STUDIES ON HOST GENOTYPE–ARBUSCULAR MYCORRHIZA INTERACTION OF RICE IN RELATION TO MYCORRHIZAL DEPENDENCY

A Thesis

submitted to the

Bidhan Chandra Krishi Viswavidyalaya

in partial fulfillment of the requirements of the

Degree of Doctor of Philosophy

in

Agriculture (Plant Pathology)

By

Ramkrishna Saha

Department of Plant Pathology
Faculty of Agriculture
Bidhan Chandra Krishi Viswavidyalaya
Mohanpur, Nadia, West Bengal
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The Viva – voce of **Mr. Ramkrishna Saha** on his dissertation entitled "**Studies on host genotype–arbuscular mycorrhiza interaction of rice in relation to mycorrhizal dependency**" submitted in partial fulfillment of the requirements of the Degree of Doctor of Philosophy in Agriculture (Plant Pathology) was held on ..... and it was recommended that the thesis be accepted for the award of the degree.

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Certificate

This is to certify that the work incorporated in the thesis entitled " **Studies on host genotype–arbuscular mycorrhiza interaction of rice in relation to mycorrhizal dependency**" submitted by **Ramkrishna Saha** in partial fulfillment of the requirements of the Degree of Doctor of Philosophy in Agriculture (Plant Pathology) of the Bidhan Chandra Krishi Viswavidyalaya, is a faithful record of bona fide research work carried out under my supervision and guidance. Results of the thesis have not been so far submitted for any other degree or diploma. Assistance and help received during the course of investigation have been duly acknowledged.

Kalyani

Dated, August 1, 2004

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Ramkrishna Saha

Place: Mohanpur

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Chapter- I Introduction

Mycorrhizas, the symbiotic associations between soil fungi and plant roots are complex biological systems. Commonest of these, the vesicular arbuscular mycorrhiza (now called arbuscular mycorrhiza or AM) formed between fungi of the order Glomales (Zygomycota) and 80% of the vascular land plants have probably existed for approximately 450 million years since the colonization of the land by ancient plants (Franken and Requena, 2002). The AM symbiosis is still now a crucial factor for most terrestrial ecosystems for the role it plays in plant nutrition, and abiotic and biotic stress tolerance. The structure and dynamics of terrestrial plant communities are also greatly influenced by the mycorrhizas (Soderstrom, 2002). Their application potential in agriculture and forestry to enhance plant production and sustainability is also under active consideration.

The arbuscular mycorrhizal fungi (AMF) are well known for their ability to improve the nutrition of plants, with phosphate being the most recognized plant nutrient supplied by these symbionts. One estimate suggests that up to 80% of the P_i taken up by a mycorrhizal plant can be supplied by the fungus (Marschner and Dell, 1994). AM fungi can also provide other macro- and micronutrients such as N, Zn, Cu and Mg (Smith and Read, 1997; Clark and Zeto, 2000). The AMF fungi, however do not always improve plant nutrition. Growth depression of plants due to AMF colonization for varied reasons and under varied environments is known (Marschner, 1995; Smith and Read, 1997; Burleigh and Bechmann, 2002).

Although AM symbiosis essentially has no host specificity, this symbiosis is said to have a high level of functional compatibility (Burleigh and Bechmann, 2002). One definition of functional compatibility is the degree to which a plant benefits in terms of promotion of P-uptake due to mycorrhiza formation by an AMF and is exemplified when a particular AMF isolate imporves P-uptake of one plant species but not another, under identical environmental conditions (Ravenskov and Jacobson, 1995). A plant species benefiting from functionally

compatible mycorrhiza formation under conditions where another species does not is said to be mycorrhiza dependent.

Variations in plant's dependence on mycorrhiza are well known for long (Gerdemann, 1975) and have been studied at morphological (e.g., Baylis, 1975), physiological (e.g., Koide, 1991) and recently at genetical (e.g., Gianinnazzi-Pearson, 1995 *et al.*,) levels. Interspecies genetic variation of the AMF in symbiosis of a host, although rare, has been identified (Pearson *et al.*, 1993, Gao *et al.*, 2001; Cavangaro *et al.*, 2001) but intra-species genetic variation in the AM fungi has not yet been shown to result in functional difference between the isolates (e.g., Sanders *et al.*, 1996).

In contrast, there have been many attempts to examine variation between AM host cultivars in their capacity for, or response to mycorrhization. Majority of research in this area was with cereals, *Zea mays* (maize), *Hordeum vulgare* (barley) and *Triticum aestivum* (bread wheat). In these and many other plant species (pearl millet, sorghum, groundnut, cowpea, chick pea etc.) host genotype variation in AMF colonization and response have been shown to occur frequently (Koide, 1995; Thompson, 1994; Barker *et al.*, 2002). Formal genetic studies with large number of cultivars of *T. aestivum* indicated that 'mycorrhizal responsiveness'genes might exist in different chromosomes of some cultivars (Hetrick *et al.*, 1995). Inter-cultivar variation in P-acquisition due to AMF colonization has been reported in double haploid genetic population of *Hordeum vulgare* (Barker *et al.*, 2002). Such studies have laid the foundations for genetic dissection of one physiological component (P-uptake) of functional AM symbiosis. Recent studies using AM defective mutants of legumes as screens, dissection of the genetic pathways for establishment of functionally compatible AM symbiosis has advanced to a great extent (Marsh and Schultze, 2001).

Importance of AM symbiosis for nutrition management of crops in low input agricultural systems requires no emphasis (Bethenfalvey and Linderman, 1992; Hoffman and Carroll, 1995; Rayan and Graham, 2002; Panja and Chaudhuri, 2004). But, the potential seems to have not been fully explored or appreciated in today's agriculture due primarily to the half a century old emphasis put on input intensive agriculture. Plant breeders in general have ignored the below ground symbiosis and the impact it may have for nutrient acquisition by plants. Beneficial

traits, like enhanced response to mycorrhization have not been exploited in strategic breeding of plants.

There are two conflicting concepts regarding evolution and genetic basis of AM dependency or responsiveness of crop cultivars. Considering AM dependency as an evolutionary trait, authors have argued that older accessions and cultivars that pre-date the heavy fertilization era might have been mycorrhiza responsive and the trait might have been lost form the modern cultivars under selection pressure of high fertilizers (Hetrick *et al.*, 1993; Smith and Read, 1997). This may not be universally true as there is evidence to show that land races, old accessions of some crops are less AM responsive than their modern counterparts (Koide, 1991).

Against this conceptual background and some preliminary work carried out in the supervisor's laboratory (Santra, 1993; Saha, 1998; Alimuddin, 2003), an investigation into host genotype – arbuscular mycorhiza interaction was carried out with selected genotypes of rice in a low nutrient soil keeping the following objectives in points of view.

i) To determine the AM dependency or responsiveness behaviour of both spatially and temporally selected and acclimatized inbred rice cultivars having widely different genetic background.

ii) Correlation of the variations in AM dependency or responsiveness of the cultivars with variations in growth and nutritional physiology and root morphological characters.

iii) Variations, if any, in the structural pattern of AMF colonization features among the differently AM dependent cultivars.

iv) Alteration in non-specific defense responses of the cultivars at early stage of AMF infection.

Results of the investigation were expected to explain the morpho-physiologial basis of intra-species variation in AMF dependency of a modern day crop and bring out information for precisely studying the genetics of mycorrhizal dependency or responsiveness for its possible ecological and economic exploitation.

Chapter– II Review of literature

Genetics of mycorrhizal dependency and variation in plant response to arbuscular mycorrhiza

The importance of mycorrhizal symbioses in nature cannot be overestimated. The significance of these plant root-soil fungus associations which existed 460 million years ago, at the early stages of land plant evolution (Redecker, 2002), in the nutrition and well being of plants is well established. Evidences have accumulated that they may also have major effects on the structure of terrestrial plant communities (Read, 1993; van der Heijden *et al.*, 1998; Hartnett and Wilson, 2002). Since this form of symbiosis was first described in the mid 19th century (Vittadini,1842), the fundamental questions surrounding these intriguing biological systems have attracted large number of researchers, as has the question of their potential application in agriculture and forestry to enhance plant production and sustainability.

One important fundamental question about the mycorrhizas that has attracted the attention of the biologists is the genetics of the symbiotic association. Is mycorrhiza a casual phenotype or there is a genetic basis of mycorhiza formation? What does the ubiquity, especially of the AMs mean for both the partners in terms of genetics and evolution of mycorrhizas and also the plants? Are there any genetic pathways of mycorrhiza development? If there what those pathways may mean for the biology of the plant partner in relation to symbiosis? These are some of the fundamental questions that are sought to be answered now, taking advantage of the progressive advancement of molecular genetics as a tool to understand the intricate questions of biology. Identification of the primary genetic determinants or the pathways controlling the development of mycorrhizal symbiosis and its metabolic activity is expected to open the door to understanding the ecological fitness of the symbiosis (Martin, 2001). Understanding the pathways may also provide insights into the more recently evolved interactions between plant roots and other microorganisms, which may have constrained the capacity of plants to evolve protective responses in roots to non-mutualistic interactions (Barker *et al.*, 2002). In this brief review attempt has

been made to summarize the available information on the evolution and genetics of mycorrhizal symbiosis with particular reference to arbuscular mycorhiza.

Mycotrophy, mycorrhizal dependency and mycorrhizal responsiveness

In mycorrhiza literature three collateral terms have been often used to describe the essentiality or otherwise of plant's dependence on mycorrhiza. These are mycotrophy (Rommel, 1939), mycorrhizal dependency (Gerdemann, 1975) and mycorhiza responsiveness (Mosse, 1973). The term ' mycotrophy' coined first in the sequence is etymologically relevant to the cases of mycorrhiza dependence where the fungus supplies carbon to the plant partner which is also a heterotroph - the mycohetertrophic plants, like the achlorophyllous orchids (Leake, 1994). However, the term is often used to describe the nutritional dependence of autotrophic plants on mycorrhizas, where the energy (carbon) transfer is reverse, as is required to be, from autotroph to the heterotroph. There is, however, an underlying unity between these two plant types of mycorrhizas. In the case of the heterotrophic plant mycorrhizas the fungal partner parts with some of the carbon it derives from another autotroph either by necrotrophy or biotrophy to the heterotrophic plant partner, so that carbon flow from the autotoph to the heterotroph remains fundamentally unchanged in heterotophic plant mycorrhizas also. Baylis (1975) used the term 'mycotrophy' in the restricted sense of deriving a growth stimulus by the autotrophic plants due to mycorrhiza formation, obviously having its basis on improved nutrition, though not of energy substrates. Usage of the term, however, is not much in vogue now and the latter two terms seem to have replaced the former.

The term 'mycorrhizal dependency' was first put forward by Gerdemann (1975) to stress upon variation in plant response to mycorrhiza formation against the main ecological variable, soil fertility. Dependency was defined as "the degree to which a plant is dependent on the mycorrhizal condition to produce its maximum growth or yield at a given level of soil fertility". Clearly, there is some merging of connotation between the two terms – 'mycotrophy' (Baylis, 1975) and 'mycorrhizal dependency' (Gerdemann, 1975) – as applied to autotrophic plant mycorrhizas. Janos (1988) recognizing the underlying homily of the two terms considered mycorrhiza dependence as an intrinsic property of a plant species or a genotype and defined it as the 'host inability for growth without mycorrhizae at a

given soil fertility'. He was in favour of use of the term 'dependency' in preference to 'mycotrophy' as apparently the trophic relation the later term connotes was misplaced. However, while mycotrophy is a subjective description, dependency is an objective measure of the relationship also.

