification in root morphology, exudation of organic acids, secretion of acid phosphates and increased ability of roots to explore different layers of soil (Schactman *et al.*, 1998; Lopez Bucio *et al.*, 2000).

Phosphorus occurs in soils not only as mineral phosphates, but also as organic compounds. Although the importance of organic P in plant nutrition is still unclear, organic P may account for 30–80% of total P in agricultural soils (Tarafdar & Claassen 1988). For organic P sources in the soil to be used, they must be first hydrolysed by phosphates. Phosphorus acquisition by lupine may involve increased secretion of acid phosphates (orthophosphoricmonoester phosphohydrolyases; EC 3·1.3·2) under P-deficient conditions, hydrolyzing mono-ester soil organic P at low pH, and thereby increasing orthophosphate availability. It has been previously demonstrated that white lupine can utilize a variety of P-sources in both sand and soil culture (Adams & Pate 1992).

A wide range of plants and plant tissues show non-specific acid phosphatase activity, with substantial differences in protein size, tissue localization, subcellular localization, and regulation of expression (for review, Duff, Sarath & Plaxton 1994). Although many acid phosphatases have been studied, the functions of these enzymes are still unclear because of the difficulties associated with determining the *in vivo* substrates for the various isoforms of acid phosphatase present in plant tissues (Duff *et al.* 1994).

Increased acid phosphatase activity in response to P-deficient conditions has been reported in a number of species (Duff *et al.* 1994; Tadano & Sakai 1991). Both extra- and intracellular acid phosphatases appear to be ubiquitous in plants. Intracellular forms occur in all plant tissues in both vacuolar and cytoplasmic locations. Localization of extracellular acid phosphatases is typically at the root apical meristem, on the outer

surface of root cells. The role of these secreted enzymes is generally suspected to be mobilization of organic P from soils (Duff *et al.* 1994; Goldstein 1992). The pH of the rhizosphere surrounding P-stressed lupin plants typically ranges between four and six due to citric acid excretion, creating an environment suitable for extracellular acid phosphatase activity. Most plant intracellular acid phosphatases are assumed to be localized in the vacuole, because of their low pH optimum of 5–6 (Nishimura & Beevers 1978). Plants also store 'extra' P in the vacuole and compartmentalized acid phosphatases may be involved in the utilization of P reservoirs or P-containing compounds (Duff *et al.* 1994).

In view of these facts, an investigation was carried out with a major objective of identifying pigeon pea genotypes with higher Phosphorus uptake efficiency by using Acid phospatase as a marker for P deficiency. To envisage these objectives 36 pigeon pea genotypes (medium duration) were selected and grown with different forms of P and without P under greenhouse conditions. Based on their leaf P content and enzyme activity, six contrasting genotypes were selected to investigate the physiological or biochemical basis of P uptake and utilization.

In many low input agricultural systems, phosphorus (P) is one of the most limiting mineral nutrients for plant production. The use of genetically enhanced plants with improved P acquisition efficiency may represent the most sustainable solution to increase crop yields in these systems. This review is intended to provide a short summary on adaptation mechanisms of crop plants facing P deficiency as the starting point to develop a research approach for improving P acquisition efficiency. The natural source for improving P nutrition of plants is existing large genetic variation for traits associated with P acquisition efficiency and will therefore be emphasized.

It has been previously reported that white lupin can acquire sufficient P for growth and development from organic P sources (Adams & Pate 1992). In agreement with this data, our results have shown that there was no significant difference in the total number of proteoid roots in plants grown with organic P or inorganic P sources.

Phosphorus acquisition efficiency of cereal genotypes was investigated by using screening technique with vermiculite as the media in polythene bags (plate. 1) Sparingly soluble forms of inorganic and organic P sources have been externally provided at frequent intervals with modified Hoagland solution. The experimental approach applied here made use of the basic definition of rhizosphere soil, e.g. the zone of soil influenced by roots; providing the possibility to investigate acquisition of soil P very close to the roots of genotypes growing under defined nutritional and rhi zosphere conditions; and by maintaining equal root surface. The genotypes were kept at desired nutritional status by external nutrient supply.

Genetic variation in root growth characteristics and P uptake

Genetic variability is always considered as an advantage to improve plants for better growth and development and yardstick for the adaptation under specified or constrained conditions. From the plant nutritional point of view, this approach would be suited to soils, which experience fertilizer or nutrient constraints and those soils of mineral stress conditions that are difficult to correct or amend.