Siqueira and Saggin-Junior (2001) in recognition of this subtle difference regarded 'mycotrophy' as an intrinsic plant trait and 'dependency' as a 'measure of the degree of dependence of a mycotrophic plant species on mycorrrhiza formation for growth relative to phosphorus status of the soil', i.e., the nutrient status of its habitat. In spite of the economic overbearance of the term 'dependency', both the terms – mycotrophy and mycorrhizal dependency, are often used to signify essentiality or otherwise of mycorrhizas for a plant species to function against evolutionary and ecological variables.

'Mycorrhiza responsiveness' is a more common term generally used to quantify variation in response to mycorrhiza formation among plant taxa (Mosse, 1973), so that the designation of a plant as mycorrhiza responsive terminologically overlaps dependency in the economic sense. However, it may be consistent with the nuances of terminology to suggest that while dependency is the cause, responsiveness may be the consequence. Janos (1988) in suggesting for distinguishing dependency (as a trait) from responsiveness (as a measure) maintained that the difference in growth response between mycorrhizal and non-mycorrhizal plants to inoculation is an expression of a plant's responsiveness to mycorrhiza under a given environment, rather than its dependency. Siqueira and Saggin-Junior (2001) maintained that while dependency refers to a plant's inherent nutrient(s) absorptive ability, irrespective of requirement or demand, responsiveness relates to its internal nutrient demand in relation to growth rate under a given environment. So far as derivation of benefits by plants due to mycorrhiza formation is concerned, some authors use the term 'responsiveness' in preference to 'dependency' (Alexander, 1989; Koide, 1991), as the former is more utilitarian in content.

Niceties of terminology apart, there exists a significant behavioural difference among plants in the essentiality of mycorrhiza formation for them to function to their optimum in natural communities or culture. On a global basis, mycorrhizas occur in 83% dicotyledonous and 79% of monocotyledonous plants and all gymnosperms are reported to be mycorrhizal (Wilcox, 1991). How

essential are the mycorrhizas for an individual plant species to function in its natural environment? Plant function in natural communities or in culture is a matter of evolution and ecology, so that understanding the basis of mycorrhizal dependency or mycotrophic behaviour of plants in evolutionary and ecological contexts is essential to exploit 'dependency' or 'responsiveness' economically. Genetics may be the keystone for such understanding.

Evolution of mycotrophy or mycorrhizal dependency

Mycorrhizas, especially the endophytic vesicular-arbuscular mycorrhiza, have a long evolutionary history. Evolution of eukaryotic cells is thought to have resulted from the repeated development of mutualistic symbioses between prokaryotes (Margulis, 1981). The ancient tradition of mutualism between prokaryotes was maintained in the early eukaryotes, so that when green plants first invaded the land, devoid as they were of roots, their ultimate success of establishment and colonization might well have been due to their establishing an intimate and mutually beneficial relationship with mycelial fungi (Pirozynski and Malloch, 1975). Fossils of Devonian plants (*Rhynia, Asteroxylon*) dating back to about 400 million years have been found to contain well preserved fungal structures virtually indistinguishable from that seen today in the roots of many healthy plants (Kidston and Lang, 1921). Similar structures occur in the rhizomes of a carboniferous fern (Andrews and Lenz, 1943) and have been recorded in the underground parts of many Palezoic, Mesozoic, and Cenozoic plants. Some of these structures resemble the structures of the present day *Endogone* (Endogonales, the earlier named order for the AM genera) (Nicolson, 1975). Kidston and Lang (1921) considered that these associations were indeed mycorrhizal. Later, Boullard and Lemoigne (1971) by re-examining some of these materials also came to the same conclusion. Structures identified as arbuscules have been found in the fossils of *Agalophyton major*, the early Devonian land plant (Remy *et al.*, 1994). *Agalophyton* is thought to be in an intermediate position between the bryophytes and early vascular plants. Spores and hyphae strongly resembling today's AM fungi were recently found in 460-million-years-old dolomite rocks from the Orodovician of Wisconsin (Redecker *et al.* 2000). This is the earliest evidence for the existence of fungi also. The Orodivician

spores are remarkably similar to today's *Glomus* type spores and have a size of 40-95 µm. Their hypahe are non-septate and 3-5µm wide.

These and similar other evidences have led to the conclusion that mycorrhizas, akin to the present day (V) A-mycorrhiza existed at the early days of land plant evolution. As new plants evolved and occupied new habitats, mycorrhizas could have evolved collaterally, so that they became widespread in distribution and relatively non-specific in nature (Nicolson, 1975). Mycotrophy or mycorrhizal dependency as observed today, hence, may have a basis in the coevolution of the early root endophytes of vascular plants similar to that what may have occurred between biotrophic fungal parasites, like the rusts and their hosts (Saville, 1971: Thompson, 1989). This would suggest that primitive land plants were in effect mycorrhiza dependent in the present day sense of the term, although structural organization of the association between the primitive autotrophic plant and heterotrophic fungus partners of symbiosis might not have been the same to that what we see today. Considering the importance of phosphorus in plant metabolism and that phosphorus might have been a limiting nutrient in emerging plant habitats (Walker, 1965), evolution of mycorrhizas as a plant adaptation for more efficient absorption of the element seems not improbable. In evolutionary sequence, mycorrhizas or the primitive root fungus associations predate root hairs (Baylis, 1972). This adds credence to the conjecture that 'as nodulation has been evolved as a mechanism of nitrogen fixation, (vesicular) arbuscular mycorrhiza might have been evolved as a means for more efficient extraction of phosphorus from the pedosphere' (Daft and Nicolson, 1969). Or, this may be the other way round, as the legume – *Rhizobium* symbiosis having been established no more than 65 to 136 million years ago, when the angiosperms evolved, seems to be much younger than arbuscular mycorrhizal symbiosis (Van Rhijin *et al.*, 1997). Evidences that common symbiosis related host genes are involved in regulating the early stages of AM and nodule formation in leguminous plants (Van Rhijin *et al.*, 1997; Albrecht et al., 1999), to be discussed in greater detail later, would suggest that the genes evolved for (V) AM symbiosis at early stages of land plant development, some 460-400 million years ago might have been conserved.

True or obligate mycotrophy, in the real sense of the term, as a mode of living is found in the so called saprophytic, achlorophyllous plants, of which there

are more than 400 species belonging to 87 genera of vascular plants (Leake, 1994). These plants are incorrectly described as saprophytes, as these heterotrophic plants are nourished by varied types of specialized mycorrhizal associations. Considering that mycotrophy as a term has more common use in case of autotrophic plant mycorrhizas, Leake (1994) preferred to call these plants as mycoheterotrophic.

The unique mode of mycoheterotrophic plant life evolved independently in widely different taxonomic groups of angiosperms showing convergence in particular adaptations to their peculiar mode of life. Mycoheterotrophic angiosperms are included in three families of the dicotyledons – Monotropaceae (including the closely related, Pyrolaceae), Polygalaceae and Gentianaceae, and two orders of the monocotyledons – Triuridales and Orchidales (Harley and Smith, 1983; Leake, 1994). The family Orchidaceae of the Orchidales is unique in that all species of its 795 described genera are heterotrophic and are nourished by mycorrhizal fungi during the early stage of their development. Although most of these subsequently become autotrophic, a significant minority fails to produce chlorophyll or never become fully autotrophic and remain entirely dependent on mycorrhizas throughout their life. Other than these groups of plants, partial mycoheterotrophy is reported in some ectomycorrhizal gymnosperms (Read *et al.*, 1985). Partial mycoheterotrophy may also be important for those members of the Pinaceae that are routinely associated with ectomycorrhizal fungi, which have the ability to absorb organic nitrogen (Abuzinadah and Read, 1989). There are some examples of mycoheterotrophy in Bryophytes, e.g., *Cryptothallus mirabilis* (Pocock and Duckett, 1984), and Pteridophytes e.g., *Schizaea fluminensis* (Mass, 1986), *Botrychium pumicola* (Camacho *et al.*, 1998).

In spite of the divergence of mycoheterotropic plants and diversity of the structure and organization of their mycorrhizas – predominantly endophytic, some comparing favourably with the (V) A- mycorrhiza (Leake, 1994) – myco-heterotrophy as a mode of living makes these plants truly mycorrhiza dependent. Taking lead from Trappe (1989), we can call these plants as evolutionarily mycorrhiza dependent, as these plants in order to survive as a species must have to form mycorrhiza sometime in their life cycle. It is remarkable to note that in most mycoheterotrophic plants, there is strong reduction of root function as in most mycoheterotrophic plant species roots are either poorly developed or small

or are entirely absent. With reductions in root development and either complete or partial absence of root hairs, resulting into minimum root surface area contact with soil, nutrient absorptive function of the mycoheterotrophic plants is most likely to be taken over by the mycelium of the symbiotic fungi (Leake, 1994), an attribute often considered as the basis of mycorrhizal dependency of autotrophic plants also (Baylis, 1975).

One plant order, the Ericales demands special attention in the understanding of mycotrophy or mycorrhizal dependency in the evolutionary context. The order Ericales that includes the already mentioned dicotyledonous achlorophyllous mycoheterotrophic plant families is wholly mycorrhizal (Harley and Smith, 1983). Besides the mycoheterotrophic herbs, the autotrophic woody shrubs and trees of the order, inhabiting strongly nutrient deficient, peaty or acidic soils, the heathlands, are also wholly dependent on mycorrhizas for their survival and form the unique group of nitrogen mineralizing ericoid mycorrhiza. The order is unique and interesting as almost all recognized forms of mycorrhizas, from typical endophytic mycorrhiza to ectomycorrhiza through the ect-endomycorrhizas, are noticed in the herbs, shrubs and trees belonging to the different families of the order (Harley and Smith, 1983). The order preserves the underlying unity in structural diversity among the mycorrhizas of today, which apparently came into existence as non-descript endophytes in the underground parts of the primitive land plants. It is in the fitness of the evolutionary trends of the fungus kingdom that the Basidiomycetes (and some Ascomycetes) replaced the primitive Phycomycetes (Zygomycetes) as mycobionts of ectomycorrhiza and the (vesicular) arbuscular mycorrhiza caused by the members of the order Glomales (Zygomycetes) may now represent the more primitive host-endophyte association, evidence of which abound conspicuously in the fossils of the early land plants. Primitivity of the (V) A-mycorrhiza gets further credence from the fact that origin of its fungal partner, the Glomales, dates back to 353-462 million years ago (Simon *et al.*, 1993), roughly coinciding with the time of appearance of the land plants. The ectomycorrhizal fungi probably evolved from saprophytic fungi after the Paleozoic era and still preserve the saprophytic capacities. Phylogenetic studies using molecular markers suggest that the holobasidiomycetes, (to which the most ectomycorrhizal fungi belong) radiated 130 million years ago and

despite the lack of paleontological data, it is speculated that the ectomycorrhizas may have a Mesozoic origin (Selosse and Le Tacon, 1998).

We may end the synthesis stating that arbuscular mycorrhizal dependency is an evolutionary trait of vascular plants acquired during early stages of land plant evolution and conserved in many families to satisfy the requirements of their survival through improvements in the efficiency of acquisition of scarce plant nutrients, like phosphorus.

Genetics of mycotrphism or mycorrhizal dependency

Genetic basis of mycorrhizal dependency or mycotrophic behaviour of plants has remained as an enigma for the biologists. The (V) A – mycorrhizal fungi in establishing mutualistic relationship with the hosts behave as obligate biotrophs showing structural resemblances with compatible (susceptible) interactions between the parasitic fungi, like the rusts and their hosts. Like the later, the arbuscular mycorrhizal fungi also do not have any saprotrophic development. The most striking difference, however, is the lack of specialization or host specificity among these fungi. While the biotrophic parasitic fungi have a narrow host range and a multitude of races, the mutualistic arbuscular mycorrhizal fungi in establishing a biotrophic relationship with their hosts have a wide host range and practically no races. The contrast raises an enigmatic situation regarding the genetic relationship between the arbuscular mycorrhizal fungi and their hosts, i.e. the seemingly co-evolved partners of symbiosis, as most known cases of either antagonistic (parasitic) or mutualistic biotrophism (including the legume – *Rhizobium* symbiosis) are restricted by host genotypes either at species or variety level. This is not to suggest that there is no variation in response in host genotypes x AM fungi interactions. Variation in response due to mycorrhiza infection has been frequently observed among different genotypes of many plant species, like, wheat, pearl millet, sorghum, maize, cowpea, chickpea, groundnut, etc. (Thompson, 1994). However, such variations are more due to plant phenotypes, not directly related to mycorrhizal symbiosis than any mycorrhiza specific genes or genotypes *per se*. However, it may be pertinent to add here that sustained research on the genetics of mycorrhiza formation over the last few years has brought out facts which suggest that plant response to mycorrhizas may depend on the genomic background of the fungus and the plant

and how these genomes interact with their environment (Franken and Requena, 2001). Presence of genes (QTLs) for mycorrhiza responsiveness is being hinted (Barker *et al.*, 2002)

The genetic basis of host – (biotrophic) parasite interactions is commonly explained by complimentarity between the host genes governing resistance (or susceptibility) and parasite genes governing virulence (or avirulence). The 'gene for gene' relationship (Flor, 1956) is the basis explaining host restriction or race specialization of the biotrophic parasites also. Apparently, the hypothesis is at cross with the arbuscular mycorrhizal symbiosis, although the other common host specific legume – *Rhizobium* symbiosis broadly fits to the 'gene for gene' relationship (Triplett and Sadowsky, 1992). But, in spite of the evident differences, so far as host relations are concerned, there may be a close underlying unity between the biotrophic parasites and the endophytic (V) A – mycorrhizal fungi (Van der Plank, 1978). Host specificity or race specialization in biotrophic parasites is determined by alleles for resistance in plants while alleles for susceptibility determine the success of parasitism. The apparent lack of host specificity or race specialization of the biotrophic arbuscular mycorrhizal fungi can be explained by the absence of resistance allele(s), that is, the presence of susceptibility alleles in plants against them. A resistant gene (allele) selects its own race and rarity of resistant genes in plant populations against (V) A-mycorrhizal fungi means rarity of (V) AM fungal races. Just as the resistant alleles in plants against the parasitic fungi are a rule, the susceptibility allele(s) may be the rule for arbuscular mycorrhizal fungi. Selection pressure in parasitic interactions leads to mutations for gain in resistance, whereas in mutualistic mycorrhizal symbioses for loss in resistance, i.e., gain in susceptibility. The recently described AM-negative or mycorrhiza resistant mutants of some legume plants and tomato (Harrison, 1999; Franken and Requena, 2001; Barker *et al.*, 2002) support the concept that plants possess susceptibility alleles for AM symbiosis and over evolutionary time the selection pressure for the arbuscular mycorrhiza was for susceptibility as opposed to the selection for resistant alleles in case of biotrophic parasites. It is significant to note in this context that one such AM defective tomato mutant (*rmc*) is colonized by some AMF species but not others (Gao *et al.*, 2001), which suggests the possibility of host restriction by resistant alleles for the AMF also.

The concept fits to the general observation that, except a few families (Fitter and Moyersoen, 1996) most plant families are susceptible to arbuscular mycorrhizal fungi. But, the non-mycotrophic nature or plant - (V) AM fungus incompatibility in some families (e.g. Chenopodiaceae, Crucifereae) might not have evolved as a consequence of direct selection against (V) AM fungi as might have happened in the evolution of resistance against parasitic fungi (Koide and Schreiner, 1992). Over evolutionary time, the association of AM fungi with temporally or spatially unpredictable plant species might have discouraged the evolution of highly host specific AM fungal species (Hoeksema, 1998), so that all such fungi have remained as 'avirulent' in the sense that no 'virulence' is needed for them to infect the hosts which are all 'susceptible'. This is a situation analogous to the prevalence of race 'O' of *Phytophthora infestans* during the days when no *Solanum tuberosum* clones had any resistance (R genes) against it (the days of the Great Irish Famine), neither there were any so called 'virulent' races of *P. infestans* (Niederhauser, 1959). The speculation gets credence from the previously mentioned observations about mutational loss of mycotrophy or mutational origin of mycorrhiza resistance (Myc $^-$ phenotype) in otherwise mycotrophic plants (Franken and Requena, 2001), which go to corroborate the concept about existence of genes for 'susceptibility' in hosts and 'avirulence' in the mycobiont as the basis of near universal host-AM fungus compatibility or mycotrophy in the plant kingdom. There is ground for further speculation that the AM fungi may have lost an essential function to the ancestral land plant genome, thus exchanging the ability to replicate independently and their fitness to survive in the congenial niche provided by the roots (Barker *et al.*, 1998). This is analogous to the proposed loss of genetic content by the chloroplast and mitochondrial genomes during endosymbiotic evolution and shall explain the lack of saprotrophic or asymbiotic development of the AM fungi in soil. However, the recent claim that an AM fungus accelerates the decomposition of and acquires nitrogen directly from the decomposing organic material (Hodge *et al.*, 2001) challenges our more than one century old belief about obligate biotrophy of the AM fungi. Fortin (1990) had conjectured that the AM fungi during the process of evolution had lost some genes, which the host plants would be able to supplement and vegetative development of the fungus would require transfer of

some short cycle genetic information from the host to allow its establishment in symbiosis.

The genetic basis of mycorrhizal symbiosis is receiving prominent research attention since the last 10-12 years. Advances in molecular biology have provided the scope for and impetus to study the possible mechanisms of genetic control of AM symbiosis at molecular level (Gianinazzi – Pearson *et al.*, 1995; Harrison, 1999; Franken and Requena, 2001; Barker *et al.*, 2002). Although the results are still fragmentary, evidences have emerged that AM development in responsive / compatible plant roots is controlled and regulated by a number of genes in both the organisms (Martin, 2001; Barker *et al.*, 2002). Some information is now available about the structure and function of some of these genes, particularly the symbiosis related host genes regulating arbuscular mycorrhiza formation in the compatible species.

Genetics of AM recognition at pre-penetration stage

The first point about genetic regulation of mycorrhizal symbiosis, as with any other host – microorganism interaction, is the possible signal transduction mechanism(s), which lead to reciprocal recognition between the partners of symbiosis during the different stages of infection leading to mycorrhiza formation. These signals are likely to be exchanged between the AM fungus and the host plant in the rhizosphere, at the point of host-symbiont attachment, or within the root itself (Koide and Schreiner, 1992) via diffusible chemicals or direct cell to cell interaction.

Arbuscular mycorrhizal infection is initiated from either spores or previously colonized root pieces – external or internal hyphae, vesicles, etc. AM fungal spores germinate normally on water but the germ tubes fail to grow and branch, unless in the vicinity of plant roots (Bonfante and Perotto, 1995). The failure of germ tube differentiation to active penetrating hypha in absence of roots may not be due to failure of DNA replication during germination and germ tube growth as presumed earlier (Burggraaf and Beringer, 1989). AM fungus spores have been shown to possess full DNA replication machinery with evidence of actual replication in absence of host roots (Bonfante and Perotto, 1995). It is unlikely that germination of AM fungal spores is under the control of any host specific inducer or elicitor molecule as these are seen to germinate freely in

axenic culture and equally in presence of host and non-host roots (Giovannetti and Sbrana, 1998). Examining their own data and the available evidence, the last named authors commented that at spore germination stage the AM fungi are unable to distinguish between hosts and non-hosts. But, pre-colonization processes subsequent to spore germination may be well under the control of cell genes regulated by signal or regulatory chemicals of host origin (Gianinazzi-Pearson *et al.*, 1995). A variety of soluble and volatile organic compounds of plant origin are available to the mycorrhizal fungi in the rhizosphere which may act as non-specific spore germination stimulators, nutrient source, chemotactic attractants and even regulatory signals for further morphogenesis and differentiation of the germ hypha before actual penetration (Gianinazzi-Pearson et al., 1989; Koide and Schreiner, 1992). Chemicals of microbial origin in the rhizosphere may also stimulate spore germination and subsequent development of germ tubes (Azcon-Aguilar *et al.*, 1986), most likely in non-specific manner.

The germ hypha of AM fungus spores undergo morphological differentiation – growth and branching, bending, swelling and production of fan like structures (Powell, 1976) or tufts (Glenn *et al.*, 1988) in close vicinity of the host roots before attachment and penetration. Root exudates of compatible hosts have been shown to cause many of these differentiations (Becard and Piche, 1992; Giovannetti *et al.*, 1994), but non-host root exudates or extracts do not stimulate hyphal growth, branching, and chemotaxis (Barker *et al.*, 1998; Giovannetti and Sbrana, 1998). These evidences would suggest that morphological differentiation of germ hypha of the AM fungus spores in the rhizosphere is under the regulatory control of host-derived chemicals. The chemical signals that have been shown to influence the hyphal responses include a variety of flavonoids and phenolics in common with that of other plant-microbe interactions (Becard *et al.*, 1992; Douds *et al.*, 1996; Harrison, 1997). Phenolics at low concentrations are important transcriptional signals in many plants – soil microbe interactions (Peters and Verma, 1990) and may activate the AM fungus cell cycle in the rhizosphere also (Bonfante and Perotto, 1995). Bonanomi *et al.* (2001) have shown that a chalcone synthase (CHS) encoding gene, *Mt-chs1* is up regulated in *Medicago truncatula* roots at a very early stage of root contact with *Glomus intraradices* even before the formation of appresorium. CHS is the enzyme catalyzing the first step committed to flavonoid biosynthesis. Flavonoids

can act as antinmicrobial compounds (e.g., medicarpin in *M. sativa*) (Harrison and Dixon, 1994), but also play a role as signaling molecules for induction of many pre-penetration morphogenetic processes of AM fungi in the rhizosphere (Tsai and Philipps, 1991).

If chemical cues, like the phenols are involved in regulating the pre-colonization responses of the AM fungi, it becomes necessary to hypothesize that the signal molecules necessary for the different stages of pre-colonization morphogenesis are produced by all mycotrophic plant species (Smith and Gianinazzi-Pearson, 1988). Alternatively, a variety of chemicals may be capable of initiating the same signal transduction pathways leading to the formation of symbiosis in different plant species by the same AM fungus. Non-impairment of symbiosis in flavonoid deficient mutants of mycotrophic maize (Becard *et al.*, 1995) raises doubt about the involvement of flavonoids as a unique signal molecule for pre-colonization responses of germ hypha in the rhizosphere of all mycotrophic plant species. It seems that signaling for AM fungus – host recognition at the pre-colonization phase is a part of the larger phenomenon of plant – microorganism recognition in the rhizosphere.

Attempts have been made to study the basis of incompatibility between non-mycotrophic plant species and the AM fungi, expecting that the apparent non-host resistance may explain the nature of signaling processes used by the mycotrophic species at the pre-colonization phase. Results of these studies do not allow any generalization, as more than one mechanism may be involved in the incompatibility behaviour of the different non-mycotrophic plant families. Presence of antifungal compounds or inhibitors (possibly derived from glucosinolates) in root exudates, as in *Brassica*, is one possible mechanism behind the incompatibility of non-mycotrophic plants. But, the same may not be applicable in other cases, like that of the members of the Chenopodiaceae (Schreiner and Koide, 1993). Recognition failure due to absence of the chemical cues or signal molecules is another likely mechanism and is probably involved in the case of the non-mycotrophic Chenopodiaceae. AM fungi have been shown to colonize non-mycotrophic hosts in close vicinity of a previously colonized mycotrophic host which would show that chemical cues needed for early recognition in the rhizosphere may be absent in non-mycotrophic hosts and once these are provided (by the colonized mycotrophic hosts) the non-mycotrophic

hosts may be freely colonized by the AM fungi (Koide and Schreiner, 1992). It is possible that the different evolutionary scenarios in plant kingdom have led to a range of mechanisms responsible for the non-mycotrophic status of these plant families.