It is well known that root growth characteristics play an important role in uptake of nutrients. In addition, the growth and development of above ground biomass is largely dependent on nutrient uptake and its translocation to the shoot. Hence, higher the root growth, more the surface area and more accessibility of nutrients, which is reflected in total biomass and ultimately the yield. Hence different growth parameters were measured under sufficient and deficient P conditions. The results of these experiments are briefly discussed here.

In the present investigation genotypes showed significant variability on a positive scale in several growth parameters viz, root volume, plant height, root and shoot biomass, R-S ratio under added P conditions (Table.2, 3,4 and 5). Several workers have reported similar genetic variability for plant growth characteristics. Fagaria *et al.* (1988) found plant height is more sensitive parameter in rice cultivars to added P. Adu Gyami *et al.* (1989) reported dry matter distribution in stem and leaves increased with high P

application. Glass and Perley (1980) reported that differences in whole plant weights between organic and inorganic could be explained by nutrient status. Differences in leaf dry weight which form important part of shoot dry weight and also source to sustain growth caused drastic changes in total dry matter of plant under phosphorus deficiency.

Total dry matter

Pigeon pea genotypes showed a large variation in TDM (Table.6).TDM was more in normal condition compared to other treatments High P uptake types did not show large decrease in shoot growth than low p uptake types TDM was more in high P uptake types and TDM was less under deficient conditions for both types of genotypes. (plate). These results are in agreement with Lio hang et al (2002) who observed the effect of P starvation on leaf area as consistent and also several other authors reported a rapid and severe effect of P deficiency on leaf growth (Rao and Terry, 1989).

Similarly, Brevedan *et al.* (2002) observed that low P treatment reduced shoot growth significantly and the main reason being the reduction in leaf expansion to an extent of 70%. The effect of low P was comparatively less on the root growth. The reduction in total biomass is due to both the reduced leaf expansion and movement of photosynthates to leaf and other parts of the shoot. Another possibility for reduced shoot biomass under P deficiency could be due to the fact that the more photosynthates are diverted towards the root in an attempt to acquire more P from the rhizosphere. This is happening at the cost of shoot growth, which is affected.

The relationship between shoot P and total dry matter produced (Fig.10) as reveals that as shoot P content increases the total biomass accumulation also increases and Fig.18 indicates the a good relationship between root dry weight and total plant P accumulated among 36 pigeon pea genotypes. These results are in consistence with the findings of Adu-gyami *et al* (1989) that shows that dry matter production is positively correlated with P uptake both under Fe-P and Ca-P treatments. Further they have reported a positive correlation between P uptake and root dry matter production.

Genetic variability for P uptake and utilization

Genetic variability for any trait is known to exist in crop plants. As far as the variability and utilization of P is concerned, it is well known that availability of P in the soil is very low since it is present in bound form. Hence it would be essential to decide breeding strategies for development of appropriate genetic stocks that would utilize the bound P efficiently. One of the emphasis of this investigation was to look for the genetic variation in pigeon pea genotypes for P uptake and utilization with an idea to identify high P uptake genotypes.

A major factor contributing for increased P uptake in tomato was apparently the greater affinity of the absorbing sites for H_2PO_4 on the roots (Cress *et al.*, 1979). The efficient tomato lines produced almost 100 per cent more dry matter than inefficient lines though the latter had higher mobility for P with in the plant under phosphorus stress (Clarkson and Scattergood, 1982).

Data indicated that P content of roots and leaves varied significantly in organic inorganic,normal and deficient condition with varied root and shoot characteristics (Table 7,8,9, and 10). However, genotypes like ICP 3226, ICP 8477 and ICP 12764 accumulated more leaf P in organic condition (Considered as high P uptake types, Fig.1 I, Table.8A and 8B). Similarly root P content also varied among genotypes (Table.4). Similar differences in P absorption and translocation among cultivars were reported in bean (Lindgren et al, 1977 and White et. al, 1976). Large variation in rates of P absorption among the 36 lines of *Phaseolus vulgaris* was reported.

Phosphorus efficiency is a multifaceted trait, which is influenced by a range of environmental factors. Generally these factors can be divided into two components.

(i) The efficiency of phosphorus acquisition by the plants from the soil, which is deficient in P (-P) (acquisition efficiency).