The next important event of recognition between host – AM fungus occurs at the root surface through the formation of appressoria. It has not yet been determined whether any pre-contact stimulus is a prerequisite for appressoria to form and there is, indeed, no definite clue as yet to what triggers appressoria formation in compatible mycotrophic plants (Douds *et al.*, 1996; Harrison, 1997). Giovanetti *et al.* (1993) had earlier shown that the formation of appressoria is induced by root exudates of compatible host plants, whereas root exudates of non-mycotrohic plants, such as lupin, stimulate hyphal elongation but not the formation of true appressoria by *Glomus mosseae*. Investigation by Nagahashi and Douds (1997) indicated the possibility of involvement of some chemical signal(s), located in the epidermal cell walls of compatible host roots in appresorium formation by the AM fungi. These authors have shown that formation of appresoria of *Gigaspora margarita* can occur *in vitro* on purified epidermal cell walls of the host (carrot) root but not on the walls of a non- host (sugar beet) root suggesting the participation of signal molecules of host epidermal cell wall origin in elicitation of appresorium formation. These putative signal molecules are different from those promoting hyphal branching and are possibly some carbohydrates (Harrison, 1999). Carbohydrate molecules are known to act as signals for pre-penetrational recognition of intact host surfaces in a number of plant-fungus interactions.

Once the recognition between the mycotrophic host plant and the AM fungus has taken place on the root surface, as depicted by the formation of appressoria, the series of regulatory events that follows for the development of mycorrhiza are those related to penetration and subsequent inter- and intracellular morphogenesis of the fungal elements in the root cortical cells of the host. Investigations relating to these regulatory events are progressing in three separate domains: (i) biochemical and molecular basis of the tissue colonization process including transfer of nutrients, (ii) cellular events of plant and fungal morphogenesis during the colonization process, and (iii) developmentally

regulated changes in gene expression in the host and the fungus at different stages of infection. Although the results of these investigations are still inconclusive, some of the recent observations throw light on the genetic basis of mycorrhiza development in mycotrophic plants. Information pertaining to the genetic regulation of the colonization process and changes in gene expression in the partners during symbiosis, in so far as these are related to genetics of AM development is summarized here.

Practically no information is available about the adhesion process between the appressoria and root surface, leading to penetration of the intact surface by AM fungi. Since the appresoria that developed on the purified epidermal cell wall did not form a viable infection hypha and did not penetrate the wall, processes subsequent to appresorium formation are thought to require an intact cell (Harrison, 1999). Basically, there are two models for penetration of intact host surfaces by pathogenic fungi – the mechanical process, exhibited by the biotrophic fungi, penetrating host surfaces by hydrostatic pressure exerted by the appressoria, and the enzymatic process exhibited by the necrotrophic fungi penetrating through partially degraded cell walls. Arbuscular mycorrhizal fungi, as strict biotrophs, are believed to penetrate the root surface mostly by mechanical force (Bonfante and Perotto, 1995), with very limited facilitation by hydrolytic enzymes, if at all, as only limited amounts of cell wall degrading enzymes are produced by the AM fungi (Garcia-Romero *et al.*, 1991). Once inside the cell, hyphae progress by growing between root epidermal cells rather than by crossing their outer walls. Subsequently, most AM fungi produce intercellular hyphae which run within air channels (Brundrett and Kendrick, 1990), and then cross the wall of the cortical cells to become intracellular, producing penetration pegs and causing only limited and subtle changes in the structure of host cell wall. The slight modifications of cell wall suggest that they may produce at this stage limited amounts of cell wall degrading hydrolytic enzymes (Bonfante and Perotto, 1995). There is possibly some xyloglucan hydrolysis during cell-to-cell penetration by the AM fungi (Maldonado-Mendoza *et al.*, 1998). AM fungi are known to produce exo- and endo-glucanases, cellulases, xyloglucanases, and pectolytic enzymes including polygalacturonase (Harrison, 1999).

Genetics of AM recognition and mycorrhiza development at penetration and post-penetration stages

Recently discovered plant mutants on which the AM fungi can form appresoria but can not penetrate to develop further in the host and also those where there is colonization failure after penetration have helped advancing our knowledge regarding the genetic control of mycorrhiza formation at the penetration and post-penetration stages. To date an array of mycorrhiza negative (Myc$^-$) mutants has been described, mainly in legume hosts, most of which are defective in the earliest stage of AM formation and block fungal penetration of the root epidermis (Marsh and Schultze, 2001). These AM defective mutants are called Pen $^-$ (penetration negative) and are cited as direct evidence that host genes control actual penetration of the epidermis by the otherwise compatible AM fungi. Such mutants where AM development is blocked at penetration stage were first described from *Pisum sativum* and *Vicia faba* (Duc *et al.*, 1989), then subsequently in *Medicago sativa* (Bradbury *et al.*, 1991), *M. truncatula* (Sagan *et al.*, 1995), *Phaesolus vulgaris* (Shirtliffe and Vessey, 1996), *Lycopersicon esculentum* (Barker *et al.* 1998) and *Lotus japonicus* (Senoo *et al.*, 2000a). Common to the Pen $^-$ mycorrhiza defective mutants of all hosts is the formation of complex hyphal branching, multiple and unusually swollen abnormal appresoria as the response of the AM fungus to host resistance to penetration (Marsh and Schultze, 2001). These responses are viewed as the consequence of continued but unsuccessful attempts by the fungus to penetrate the root surface. In pea, 21 such Pen $^-$ mutants have been described which belong to at least six loci or complementation groups (Marsh and Schultze, 2001). In *Medicago truncatula*, the model legume for AM studies, the Pen $^-$ phenotype is linked to three separate loci, named *dmi1, dmi2,* and *dmi3*. (Catoira *et al.*, 2000). These observations have indicated that entry of the AM fungi into host root after appresorium formation is under complex genetic control of the host (Harrison, 1999). There is also one suggestion that both host and AMF genes play roles in recognition at this stage, as formation of multiple abortive appresoria following penetration failure of two alfalfa mutants, MN-NN1008 and MN-IN3811 occurs with one AMF species (*Glomus versiforme*) but not another (*G. intraradices*) (Bradbury *et al.*, 1991).

As regards the mode of action of these genes, evidence suggests that plant defense responses and signaling events are altered in the Pen $^-$ mutants. In one such Pen$^-$ mutant of pea (P2) at *sym30* locus (Gianinazzi-Pearson, 1996), cell wall thickening and deposition of callose and phenolics by epidermal and hypodermal cells was observed at the point of contact with abortive appresoria (Gallotte *et al.*, 1993). The level of endogenous salicylic acid and transcript levels of a number of defense related genes in P2 roots were higher than the wild type roots (Blilou *et al.*, 1999; Ruiz-Lozano *et al.*, 1999). In the Pen $^-$ *dmi 1* mutant of *M. truncatula* there is strong induction of the chalcone synthase encoding *Mt-chs 1* gene at very early stage of root contact (Bonanomi *et al.*, 2001). These evidences have shown the possibility that in such Pen $^-$ mutants defense responses have been elicited at the point of host attachment with the appresoria causing the plant cells to behave as if the invading AM fungus is a pathogen. This has allowed interpretation of the mutated genes as likely suppressors of plant defense response genes (Harrison, 1999).

In addition to the above phenomena which may be related directly to Pen $^-$ phenotype, a variety of other effects are seen in these mutants. Notable among these is the defective calcium spiking of root hairs in many such Pen $^-$ mutants, like the *dm1* and *dm2* in *M. truncatula* and *sym8* and *sym9* in *P. sativum*. Calcium spiking in root hairs is one of the earliest responses of *Rhizobium* nodulation factors (Wais *et al.*, 2000; Walker *et al.*, 2000). This raises the question as to whether calcium spiking is stimulated by AM fungal signals also and at least some of the mutant alleles are responsible for recognition of the AMF signals by the host (Marsh and Schultze, 2001). There is additional evidence form the *sym8* pea mutants (E 140, R 19 and R 25) that the gene product SYM8 is essential for the induction of early nodulation (*ENOD*) genes, *PsENOD5* and *PsENOD12* in roots inoculated with *Gigaspora margarita* and *Rhizobium* (Albrecht *et al.*, 1998), suggesting for at least partial homology of the early recognition process for arbuscular mycorrhiza formation and *Rhizobium* nodulation of legume plants. The Pen $^-$ *rmc* mutant of tomato (Barker *et al*, 1998) becomes interesting in the context, as being a non-legume its mutated gene may be apparently specific to AMF symbiosis without any relation with the regulatory processes induced by the early nodulation genes for AM development in the legumes (Marsh and Schultze, 2001).

The genetic basis of subsequent tissue colonization process and morphogenesis of functional mycorrhiza where early studies and empirical observations suggested the regulatory roles of host genes is now being precisely studied by employing both mutation and molecular biology tools. Direct evidence in support of host genes controlling arbuscular mycorrhiza development at post-penetration stage was provided by the early studies with some legumes, which carried mutations for absence of nodules (Nod $^-$ phenotype). It was first shown that the Nod $^-$ mutants of pea (Duc *et al.*, 1989; Gianinazzi-Pearson *et al.*, 1991) and alfalfa (Bradbury *et al.*, 1991) were Myc$^-$ (absence of AM colonization) also. Most of the Myc $^-$ legume mutants isolated till date share the common origin from the pre-existing Nod $^-$ mutant pool. The arbuscular mycorrhiza negative (Myc $^-$) legume mutant lines (including the Pen $^-$ mutants) described till now represent nearly 40 mutations in at least 20 separate loci and constitute roughly the half of the total Nod $^-$ loci from which they were selected (Marsh and Schultze, 2001). In 21 of the 45 Nod $^-$ mutants of pea and vetch diallelic crosses have shown that the Myc $^-$ and Nod$^-$ phenotypes cannot be uncoupled indicating that both the phenotypes are derived from mutations of the same genes (Gianinazzi-Pearson *et al.*, 1991). This has shown that these legume mutants are affected in genes that play a role in both arbuscular mycorrhiza formation and *Rhizobium* nodulation and at least a part of the gene products required for AMF and *Rhizobium* colonization at the early stage are shared by the two distinct symbioses. The remaining Nod $^-$ mutant lines carry mutations specifically for *Rhizobium* symbiosis but do not alter mycorrhiza formation.

In contrast to Pen $^-$ mutants in which the infection process aborts following appresorium formation, a large collection of mutants have been described mainly in *Lotus japonicus* which are blocked prior to cortex invasion (Coi $^-$). These are also called late Myc $^-$ in the sense that mycorrhiza formation is blocked at late developmental stage (Wegel *et al.*, 1998; Bonfante *et al.*, 2000; Parniske *et. al.*, 2000). Complementation analysis has revealed that mutations in at least 6 distinct loci (*Ljsym2, Ljsym3, Ljsym4, Ljsym5 Ljsym23,* and *Ljsym30)* result in the Coi $^-$ phenotype in *L. japonicus* characterized by arrested hyphal development following epidermal cell penetration. Bonfante *et al.* (2000) showed that fungal penetration of one or more epidermal cells prior to intercellular development is a prerequisite for normal colonization and arbuscule development. In the above

named mutants epidermal cell penetration leads to death of both the host cell and fungal hyphae (Marsh and Schultze, 2001), a phenomenon reminiscent of the hypersensitive reaction in the incompatible host-pathogen interaction.