(ii) The efficiency with which native phosphorus (in the plant) is utilized to produce yield (P-use efficiency).

The extent of variability in crop plants to acquire soil P is influenced by both genotypes and environmental factors (Nielson, 1983). Crop response to P fertilizer depends on genetic and physiological characteristics of the plant that help efficient P uptake and utilization, as well as soil and environmental conditions that affect P availability (Bieleski, 1973). These differences are under genetic control but their expression may be altered dramatically when grown under varied environments.

In the present study, genetic variability in P uptake and utilization efficiency among pigeon pea genotypes was studied (Table.6). Genotypes ICP 3226, ICP 8477 and ICP12764 showed high P uptake efficiency (fig.12). Similar differences among the cultivars in P uptake was documented by Gowley *et al.* (1994) and was mainly attributed to root characteristics.

The strategies that plants adopt for high uptake of nutrients depends on species, environment and some times cultivars. High P uptake genotypes identified as P efficient had high total dry matter production and high root biomass under organic and deficient condition (Table.5 Table.8). Similarly, Wissuwa (2003) reports that genotypic differences in P uptake from P deficient soils may be due to high root growth or high external root efficiency. Itoh and Barber, (1983) revealed that in tomato cultivars P uptake efficiency was influenced by the amount of root surface area per plant, rate of P uptake, kinetics of roots in addition to root hairs.

In the present investigation it was observed that the uptake efficiency increased under +P conditions compared organic, inorganic and deficient conditions whereas the utilization efficiency increased under –P conditions (Fig.). Similar observations made by Baliger *et.al.*(1987) that breeding for Phosphorus use efficiency (PUE) is considered easy in a crop which has wide genotypic variation in PUE and which is stable in adaptation in terms of responses to low P availability in different soils. Sustainable diversity in PUE parameters was found in maize, rice, field bean. Further, Weineke (1990) showed higher efficiency of P absorption in SC 33-9-8-E (a maize cutlivar) and attributed it to higher allocation of photosynthesis to roots and also higher remobilization capacity of the plants from root and leaves. Fawole *et al* (1982) screened French bean cultivars for efficient P utilization under stress conditions and isolated the efficient

cultivars. Hedley *et al* (1994) observed differential internal P efficiency (Shoot dry weight per unit total plant P), in upland rice cultivars.

On the similar line, the results indicate a close relationship between root dry weight and total P accumulated in the plant (Fig.). In a study conducted by Foehse *et al* (1988) it was reported that length of root hair is closely related to the P content of the root and shoot but formation of root hairs did not depend directly on P concentration at the root surface but on the P content of the plant. It was found that under different conditions, contribution to P uptake by root hairs was upto 90% of total uptake as revealed by the study of Foechse *et al.* (1991).

Variations among pigeon pea genotypes were observed in the leaf p shoot P, root P and total plant P in the present investigation indicates existence of genetic variability in uptake of P and utilization (Table. and). Similar study conducted by Krishna (1997) who suggests that the genetic variation for P uptake and utilization among peanut (*Arachis hypogea* L.) genotypes exists. Further, he reported that genotypes with similar physiological maturity differed significantly in their P efficiency ratio in terms of total P per plant, root P, shoot P, and seed P. Several traits contribute to the total P efficiency of the genotype including root length, rate of P uptake per unit root lengths, leaf and pod characters such as P accumulation and dry matter/yield produced per unit P absorbed (i.e. P efficiency ratio).

Genetiv variability for p uptake and utilization

Despite the important implications of P uptake and its utilization efficiency (PUE) a little is known about on the physiological and molecular events responsible for efficient absorption of P and effect of P availability on adaptive responses in root system. Differences in P uptake among 36 pigeon pea genotypes with similar duration in the present study can be attributed to root characteristics and altered plant metabolism to cope with P stress.

Physiological basis

Root characteristics

The above study indicated that roots find their own way of adjustment to stressed environment using phosphatase activity but there are other mechanisms through which roots adapt to low P conditions like root activity and architectural properties. In a study by Lindgren *et al.* (1977) using excised roots for identifying differences in rates of P absorption by 59 lines of *Phaseolus vulgaris*, large variations in P absorption rates between lines were noticed. They concluded that high variance for P absorption by excised roots was due to high environmental

effect. Larger root system provides greater root soil contact which was particularly important for uptake of P and other less mobile ions. They concluded that P uptake was often closely related to root length (Atkinson, 1991)the root architecture response to P deprivation should be of great adaptive significance because soil P levels are in the range of 0-20 μm (Holford, 1997) and is highly specific. (Bates *et al*, 1996). Root size and its distribution is important for P acquisition (Noord- wijk et al., 1990) and crop genotypes may differ in root size (Schjrring and Nielsen, 1987).

In a number of plants including bean, lupin, tomato and *Brassica nigra,* low P availability modifies important root architecture traits such as root branching, total root lengths, root surface hair formation (Dinkelatar, 1995, Carswell *et al.*, 1996: Bores *et al.*, 1999). These physiological adaptations are believed to lead toward enhancing P uptake capacity of the root system. Root growth is generally known to increase slightly under P deficient conditions. Baker *et al.* (1979) in their P uptake study highlighted the importance of deep rooting in corn hybrids rather than other P absorption characteristics of the roots. The study revealed that P accumulation in leaves of a deep rooting variety was more compared to others. While,. With this background, the following root chacteristics were examined in the selected pigeon pea genotypes.

Root dry weight

Pigeon pea genotypes showed significant variability in root dry weights in organic and inorganic conditions (Table.2). Selected genotypes like ICP3226,ICP8477 and ICP 12764 produced more root dry weight under P deficiency compared to other forms of P indicating the intrinsic ability of these genotypes to increase the surface area of roots to access more P (Table.9 and fig 16A). This is one of the important adaptative strategy plants have evolved to take up more P during P stress conditions. Similar observations made by Wissuwa (2003) showed genotypic differences in P uptake from P deficient soils which was attributed to high root growth or high external root efficiency. Increasing root fineness or high external root efficiency for root dry matter production by 18.7%(Table.11) was sufficient to increase P uptake. Liao Hong et al. (2002) Observed genetic variation in root morphology among different genotypes in responses to P availability. In our study the P efficient genotypes appeared to have larger, finer and long root systems than P inefficient genotypes. Low P uptake genotypes did not showed any difference in root growth between organic and inorganic p as compared to high P uptake genotypes, which showed significant differences between different forms of P treatments. (Plate.3).

Nielson *et al* (2001) assessed the importance of increased allocation of carbon to roots for adaptations of plants to low P availability in common bean. P-efficient genotypes allocated fraction of their biomass to root growth especially under low P conditions. Efficient genotypes had lower rates of root respiration than inefficient genotypes, which enabled them to maintain greater biomass allocation than inefficient genotypes without increasing overall root carbon costs. Similar results for biomass were observed in the present investigation, where efficient genotypes like ICP 8477 and BRG-2 allocated more biomass to the plant roots under P deficiency (Table.11)

Root length

Root length varied significantly among genotypes grown in organic inorganic and normal condition in all the experiments conducted. Mean root length was increased among 36

pigeon pea genotypes under –P conditions (Fig.3). High P accumulating genotypes showed high root length (Table. and Fig.) under organic and P deficiency conditions there by increasing surface area that helps to access more P under P deficient conditions. Such genotypes invest more biomass in root growth and metabolism under P deficiency so that plant acquires the required.

Krishna (1997) observed significant differences among 25 genotypes of groundnut for total P efficiency of the genotypes including root length, rate of P uptake per root length. P efficiency was interpreted as higher uptake of certain genotypes which depended more on increased root length and increased exploration of soil, while others depended on greater P absorption rate and total P uptake per unit length. He coneluded that selection for higher P absorption from soil was correlated with better root traits P.

Clarkson and Hanson (1980) showed that higher root length may be the basis of higher nutrient absorption both in deficient and sufficient levels of P yielding higher and differential plant dry weights in genotypes.

Sun *et al.* (2002) studied morphology of root systems of different wheat (*Triticum aestivum* L.) genotypes under low P stress to determine the effect of external factors. The length of root axis and number of lateral roots sharply increased under P stress. The number and length of root axis were significantly different under different levels of P supply.)

Root size and root volume

Yong *et al.* (2002) studied the effect of localized supply of P on root morphology and architecture of two rice varieties *viz.*, Azucena and IR1552. They found Azucena as more responsive to localized P supply due to more plasticity in root morphological and architectural adaptation. The increased root length was observed with high P supply, whereas the adaptation for low P was due to cluster-roots like fine roots. Results also indicated that the P deficiency signal comes from the low-P side, while enhanced root growth is realized on the high P side. They concluded that the induced changes in root morphology and architecture by localized supply would have physiological significance

that P uptake by plants can be achieved by allocating a portion of the roots to the high P zone.