Besides the Coi $^-$ phenotype, a number of genetic lesions have been identified which affect intercellular hyphal proliferation to varying degrees. These include phenotypes in which inner cortex invasion does not occur (Ici $^-$) as in the *mcbee* (mycorrhiza colonization blocked between epidermis and exodermis) and *mcbex* (mycorrhiza colonization blocked in exodermis) mutants of *L. japonicus* (Senoo *et al.*, 2000b). In other mutants, normal colonization of inner cortex is not followed by arbuscule formation (Arb $^-$), as exemplified by the R69 mutant of *P. vulgaris* (Shirtliffe and Vessey, 1996) and the Nod$^+$/Fix $^-$, MN-IN3811 mutant of alfalfa (Bradbury *et al.*, 1991). Resendes *et al.* (2001) have recently described another mycorrhiza defective phenotype in a low nodulating mutant of pea (E107) which is low pen, low coi but Arb $^+$. Molecular basis of these mutant phenotypes have not been described yet.

Compared to these, two mutants have been described in which the phenotype appears to be restricted to an effect on arbuscule development (Ard $^-$). In RisNod24 mutant of pea, following normal hyphal development in the cortex only truncated, abnormal appresoria are formed (Gianinazzi-Pearson, 1996). The RisNod24 mutant is mycorrhiza defective only in the later stage of colonization. In contrast, the *mcbco* (mycorrhiza colonization blocked in the cortex) alleles of *L. japonicus* form arbuscules which are normal in appearance but senesce prematurely (Senoo *et al.*, 2000b). Increased expression of the gene *Psam4* which is predicted to encode a proline rich protein has been reported in the RisNod24 mutant (Lapopin *et al.*, 1999). Proline rich proteins (normally down regulated in AM) are associated with defense against plant pathogens.

A clear picture of post-infectional regulation of AM symbiosis by the host genes and the possible pathways of signal transduction for expression of the genes controlling symbiosis was provided by a study again with a legume where molecular basis of nodule formation is somewhat better understood. *Rhizobium* nodulation in legumes is under the control of a series of genes where the early nodulin (*ENOD*) genes are characteristically expressed during the early stages of nodule morphogenesis. Van Rhijn *et al.* (1997) have shown that transcripts of two early nodulin genes of alfalfa (MsENOD 40 and MsENOD 2) are found in non-

nodulated mycorrhizal roots, but not in non-AM colonized roots or in roots infected with the fungal pathogen, *Rhizoctonia solani*. The same two early nodulin genes are expressed in uninoculated roots upon application of the cytokinin, 6-benzylaminopurine. Increased accumulation of cytokinin in alfalfa roots linked with induced expression of the two early nodulin genes have been demonstrated to occur in AM symbiosis, nodulation, but not in response to pathogen infection. Mycorrhizal roots were shown to contain higher levels of trans-zeatin riboside (cytokinin) than non-mycorrhizal roots. These results have been interpreted to suggest that the early nodulin genes of legumes are symbiosis related genes and there are commonalties in signal transduction pathways between phosphate acquiring mycorrhiza and nitrogen fixing nodules. The signal transduction pathways for *Rhizobium* nodules and arbuscular mycorrhiza seem to be evolutionarily conserved and plant hormones, like the zeatin riboside-type cytokinin may trigger for early nodulin gene induction in both nitrogen fixing and mycorrhizal interactions. These evidences would suggest an underlying unity in the genetic pathways of symbiosis development between the two symbiotic systems - arbuscular mycorrhiza and *Rhizobium* nodulation.

The commonality of genetic pathways between the early stages of AM and *Rhizobium* symbiosis has been indicated by a few more evidences also. Balestrini *et al.* (1999) showed that the gene *PsNlec1*, which encodes a lectin like glycoprotein and is expressed in the root nodules of pea, was also detected in roots colonized by AM fungi. In both symbioses, gene expression was limited to infected cells possessing fully differentiated symbiotic structures. Fruhling *et al.* (1997) showed the induction of a novel leghaemoglobin gene *VFLb29* in mycorrhizal broad bean roots but its functions could not be understood. Morandi *et al.* (2000) have shown that supernodulating mutants of *Pisum sativum* and *Medicago truncatula* showing hyper nodulation as compared to the wild types were hyper responsive for AM colonization also. Similarly the low nodulating pea mutant E107 was found to be low in AM also and control of both the low symbiosis phenotypes was present in shoot as compared to the other AM defective Nod $^-$ mutants where control of the two phenotypes (Nod $^-$ / Myc $^-$) was present in root (Resendes *et al.*, 2001).

These evidences point out that morphogenesis of functional arbuscular mycorrhiza in mycotrophic plants is under the control of a number of host genes

which are expressed at different stages of mycorrhiza development in a coordinated manner. These genes, identified overwhelmingly from legume hosts are empirically called symbiosis related (SR) or *sym* genes and have a general spectrum of action in relation to symbiosis, as their mutations affect two types of symbiotic plant – microbe interactions, *Rhizobium* nodules and AM mycorrhiza in leguminous hosts. The SR (*sym*) genes in pea, however, do not regulate root infection by pathogenic microorganisms which further establish the specificity of SR gene function in symbiosis only (Bonfante and Perotto, 1995). Although most of the Myc $^-$ legume mutants so far described are in some way or other Nod $^-$ also, not all Nod $^-$ mutants are Myc $^-$. By analogy, we can expect that there may be a number of AM mutants (not identified yet, except a few), which will be specific to mycorrhizal symbiosis only, not alone in legumes but other non-leguminous plants also (Marsh and Schultze, 2001). It is also observed that the *sym* genes, till now identified in legumes, that are required for interaction with both AM and *Rhizobium* as symbionts, are most probably widespread in the plant kingdom and not specific for the legumes (Albrecht *et al.*, 1998). Reddy *et al.* (1998) observed that *Rhizobium* Nod factors can activate a *Medicago* ENOD 12 promoter in transgenic rice which showed that *Rhizobium* Nod factors can be recognized by a non-legume also. Earlier, sequences of the *ENOD40* legume gene, which is expressed in AM symbiosis, were reported to be present in a non-legume, namely, tobacco (Van de Sande *et al.*, 1996). Reddy *et al.* (1999) examining 80 accessions representing 23 species from the genus *Oryza* reported the universal presence of the homologues of early nodulin genes (*ENOD*) and observed that biological functions of the early nodulins (ENODs) may be diverse, and not restricted to nodule organogenesis alone. A concept is gradually gaining ground from these evidences that there exists a set of fundamental plant genes, the functions of which are utilized in various combinations to effect novel outcomes, like *Rhizobium* nodulation and AM symbiosis (Barker *et al.*, 1998).

Regulation of nutrient transporters in arbuscular mycorrhiza

In response to persistent P_i - deficiency, plants have evolved several mechanisms to enhance P_i –mobilization and its subsequent acquisition. Secretion of phosphatases, organic acids, and protons increase the mobility of soil P_i (Duff et al., 1991) while enhanced root growth and modification of root

morphology increases the root surface area with which to explore a larger volume of soil (Lynch, 1995). Majority of the terrestrial plants forming arbuscular mycorrhiza benefit from the P_i acquired via these fungi (Harrison, 1999). The genetic basis of the P_i –acquisition mechanism of the mycorrhizal plants is being studied to know how the improved nutritional physiology of mycorrhizal plants is regulated.

There is a considerable concentration gradient of inorganic orthophosphate (P_i), the plant accessible form of P, between the soil solution and plant / fungal cytoplasm. In the former concentrations rarely exceed 10 μ*M* (Bieleski, 1973), whereas in plants the cytoplasmic concentration is probably around 10m*M* (Mimura, 1999). To absorb P_i under these conditions plants and soil microorganisms have developed specialized energy dependent transport systems (proteins) at the root/soil interface for uptake of P_i (Schachtman *et al.*, 1998). Two such transport systems having high affinity (*Km* 3-7 μM and low affinity (*Km* 50 - 330μM) for soil P_i are known. Both high-affinity (high soil P_i repressible) and low-affinity (constitutive) P_i –transporter genes have been identified, cloned and sequenced from plants and hyphae of AM fungi (Veersaw *et al.*, 2002). Genetic regulation of these transporter systems under AM symbiosis are being actively studied now.

Study of plant P_i —transporter gene expression in AM was initiated as soon as the first of these genes were cloned from plants (Smith et al., 1997). It has been found that high-affinity P_i –transporter genes of plants are down regulated in mycorrhizal roots not receiving high levels of P-fertilization (*Liu et al.*, 1998; Rosewarne *et al.*, 1999). The same genes were also down regulated in non-AM colonized roots receiving high levels of P-fertilization. *MtPT1* and *MtPT2* are two such high-affinity P_i – transporter genes which are expressed in roots but not in leaves of *M. truncatula* and are expressed in high order under P_i starvation. Transcript levels of these two genes were seen to decrease during the development of AM symbiosis with either *Glomus versiforme* or *G. intraradices.* Such decline was detected at very early stage of colonization and continued during development of the symbiosis. A similar response was observed for the P-starvation-inducible plant Mt4 gene in mycorrhizal roots of *M. truncatula* in response to a signal from the fungus (Burleigh and Harrison, 1997). The down regulation of the transporter genes following AM colonization may result from

signals from the fungus or may be an indirect consequence of increased P_i levels in the plant (Chiou *et al.*, 2000; Liu et al., 1998). These and similar such observations have led to the suggestion that plant P_i – transporter genes are down regulated by AMF colonization due to the improved P-nutrition of the plant resulting from symbiosis (Burleigh and Bechmann, 2002). In this model, a plant perceives an increase in cellular concentrations of P_i due to both its own uptake and fungal supply, and correspondingly reduces the expression of its own transporter genes to maintain an optimal level of P within the plant.

It is now reasonably argued that the down regulation of P-starvation-inducible plant genes within mycorrhizal roots is primarily an indirect effect as a result of the transfer of P_i from the fungus to the plant (Burleigh and Bechmann, 2002). Examination of the spatial expression patterns of *MtPT1* have revealed that the transcript and the encoded protein were localized specifically in the epidermal cells and root hairs and the protein was present in plasma membrane of roots grown under low phosphorus conditions (Veersaw *et al.*, 2002). This would suggest that this transporter gene plays a role in uptake of P_i from soil solution. The tomato P_i transporter *LePT1* transcript and protein showed a similar expression and subcellular localization. But, transcripts of this gene were also found in cells containing arbuscules, stele and leaf parenchyma cells. Rausch *et al.* (2001) reported that under limiting conditions of P-availability a P_i –transporter gene *StPT3* is up regulated in arbuscule containing root cortical cells of potato with simultaneous down regulation of the two common transporter genes*StPT1 and StPT2*. These observations would show that different plant transporter genes may have distinct physiological roles during different stages of symbiosis (Rosewarne *et al.*, 1999; Versaw *et al.*, 2002). Evidence is now also available to show that besides P_i –transporter genes, expression of other plant nutrient transporters, like that of Zn and NO_3 are also down regulated during symbiosis (Burleigh and Bechmann, 2002).

Changes in expression of defense response genes

Possible activation of defense related host genes under AM symbiosis happens to be one major area of interest in exploring the molecular basis of AM development. Perhaps, the knowledge gained becomes important to understand the often claimed host resistance to pathogens induced by mycorrhiza

(Schoenbeck, 1979; Giovannetti *et al.*, 1991; St- Arnaud *et al.*; 1996; Graham, 2001). It is now well known that plants rapidly activate their defense genes, when challenged by incompatible fungi, whereas such responses are weaker and delayed when the plant and the fungus are compatible. Considering that AM fungus - mycotrophic host combinations exhibit universal compatibility, structural and biochemical responses akin to host defense, as in case of host-pathogen interactions are likely not expected in arbuscular mycorrhiza. But, there is disruption of the middle lamella during intracellular AM fungus growth, which being a wounding event is expected to trigger the host's defense responses. The genes which are generally responsible for host defense against parasitic microorganisms are those encoding: fungal cell wall degrading enzymes, such as, chitinases, ß-1, 3 glucanases; flavonoid and phytoalexin biosynthesis pathway enzymes, such as, phenylalanine ammonia lyase, chalcone synthase, chalcone isomerase; proteins like hydroxyproline rich glycoproteins which together with ß-1, 3 glucans reinforce cell walls.