Root hairs and lateral roots assist the acquisition of P by exploring a greater soil volume and by increasing the absorptive surface of the root. The formation of a highly branched root system, in response to nutrient starvation, may be a consequence of allocation of more carbon and energy resources to root system capable of exploring large areas of upper soil layer, where nutrient rich patches are normally present (Stitt and Rudigh-Scheible, 1998).

Similar results were obtained in the present investigation where root volume also increased under P deficient conditions. Further genotypes showing higher P uptake under P deficient conditions showed increased root length and also volume. the leaf P content in all these genotypes was high, but high P uptake genotypes which is high P uptake types had more root weight and root length and in organic and deficient conditionand higher leaf P (plate.3 and Table 9 and 11). These could be due to the contribution of other factors like excess root exudation and high acid phosphatase activity (Table.14).

Root to shoot ratio

The root/shoot ratio increases with decreasing the P supply, a situation mainly due to the greater reduction in the growth rate of the shoot. The P concentration in both the shoot root decreases with decreasing P supply although the weight decrease is more marked in the root. As the experimental P supply is reduced, the root keeps growing at similar rate progressively with less P and total dry weight of the plants was reduced.It is obvious that under P deficient conditions ratio of root to shoot increases generally due to increased root growth.

Root length, radius, surface area, root to shoot dry weight ratio and root hair densityare important morphological parameters of root for uptake of any nutrient and also for P. These root characteristics may be affected by soil properties (Prummel, 1979).

Similar increase was noticed in the present work there was increased root to shoot ratio under deficient condition compared to other forms (Table.3). Many authors observed higher root to shoot ratio under P deficiency and plants are known to allocate higher amounts of carbohydrates to roots and higher sugar (Cakmak et al., 1994; Paul and

Stitt, 1993). Some pigeon pea genotypes have root characteristics that make them P efficient, obviously lengths of root, their spread in the soil and morphology together contribute to efficient uptake of P. The increase in root to shoot ratio was observed in the present investigation was in genotypes like ICP 3226,ICP12764 and ICP8477 which showed higher root to shoot ratio. A positive relationship between root dry weight and P uptake indicates the importance of root volume and root surface area in the acquisition of P from soil. Root to shoot ratio was higher in P deprived plants, which indicates that shoot growth was more severely reduced than root growth.

In all the efficient genotypes, desirable root parameters increased under P deficient conditions. Between genotypes ICP 3226,ICP8477 and ICP12764, genotype had higher root length and root weight and root volume. Clark(1990) who showed differences among sorghum genotypes for distribution of P between roots and shoots reported that the genotypes considered to be the most efficient had lower shoot / root ratio and the ones, which have high root to shoot ratio, high root length accounted for high P uptake. Similar trend was noticed in the present investigation, wherein the uptake of P was higher in the genotypes producing long and denser roots. Root to shoot ratio was higher on P deprived plants (Table.), which indicates that shoot growth was more severely affected than root growth. One of the reasons for increased root to shoot ratio could be increased allocation of carbon to roots as observed by Nielson et al (2001) in common bean.

Biochemical basis

Of the several mechanisms, plants adopt to acquire needed P under P deficient conditions, by increased activity of phosphatases are considered to be most important.

Acid Phosphatase activity

The ability to study temporal and spatial responses to P and other macronutrients in plants is often limited by the lack of appropriate molecular tools. Bioavailable P, which is critical to plant growth, is often present in limited amounts in soils. When plants have an adequate supply of P and/or absorb it at rates that exceed demand, P is usually stored in

organic compounds (e.g. phytic acid) in the vacuoles or in the cytoplasm of the leaf cells (Lee and Ratcliffe 1993; Schachtman et al., 1998). Plants usually absorb P from soil in the form of soluble orthophosphate anion (Pi, H2PO42, or HPO422), which is often present in limited amounts. In contrast, soils often contain large amounts of insoluble organic and mineral P compounds (Goldstein et al., 1987). One of the most profound responses of plants to P deficiency is the induction of APases capable of extracting inorganic phosphate from the organic compounds.

It is well documented that acid phosphatase activity increases under P deficient conditions. The acid phosphatase activity of Pigeon pea was determined by P-n p nitro phenol method. There was a considerable increase in root acid phosphatase activity in .high P uptake genotypes like ICP 8477, ICP 3226and ICP12764under organic condition. The activity of phosphatase secreted, increased with decrease of P concentrations in nutrient solution (Fig.21). The activity was comparatively low in low P accumulating types like , ICP 4557, and ICP 8863,ICP7025 than in high P accumulating types. High P uptake types showed increased phosphatase activities under organic and P deficiency conditions. There is strong correlation between root acid phosphatase activity and leaf P accumulation. For phosphates to be available to plants from phytate, it must be hydrolysed by phosphatase (Richardson et al.,2000). Plant roots with a high phosphatase activity have great potential to utilize soil organic phosphorus (Helal,1990). Under conditions of P deficiency, acid phosphatase secreted from roots was increased (Nakas et al., 1987; Liet al., 1997; Hays et al., 1999). In the present study, it isclear that pigeon pea root was able to secret greater amounts of acid phosphatase in vermiculate culture and increased hydrolysis of phytate. As a result, pigeonpea could utilize phytate as effectively as KH2PO4.

Other studies also indicated that hydrolysis of organic-P can be mediated by root-born phosphatase (Tarafdar and Claassen, 1988) and/or microbial phosphatase (Doumas et al., 1986; Haussling and Marschner, 1989; Mousain and Salsac, 1986). Phosphatase activity near roots is affected by P status of plants (Doumas et al., 1986; Helal and Sauer beck, 1988; Silberbush et al., 1981). To minimize the later, we maintained equal supply of phosphorus and other nutrients to the genotypes from the external nutrient solution

Bio-available P, which is critical to plant growth, is often present in limited amounts in soils. When plants have an adequate supply of P and absorb it at rates that exceed demand, P is usually stored in organic compounds (e.g., phytic acid) in the vacuoles or in the cytoplasm of leaf cells and released and utilized (Lee and Ratcliffe 1993; Schachtman *et al.*, 1998). Plants absorb P from soil in the form of soluble orthophosphate anion (P; H_2PO_4 or HPO_4^{-2}), which is present in limited amounts. In contrast, soils contain large amounts of insoluble organic and mineral P compounds (Godstain *et al.*, 1987) one of the profound responses of plants to P deficiency is the induction of acid phosphatases capable of extracting inorganic phosphate from organic compounds. In the experiment conducted during the present investigation it was observed that enzyme activity was more in organic and deficient condition compared to other forms progressively higher secretion of acid phosphatase activity (Table.14 and Fig.19, 19A).

Series of experiments conducted by Tadana and Sakai (1991) revealed that P-stressed lupin roots have 20 times more acid phosphatase activity than +P control based on root dry weight. Table.14 indicates that by 35 days in P deficient conditions there is approximately two-fold increase in acid phosphatase activity of root extracts. Although acid phosphatase have been studied in a number of different tissues and species the functions of these enzymes are still unclear because of difficulties associated with determining the in vivo substrates for the various isoforms of plant Acid Phosphatases.

A good inverse relationship between P content in root and activity of root acid phosphatase secreted during growth (Fig.21). At a given growth stage the activity of root acid phosphatase increased with decreased P content in the roots of Pigeon pea genotypes particularly in high P uptake types (Table.12 and 14,Fig.21).

However, ability of roots to secrete acid phosphatase differed among selected contrasting genotypes. Acid phosphatase secreted by roots may hydrolyze organic P compounds in the rhizosphere and liberate inorganic P (Haussling and Marshnar, 1989). In vermiculite culture, all genotypes were able to take up sufficient P for adequate growth from the nutrient solutions. (Data not shown). Withdrawing P supply for 15 days did not

produce any visible symptoms of P deficiency. However genotypic differences in P uptake of different forms of P by young roots and their phosphatase activity was observed (fig.19).

The practical significance of root phosphatase for nutritional efficiency of plants under field conditions is not yet clear. However, plant roots with high phosphatase activity obviously have the potential to utilize soil organic P. It is well known that availability of P in soils and inorganic P, applied through fertilizer in many areas such as alkaline and calcareous soils in semi arid regions is low. Under such conditions the nutrition use efficiency of plant varieties with high root phosphatase activity needs to be investigated.