There have been many investigations during the 1990s on the expression of these defense-related genes under AM symbiosis. Thus far, the evidence that mycorrhizal colonization conditions systemic resistance to other pathogens, such as through up-regulation of pathogenesis related proteins, has remained controversial (Blee and Anderson, 2000; Graham, 2001). The main conclusion that is made from these studies is that although small and transitory increases in the expression of these genes do occur, there is no case for any significant or extended induction of a defense response by inter-or intercellular growth of the AM fungus in mycotrophic plants (Harrison, 1997; Barker *et al.*, 1998). Bianciotto *et al.* (1998) with transgenic tobacco plants expressing antifungal 'defensin' protein against pathogens, have shown that constitutive expression of such defense related gene(s) may not interfere with AM symbiosis. Whether the commonly observed transient increase in some of these defense genes is the result of the lack of elicitation of plant response by AM fungi, or because of the plant mechanisms that can counteract the possible elicitors is not well settled yet. It is possible that AM fungi initially produce elicitors, which cause the observed transitory induction of several defense – related genes. Production of these elicitors may be later suppressed by the signaling molecules, which might act as suppressors (Bonfante and Perotto, 1995). Suppression of defense related genes

can also occur at later stages of AM development (Graham, 2001). Phytohormones, whose concentrations change in mycorrhizal roots, might act as long distance signal molecules for such purposes (Bonfante and Perotto, 1995). The fact that a mild induction of defense response genes continues to occur as the AM fungus grows through the root, rather than only at the appresorium, suggests that the AM fungus does not elicit a general signal through the root system to completely suppress plant root defenses. The recognition process must then be initiated with each new cell contact to result in suppression of the defense response (Barker *et al.*, 1998). That the AM infected root cells are not sensitized to become hypersensitive to subsequent pathogenic root infection supports the observation of a lack of any general signal for root defense against AM colonization.

Although the genetic pathway of AM symbiosis is shared in part by other similar root-microbe symbioses, there is no evidence of enhanced resistance to pests or pathogens in any legume or any other mycorrhiza defective mutants, or conversely, pest and pathogen resistance genes shown to confer resistance to mycorrhiza formation (Barker *et al.*, 2002). On the contrary, two reports have shown that two separate AM resistance mutations in *Lycopersicn esculentum* did not alter host response to other microbes tested (Barker *et al*, 1998; Schwartz – David *et al.*, 2001). Interestingly, another report has shown that several *Hordeum vulgare* mutations leading to increased susceptibility to *Erysiphe graminis* are correlated to increased resistance to AM symbiosis (Ruiz-Lozano *et al.*, 1999).

The mycorrhiza defective legume mutants, especially the Pen$^-$ and Coi$^-$ mutants described earlier are examples of plants gaining resistance against colonization by AM fungi. These mutants are also the tools for making fundamental insights into the genetics of host-parasite interactions related to resistance / susceptibility. Recent ultrastructural observations of the interaction between the Myc$^-$ host mutants and AM fungi are suggestive of elicitation of responses akin to plant disease resistance responses. Observations include cell wall thickening and deposition of wall reinforcing substances, cytoplasmic emptying, or increased cell wall autofluorescence localized to the site of attempted penetration by the AM fungus (Bonfante *et al.*, 2000; Gao *et al.*, 2001; Peterson and Guinel, 2000; Senoo *et al.*, 2000b). Given this resemblance of mutant host-AM responses to disease resistance responses of plants it might be

expected that the outcome stems from an equivalent defense response signal transduction pathway. However, the nature of biological interaction of the Myc$^-$ host mutants is the converse of the plant disease resistance or R genes. The early symbiosis negative host mutations studied till now are all recessive suggesting for loss of function is the cause for the loss of host mycorrhizal capacity. In contrast, loss of function of a receptor –kinase pathway disease resistance gene enables the pathogen to grow in the susceptible (r) host (Barker *et al.*, 2002). Therefore, if receptor kinase type function is to operate as the basis of myctrophic host –AM recognition at early stages of symbiosis, then triggering of the defense response pathway either does not occur, or is repressed by other plant and / or fungal genes. That would explain the lack of any significant defense response in compatible host-AM interactions.

There are many more areas of research where advanced tools of molecular biology, cytology and cytochemistry are being utilized to elucidate the mechanism(s) of development of this ancient form of symbiosis. The way these investigations are progressing generates optimism that a clear picture of the genetic pathway(s) of arbuscular mycorrhiza development may soon be available with implications for understanding of not AM symbiosis alone but also other plant-microorganism interactions in general and plant disease resistance or defense mechanisms in particular.

Chapter-III Materials and Methods

Experimental work presented in the thesis was carried out in the Mycorrhiza Research laboratory of the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal. Materials used, methods followed and details of individual experiments are given below.

1. Soils, their source and properties

Two different types soil were used throughout the course of investigation for all experimental purposes. One was laterite soil, collected from the University Experimental Farm of the Red-laterite Zone Research station, Jhargram, West Midnapore, West Bengal; the other was an alluvial soil, collected from barren uplands of the University Farm at Kalyani (New Alluvial Zone), West Bengal. Soils were collected from selected locations from 20 cm. upper surface. Riverine sand was mixed in varying proportion with either of these two soils for bringing appropriate texture of the potting mixture for seedling raising, multiplication and maintenance of AMF cultures etc. Wherever necessary well rotten cow dung compost was added to the soil to bring appropriate structure of the potting compost. Soils, sand and compost were preserved on a covered cemented floor in the nursery and kept covered with polythene sheet. Physical and chemical properties of the soils used are mentioned below.

Physical and chemical properties of the experimental soils

Location and soil type	Sand	Silt	Clay	pH	Organic C%	Total N %	Available P (ppm)	Available K (ppm)
Sandy loam laterite soil, Red-laterite Zone, Jhargram	56.7	8.0	35.3	5.8	0.27	0.03	4.7	13.5
Silty loam Alluvial soil, New Alluvial Zone, Kalyani	40.4	36.4	23.2	6.8	0.49	0.05	14.8	25.0

2. Rice varieties and source

Eight varieties of rice were used for all experiments throughout the investigation. These varieties were selected from a collection of large number of varieties obtained from different sources, on the basis of their growth habit in the above two soils in the nursery. The selected varieties belonged to 3 categories – land race selections, improved traditional varieties and modern high yielding varieties which were widely different in their genealogy. Descriptions of the varieties are given below

The selected rice varieties used in the investigation

Name of the variety	Collected from	Type of variety
Black Gora	Central Upland Rice Research Station, CRRI, Hazaribag, Jharkhand	Landrace selection
ARC 12737	Assam; through CRRI, Cuttak	Landrace selection
Yamuk	North-Eastern Research Complex, Regional Station, ICAR, Arunachal Pradesh	Landrace selection
Jhingasail, Dular	Rice Resarch Station, Chinsurah, West Bengal.	Improved local varieties
TN-1, IR 64, MTU 7029	BCKV Rice Research Station, Chakdah, Nadia, West Bengal.	High Yielding dwarf hybrid selections

The plant type characters as measured under field conditions of the University Farm (moderately fertile alluvial soil) and sources of the selected 8 varieties are given in Table next page.

Plant type characters and sources of the 8 selected rice varieties

Variety name	Parentage / Location	Plant height (cm)	Tiller No.	Days to flower	Days to mature	Seedling characters 20-25 days
(V1) Black Gora	Local inbred selection, Chhotonagpur Plateau; Dry upland, direct seeded	145-150	6-7	60-65	95-100	
(V2) ARC 12737	Land Race, Assam; Wetland, direct seeded	145-155	7-9	60-65	90-95	
(V3) Yamuk	Land race, Arunachal Pradesh; Wetland, direct seeded	135-140	5-6	55-60	90-95	
(V4) Jhingasail	Local inbred selection, West Bengal; Wet land, transplanted	118-120	8-10	80-85	130-135	
(V5) Dular	Dumai x Larkoch (local *'Aus'* selection), West Bengal, early transplanted/direct seeded	110-115	7-8	60-65	105-110	
(V6) Taichung Native 1	DGWG x Tsai Yuan Chung, Taiwan SE Asia, early generation dwarf; wetland transplanted	85-90	7-8	50-55	110-115	
(V7) MTU 7029	Vasista X Mashuri, Andhra Pradesh, dwarf HYV; low wetland transplanted	80-85	12-15	75-80	145-150	
(V8) IR 64	IRRI selection, wetland semi-dwarf; wetland transplanted	85-90	12-15	80-85	145-150	

3. Other plant species

For routine maintenance and multiplication of the AM fungal cultures in plant association, maize (*Zea mays* L.) Wilczek, sorghum (*Sorghum bicolor* (L.) Moench), moong bean or green gram (*Vigna radiata*(L.) Wilezck), red gram (*Cajanus cajan* Milsp.) and onion (*Allium cepa* L.) were used. Seeds of these plants were procured from the University Farm. Sun dried seeds of all plants were preserved over dry silica in desiccators.

4. Mycorrhizal inoculum – source and composition

Arbuscular mycorrhizal fungal inocula used for the experiments were obtained from the culture collection of the laboratory maintained as single species AMF cultures in root association of mycotrophic host plants. Mixed species AMF inocula constituted with root based cultures of the species *Glomus mosseae* (Nicolson & Gerdemann) Gerdemann and Trappe, *Glomus faciculatum* (Thax. Sensu Gerd.) Gerdemann and Trappe, *Glomus aggregatum* Schenck & Smith *and Gigaspora margarita* Becker & Hall were used. The species isolates were collected from herbaceous (including upland rice) and tree plants of the Red-laterite Agroclimatic Zone of West Bengal and maintained in the laboratory as single species isolates in sterile sand soil mixture under isolation. AM efficiency of these isolates in terms of root infection intensity and plant growth response were previously determined by previous workers of the laboratory (Bhattacharya 1999; Paul,2002).

5. Stains, reagents and solutions

All chemicals used were analytical grade of different standard manufacturers. Names of the manufacturers of rare chemicals are given where these appear.

Stains for mycorrhizal fungi in root association

Trypan blue (E. Merck) – 100 mg in 100 ml lactophenol

Chlorazol Black (E. Merck) - 1% Chlorazol Black E, 85% lactic acid and glycerol (1:1:1)

Forma-acetic-alcohol (FAA) solution for root fixation

Formalin – 13 ml, Glacial acetic acid – 5 ml, Ethyl alcohol (50%) – 200 ml

Alkaline H_2O_2 as bleaching agent for pigmented roots

H_2O_2 (10%) - 3ml, NH_4OH -0.3ml, Distilled water - 56.7ml

Hypochlorite solution for seed surface sterilization

5% NaOcl solution

Solutions for surface disinfection of AMF spores (Watrud, 1984)

Chloramine-T 2-5% (w/v)	3-20 minutes
Streptomycin sulfate 0.025%	20 minutes
Gentamicin sulfate 0.01%	20 minutes
Tween 20 0.05%	as surfectant

Hogland's nutrient solution for plant growing

Solution I	**Solution II**
KNO_3 -1. 02 g	H_3BO_3 -2.86 mg
$Ca(NO_3)_2$ - 0.492 g	$MnCl_2$, $4H_2O$ - 1.81mg
$MgSO_4$, $7H_2O$ -0.49 g	$ZnSO_4$, $7H_2O$ - 0.220mg
KH_2PO_4 - 0.50 g	$CuSO_4$, 5H20- 0.080mg
Distilled water – I liter	$NaMoO_4$- 0.090 mg
	Distilled water-1 liter

Before use, solution I was diluted 20 times and solution II was added at the rate of 1ml/lt. Whenever necessary, phosphorus was omitted by eliminating KH_2PO_4 from the solution. Further dilution of the nutrient solution was made as needed.

Buffer solutions

0.2 M Phosphate buffer

Solution A: 27.8 g Monobasic Sodium Phosphate in 1000 ml

Solution B: 53.65 g Dibasic Sodium Phosphate 5 hydrated in 1000 ml.

Stock solutions were mixed as follows to prepare buffer solutions for different purposes.

Purpose	pH	Solution A	Solution B	Final Volume
Soluble protein extraction from rice roots	6.8	51.0 ml	49.0 ml	200 ml
Peroxidase extraction from rice roots	7.1	39.0ml	61.0ml	200 ml

Reagents and solutions for root protein estimation (Lowry *et al.*, 1951)

Reagent A	2% sodium carbonate in 0.1N Sodium Hydroxide
Reagent B	0.5% Copper sulfate in 1% Potassium sodium tartrate
Reagent C	50 ml of Reagent A mixed with 1ml of Reagent B prior to use
Folin-Ciocalteau Reagent	The 2N commercial product (SRL) was diluted to1N with distilled water
Standard protein solution	50 mg bovine serum albumin (SRL) dissolved in 50 ml distilled water
Working standard (protein)solution	10 ml of standard protein solution was diluted to 50 ml with distilled water to give 200µg protein / ml

Reagents and solutions for estimation and gel electrophoresis of peroxidase

Spectrophotometric estimation of whole root peroxidae (Sadasivam and Manickam 1996)

A. By O-Dianisidine method

Phosphate buffer	pH 7.0
O-Dianisidine (E. Merck)	1.0 mg/ml methanol
Hydrogen peroxide solution (0.042% = 12.3 mM)	0.14 ml of 30% H_2O_2 in 100 ml distilled water. Prepare freshly.

B. By Guaiacol method

Phosphate buffer	pH 7.0
Guaiacol solution 20 mM	240 mg guaiacol (SRL) in100 ml distilled water.
Hydrogen peroxide solution (0.042% = 12.3 mM)	0.14 ml of 30% H_2O_2 in 100 ml distilled water

Gel casting solutions and buffers for polyacrylamide gel electrophoresis of peroxidase (Kahler and Allard, 1970)

Stock solution	Chemicals	Amount	pH
A. Stock Acrylamide solution	Acrylamide (SRL) Bis-acrylamide (SRL) Distilled water	14.6 g 0.4 g 50 ml	-
B. Tris – Hcl buffer	Tris (SRL) Distilled water	18.17 g 100 ml	8.8
C. Polymerising reagent	Ammonium persulfate Distilled water TEMED (Sigma)	0.15 g 10 ml Fresh from refrigerator	- -
D. Tank buffer	Tris Glycerine Distilled water	3.025 g 14.413 g 100 ml (Final volume 1 lt.)	8.2 to 8.4
E. Dye solution	Bromo-phenol blue (Sigma) Glycerol	0.1 g 1 ml	-
F. Stainer (Wettwer & Dyck, 1983)	a) O-Dianosodine b) Acetic acid c) Distilled water Hydrogen peroxide (30%) (a+b+c mixed and kept in dark. H_2O_2 added before observation)	0.1 g 1.0 ml 100 ml 0.1 ml	-

Gel preparation

The casting solutions and buffers were mixed in following proportion to cast a 16 cm x 14 cm x 1.5 cm slab gel

A. Stock Acrylamide solution 11.25 ml
B. Tris – Hcl 5.62 ml
C. Ammonium persulfate solution 3.0 ml
D. TEMED 0.15 ml
E. Distilled water 25.8 ml

6. Sampling methods and sample preparation

Soil - Soil samples were collected from field locations or pot cultures, generally from 0-15 cm soil depth. Replicate soil samples were bulked, air dried and sieved (<0.050mm), then preserved for physical, chemical and mycorrhizal analyses.

Plant - Roots were collected from whole plant harvests of experimental pots in appropriate replicates. Washed roots were processed and preserved according to the study purpose. For routine analysis of mycorrhizal infection, only finer branch roots were taken as samples. For early age seedling plants, all the branch roots of whole plant harvests in replicates were collected, bulked and preserved in FAA. Estimation of root infection intensity and assessment of histological details of AMF infection were done with sub-samples of these bulked samples. For late age plants with larger mass of roots, the whole plant root mass of a single plant was spread over squared paper and all finer roots from 3 random 1 cm^2 quadrants were collected, bulked and preserved separately for single plants for further analysis. For analysis of root development pattern, whole root mass samples of individual plants in replicates were examined immediately or preserved in FAA till use. For chemical analysis, whole root samples of individual plants or replicate bulks were oven dried, powdered, mixed well and preserved over dry silica till use. For biochemical analysis, whole roots of appropriate replicate number of plants were used fresh after thorough washing and air drying over filter paper pads. For all analyses, roots sampled from minimum 5 and maximum 15 treatment pots were considered as replicates.

For analysis of shoot mass yield and shoot nutrients content the above ground parts of the plants were harvested on per pot basis (1-5 plants/pot) and after oven drying as separate or bulked samples were powered, sieved and preserved over dry silica.

7. Soil and planting mixture sterilization

Steam sterilization - Soil, sand, organic matter and potting mixture were sterilized by autoclaving at 15 lbs psi for 1hr, for two consecutive days.

Formaldehyde treatment - Used for bulk sterilization of soil, sand, organic matter and potting mixtures. Over a polythene sheet 3 cm layer of soil was

spread and drenched thoroughly by spraying with 4% formalin solution. Three to four such layers were made, spraying each layer with formalin. The sprayed mass of soil layers was covered with another polythene sheet and kept for 20 days. The polythene cover was removed the soil was turned and mixed thoroughly and then exposed to air to remove traces of formalin for another 15-20 days. The treated soil was ready for use within 40-45 days of treatment. Treated soil was stored in large covered plastic buckets till use.

Soil solarization - For partial sterilization soil was treated by solarization during spring –summer season (March to June). In an open place of the nursery soil was spread thinly over a polythene sheet on a cemented floor and moistened with water to 60-70% WHC and then kept covered with black polythene sheet for 40-50 days with working and turning at regular intervals. Soil temperature inside the sheet varied between 55-60 °C. After removal of the cover, the soil was dried in sun, mixed and sieved and then stored in plastic buckets

8. Seed germination and plant growing

Seeds were preserved in dry silica desiccators in a cool and dry room. For germination, disease free, well sized, bold seeds in lots having equal 100-seed weight were surface sterilized with hypochlorite solution for 5 minutes. Thoroughly washed seeds were soaked in sterile water overnight. Soaked seeds were spread over moistened absorbent cotton pads in sterile Petri dish and incubated at 28 – 30 °C. Seed germination usually occurred in 24-30 hrs. Seeds just ruptured, were picked with forceps and spread thinly over desired moistened growing medium in pots and covered thinly with sand. Emergence occurred within the next 24-30 hours. When same age, similar vigour seedlings were required, any seedling emerging late after the desired number of sprouts was obtained was thinned out.

All experimental plants were grown in pots over cemented racks in a partially covered net house under ambient light, temperature and humidity. For AMF inoculation experiments, especially for the purpose of biochemical analysis, small volume plastic cups were kept covered under transparent fine polythene nets. Irrigation was provided with fresh demineralized water. Details of the methods of plant growing are mentioned under individual experiments.

9. Inoculum preparation, maintenance and multiplication

Starter Inoculum - Spores of the individual species of AM fungi maintained in the laboratory in maize root association in sterile laterite-coarse sand mixture in isolation were harvested. Standard floatation – decantation - wet sieving (Gerdemann and Nicolson, 1963) and sucrose density gradient centrifugation techniques (Daniels and Skipper, 1982) were used to isolate the AMF spores from the roots and root associated soil. Isolated spores were surface disinfected by the standard method (Watrud, 1984; Schenck and Perez (1990). Surface disinfected spores were germinated in *Paspalum* millet /maize/onion root association in small volume sterile sand-soil columns in 4-6 inch funnels kept within fine nylon mesh cages under partially controlled set - up for 3-4 weeks. Plants were irrigated with sterile water. Harvested plants were examined for AMF infection- structures and absence of non- AMF fungi. AM-Infected roots after air drying and fragmenting were used as the primary or starter inoculum for preparation and production of bulk inoculum.

Inoculum production and multiplication - Primary root based inocula of the individual AMF species were multiplied in maize root association in sterile soil-sand mixture in 2.5 liter plastic root trainer pots in the nursery for one cycle using 5 g starter inoculum for 1 plant per pot. The starter inoculum was placed below in 1 cm circular band below 2 cm of the seed. The plants were routinely irrigated with sterile water and intermittently fertilized with low phosphorus Hogland's solution. The plant assembly in batches was kept covered under fine nylon mesh cages. Roots harvested from such plants after ascertaining AM presence were air dried, mixed in equal proportion for the all the species to constitute the mixed species inoculum for mass multiplication of AM. The procedure of plant growing with inoculum was repeated for 3-4 cycles, each of 50-60 days, using the whole soil mass with roots of the previous generation mixed with equal volume of formalin sterilized soil as the growing medium. Finally, the harvested roots are chopped in to 5 mm bits, air dried and used as inoculum. Until use, the inoculum was kept in sealed plastic packets in dry, cold room at 20-22°C temperature. For large volume pot experiments, the root inoculum mixed with charcoal was used to

inoculate soil. For precision experiments, depending upon the purpose, only root inocula from different cycles of production were used.

During multiplication, all precautionary measures were taken to avoid air and soil borne contamination as far as practicable.

All root based inocula were routinely examined presence of AMF and also for infectivity status by MPN analysis by using appropriate dilutions.

Inoculum maintenance- The AM fungi either as individual species or in mixture were maintained in isolation in small volume pots in sterilized soil and whenever needed multiplied in bulk as above.

10. Mycorrhizal analysis

Processing and staining of root segments to assess AMF infection and histolological details of root-AMF association

Roots fixed in FAA for at least 7 days were stained with either Trypan Blue (Phillips and Hayman 1970) or Cholorazol Black (Brundrett *et al.*, 1994).

Trypan Blue staining

Alkaline hydrolysis of root samples was done with 10% KOH at 15 lbs psi steam pressure for 2-5 minutes. After washing, the roots were treated with 10% alkaline H_2O_2 for 5 minutes. Washed roots were treated with 1N HCl for 5 minutes. HCl treated roots were then stained with hot 0.1 % Trypan Blue solution (55-60 °C) in lactophenol for 15 -20 minutes. Roots were destained and preserved in lactophenol bnefore examination.

Chlorazol Black staining

Roots were fixed with 50 % ethanol overnight. Fixed roots were hydrolyzed with 5% KOH solution at 15 lbs psi steam pressure for 10 minutes. Washed roots were stained with Chlorazol Black staining solution at 50 °C for 1 hour. Roots were destained and kept in glycerol until observation.

Assesment of root infection intensity

The slide micrometric method of root infection intensity assessment (Kormanik and Mc Graw, 1982) was followed to asses root infection intensity of experimental plants. Stained root segments of about 1 cm length, mounted in lactophenol were observed under microscope. Root and hyphal length was

measured by ocular micrometer; number of vesicles and arbuscules were counted. Depending upon the purpose of experiment a minimum of 10 and maximum of 25 root pieces were observed and averaged. Root infection intensity was expressed as per-cent root length colonized by AMF hyphae and number and intensity of arbuscule formation.