SUMMARY

VI. SUMMARY

Phosphorus (P) is an essential part of metabolic processes that occur within the plant, such as photosynthesis, the synthesis and breakdown of carbohydrates, and energy transfer. If the soil level of available phosphorus is not adequate for these plant processes, then production will be reduced unless fertilizer phosphorus is added. P is least accessible but required most by plants and its availability in native soils is rarely adequate for optimal growth Phosphorous (P) is one of the most important elements that significantly affects plant growth and metabolism and low P availability is a primary constraint to plant productivity, particularly in weathered soils in tropics and sub tropics when P is commonly bound to iron and aluminium oxides through chemical precipitation or physical adsorption.

In response to persistently low levels of available P in the rhizosphere, plants have developed highly specialized adaptive mechanism through morphological, architectural and biochemical changes that enable more efficient acquisition and utilization of the limited P. Plants which have the ability to increase Pi uptake, either by extensive root growth or increased rate of Pi uptake or a greater ability to absorb Pi due to increased root elongation show superior growth in low P soils. Therefore, P uptake not only depends on the amount of available P in soil but also on plant properties root growth and of certain enzymes like acid phosphates activity under P deficient conditions.

Hence the emphasis in the present investigation has been to identify pigeon pea genotypes that are efficient in their ability to absorb and utilize P from different forms of P and further, understanding the physiological/biochemical basis for higher P uptake.

Genetic variability for P uptake

Significant variation in the ability of pigeon pea genotypes to absorb P from organic and inorganic sources was observed. Based on the ability of genotypes to take up P and their, leaf P and enzyme activity the genotypes were classified into following groups employing the standardized normal distribution analysis (Z-plot).

Classification

Genotypes with High leaf P (%) types and high acid phosphates activity

1. ICP 3226

2. ICP 8477

3. ICP 12764

Genotypes with Low leaf P (%) types and low acid phosphates activity

1. ICP7025

2. ICP 4557

3. ICP 8863

These six contrasting genotypes were selected from 36 pigeonpea genotypes to investigate further the basis for variation in uptake of P.

Root growth characteristics and P uptake,

In the genotypes identified as high P uptake types, increase in the root growth in terms of root length, increased root volume, root weight and root to shoot ratio was observed under P deficient conditions.

Acid phosphatase activity and P uptake

Acid phosphates activity increased dramatically under organic and –P conditions. The increase in activity of acid phosphates was observed in the high P uptake types under organic P deficient conditions compared to normal and inorganic conditions. This suggested that the ability of these genotypes to access the unavailable P through increased activity of acid phosphates.

Salient features of investigation;

1. Significant genotypic variation in P uptake was observed; indicating, that genetic improvement for higher P uptake from different forms of P through further selection is possible.

2. The higher P uptake efficiency seems to be related to better root character like, high root biomass, high root length, high root volume and altered plant metabolism like root acid phosphatase activity. These strategies are adopted by the efficient genotypes under P deficiency.

3. The efficient genotypes ICP 8477, ICP 3226 and ICP12764 showed high acid phosphatase activity and high P solubilization activity under organic and deficient forms of P.

4. The genotypes like ICP 3226, ICP 8477 and ICP12764 were identified as P efficient with moderately high root biomass and may be suitable for growth on low P sources.

5. In general, though overall growth and total biomass was low under low P conditions compared to recommended or high P condition, root biomass was slightly higher under organic and deficient P conditions compared to recommended or high P deficient conditions.

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VII. REFERENCES

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APPENDIX

APPENDIX

Typical physical properties of exfoliated vermiculite

Colour	Light to dark brown
Shape	Shaped granules
Bulk density	100-150 Kg / cum
Combustibility	Non – combustible
Sintering temperature	1150 – 125^0 deg C
Fusion point	1200 – 132^0 deg C
Cation exchange capacity	50 – 150 me / 100g
Specific heat	0.84 – 1.08kj/kg K
	0.20 – 0.26 kcal / kg K
	0.20 – 0.26 Btu/lb deg F

TYPICAL CHEMICAL ANALYSIS

Element	**Percent by weight**
Silicon di oxide	38.0 – 46.0
Aluminium tri oxide	10.0 – 16.0
Magnesium oxide	16.0 – 35.0
Calcium oxide	1.0 – 5.0
Potasium oxide	1,0 – 6.0
Ferrous	6.0 –13.0
Titanium di oxide	1.0 – 3.0
Water	8.0 – 16.0
Others	0.2 – 1.2