Assessment of pre- and post-infection AMF structures in root association

For assessment of pre-and post-infection colonization structures, mainly the Chlorazol Black stained root material was used. Whole plant root samples were spread in squared paper and the lateral branch roots from 3 random $1cm^2$ segments were separated, bulked per plant and cut into 1.5 cm pieces. Such cut root pieces were stained separately for each plant sample and 100- stained root pieces per plant sample were mounted suitably in slides for examination and data recording. Minimum 3 plants were used as replicates for a variety / date so that ultimate data were based on 3 x 100 – 1.5 cm or 450 cm root length examination for each plant variety. All pre-penetration structures were counted and measured by micrometry under low magnification and data were expressed as number or length pf AMF structures per unit root length or per unit root surface area or per unit surface length of surface colonizing hyphae. For count and estimates of internal colonization structures, a 4-point scoring system was devised from repeated observation of stained root pieces. The scoring system has been described later.

AMF spore count from soil

AMF spores were isolated from soils by Gerdemann and Nicolson's (1963) wet sieving and decanting method. The isolated spores were collected on 40μ nylon mesh and observed under stereo binocular microscope. All spore counts were based on at least 3 replications of required quantity of sample soils and expressed as number per 100 g of air dry soil.

Enumeration of infective inoculum density

Most probable number (MPN) of infective mycorrhizal inoculum density of any soil-root sample used as inoculum was assessed by Powell's (1980) 5 vial 10 fold serial dilution technique. 200-250 g diluted root-soil sample from last 5 dilutions of the series were taken in polythene tubes in 5 replicates and red gram

(*Cajanus cajan*) or onion (*Allium cepa*), were used as test plant to grow for about 40-45 days. Whole root mass from each tube were harvested and after usual processing, examined under microscope for presence or absence of mycorrhizal infections. MPN values were determined from the Statistical Table given by Alexander (1965).

Identification of AM fungi

The AM fungi were identified by measuring the morphological characters and comparing the same with type descriptions of the different species (Schenek and Perez, 1990).

11. Adjustment of available soil Phosphorus for plant response

For phosphorus demand studies available phosphorus level of the experimental laterite soil was raised to different levels with addition of single super phosphate (SSP). To standardize the available soil phosphorus concentration, 1 kg experimental soil was treated with different doses of SSP (0, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0 g SSP/kg soil) in plastic packets and incubated for one month allowing alternate wetting and drying at 25 °C. Soil samples from different treatments were then taken, oven dried and available soil phosphorus was measured by Olsen's method. A linear regression equation was obtained from these available soil phosphorus values and doses of SSP. By using the equation, the amount of SSP needed to raise the available soil P to a desired level, was determined.

The equation derived for the experimental laterite soils was:

$$P=56.2\ x+0.0493$$

Where, P=available soil phosphorus in ppm and X= g SSP/kg soil

12. Soil and plant analysis

The following standard methods were followed for physical and chemical analyses of soil and plant materials

Properties	Method in brief	Reference
Soil analysis		
pH	1: 25 soil water suspension, pH meter	Jackson, 1973
Mechanical analysis		Piper, 1966
Organic Carbon	Tritrimetry (Walkley and Black)	Jackson, 1973
Available phosphorus	Modified Olsen's method, ascorbic acid colorimetry	Watanabe and Olsen,1965
Total Nitrogen	Kjeldhal',s method	Jackson, 1973
Available potassium	Flame photometry, ammonium acetate extract	Jackson, 1973
Plant analysis		
Dry matter	Drying to constant weight at 70 °C	
Total phosphorus	Vanado-molybdophosphoric acid colorimetrc method, Tri-acid digestion	Jackson, 1973

13. Biochemical analysis

Protein estimation

Soluble root protein was estimated by Lowry's colorimetric method (Lowry *et al.*, 1951) using bovine serum albumin as standard. Whole root crude protein extract (1 g root in 1 ml) phosphate buffer (pH 6.8) was centrifuged at 10000 rpm for 15 minutes. The extract was diluted 20 times with buffer and mixed with Reagent C (Section 5) (1 ml diluted extract + 5 ml Reagent C). 0.5 ml 1 N Folin's reagent was added to the treated extract after 15 minutes shaking and incubated in dark for 30 minutes. OD of the resultant material was read at 660 nm using Spectronic 20 Spectrophotometer. Protein concentration per g dry root was determined from BSA standard curve.

Peroxidase Estimation (Sadasivam and Manickam 1996)

O-Dianisidine method

1g of plant tissue stored overnight at –20°C, was extracted with 1 ml of phosphate buffer (pH 7) by grinding in a pre-cooled glass mortar and pestle at 0-4 ° C. The homogenate was centrifuged at 18000 g at 5 ° C for 15 minutes. The supernatant was used as enzyme source immediately. 0.2 ml enzyme extract and 0.1 ml freshly prepared O-dianosidine solution was added to 3.5 ml phosphate buffer in a dry cuvette. The temperature of the assay mixture was immediately brought to 28 - 30 °C and the cuvette was placed in spectrophotometer at 430 nm. 0.2 ml 0.2 M H_2O_2 was then added immediately. The initial absorbance at 0 time immediately after addition of H_2O_2 was recorded and followed further by recording at every 30 sec interval up to 3 minutes. A blank with reaction mixture minus enzyme extract was run parallely. Enzyme activity was expressed as rate of increase in absorbance per unit time per mg protein.

Guaiacol method

Enzyme extraction was done as in as O-Dianisidine method. 3 ml buffer solution (pH 7), 0.05 ml guaiacol solution and 0.1 ml enzyme extract and 0.03 ml H_2O_2 solution were taken in a cuvette. Temperature of the mixture was raised to 25°C. After mixing the reaction mixture, the cuvette was placed in Spectrophotometer at 436 nm.

Time required in minutes (Δt) for increase in the absorbance of the assay mixture from 0.05 to 0.1 was read with the help of a stopwatch.

Enzyme activity per ml of extract was calculated as follows:

Enzyme activity units/ml = 3.18 x 0.1 / 6.39 x 1 x Δt x 0.1 (6.39 – extinction coefficient of guiacol). Activity units per ml were then converted to per mg protein by estimating the protein content of extract by Lowry's method. The estimated one unit of peroxidase was equivalent to that quantity of enzyme, content per ml. of root extract which changes the OD of the reaction mixture from 0.05 to 0.10 in 40 seconds.

Poly acrylamide Gel Electrophoresis (PAGE) of peroxidase isozymes (Kahler and Allard, 1970)

Phosphate buffer pH 7.0 was used for extraction of peroxidase isozymes. Root samples chilled at –20 °C was crushed and extracted with buffer in chilled glass mortar pestle with fine glass powder in ice buckets; centrifuged at 10000 rpm for 15 minutes and the supernatant was used as crude enzyme extract. Protein concentration in the extract was estimated by Lowry's method as mentioned earlier.

Polyacrylamide gel mixture for 16 cm x 14 cm x 1.5 mm size was prepared with the constituents, described earlier. Freshly prepared gel mixture solution was carefully poured into the chamber between the glass plates. A 13 well comb was placed on the top of the gel. A layer of small volume of distilled water was provided on top of the gel and kept for 45 minutes for polymerization.

After proper polymerization the gel was put to pre-run for 60 minutes at 4°C inside a cold chamber in a vertical slab gel apparatus (Biotech Model No. EBW-20) at a constant current of 30 mA. This was followed by washing of individual well with electrode buffer (pH 8.2-8.4). Chilled enzyme extract containing equal quantities of protein in sample buffer were loaded in each lane.

For gel running, initially 15-20 mA current was provided but later gel was allowed to run at a constant current of 30 mA for movement of the samples through the stacking gel. Gel was run until the Bromo-phenol blue dye reached the bottom of the gel.

After complete run, the gel was stained in dark with freshly prepared staining solution for 10-15 minutes followed by a quick exposure to hydrogen peroxide solution. The reaction was stopped by washing with distilled water and observed for peroxidase bands.

Gel analysis - The peroxidase gel was visualized, analyzed and documented by Ultra-Lum Inc. USA gel documentation system (Model EBW-20) with Total Lab gel analysis software.

14. Data recording, analysis and interpretation

Mycorrhiza inoculation effect (MIE%): Mycorrhiza inoculation effect for any plant growth (mass or weight) or nutrition parameter (concentration or content)

was estimated from basic data of experimental plant materials as (Treatment – control) / treatment x 100 and expressed as percentage.

Specific shoot growth rate: Specific shoot growth rate of plants under any treatment was assessed as growth rate (mg dry matter accumulation in shoot per plant) per unit time per unit mass of root.

Specific plant P-content: Specific plant or shoot P-content was assessed by estimating plant or shoot P-content per unit root mass weight.

Root branching pattern: Lateral root branching pattern was assessed by actual count and measurement of unstained but fixed roots by micrometry under low magnification stereoscopic microscope (Leica WILD M3Z). For all such purposes whole plant root samples in suitable replicates were used. Lateral root length density was calculated form total length of branch roots / volume of soil actually traversed in cylindrical thick black polythene tubes. Taking horizontal spread of branch roots in a standard diameter tube, the volume of soil traversed was found out from the maximum length of the branch roots.

Phosphorus demand and supply estimates: These were calculated from the polynomial and linear regression equations (trend lines) between *dW /dt* (rate of shoot growth as dry matter accumulation per unit time) and *dP/dt* (rate of P-uptake per unit time) and between *dP/dt* and soil available P estimates respectively, according to Koide (1991). For the purpose, plants were grown in graded levels of phosphorus containing soils described later.

Scoring of internal development of AMF structures: Accurate estimation of spread of the AMF in root cortex, especially with large number of samples was difficult. For the purpose a scoring system was devised by repeated observations of sample roots. Considering that only two dimensional views were possible from micrometry of a tubular root piece, only surface view of fungal spread in 1.5 cm root pieces of nearly equal width (0.30mm) were taken to represent the colonization. The extent of surface spread of intracellular and intercellular AMF hyphae were divided into 5 categories attaching scores in simple arithmetic series as appear in the next page.

No internal spread of AMF structures (-):	0
10 % of root surface area covered by either Intracellular or intercellular hyphae or arbuscules in the cortical cells (+)	1
11-25 % of root surface area covered by either Intracellular or intercellular hyphae or arbuscules in the cortical cells (++)	2
26-40 % of root surface area covered by either Intracellular or intercellular hyphae or arbuscules in the cortical cells (+++)	3
41 % to 60% % of root surface area covered by either Intracellular or intercellular hyphae or arbuscules in the cortical cells (++++)	4

During the relatively short experimental time period allowed for root colonization, highest root surface area covered by internal spread rarely exceeded 50 %, so that keeping the limit of spread up to 60 % was realistic for the situation. Root pieces individually showing the colonization grades as above were counted and the total number of root pieces belonging to each grade was multiplied by score value. The summation of scores of all the root pieces examined was divided by the total number of samples to arrive at the relative score of internal colonization by either intracellular or intercellular hyphae or arbuscules. For the purpose a minimum of 3 x 100 – 1.5 cm root pieces were examined.

Estimation of peroxidase activity from PAGE:

Enzyme activity was measured as unit peroxidase per mg of root protein. Known amount of peroxidase (peroxidase unit per mg of root protein as analyzed by guiacol method) was loaded in the gel. Peroxidase bands obtained by PAGE were visualized under visible light illuminator and documented by the gel-documentation apparatus. Quantitative analysis of the bands were done by the Total Lab software. Unit peroxidase present in different bands were then calculated from the total unit of peroxidase loaded.

Statistical analyses and interpretations:

ANOVA – Single factor CRD analysis was performed for determining the statistical significance of differences among the varieties for the measured traits (Gomez and Gomez, 1984).

t Test – To determine the significant difference between two means 'Fisher's t test' was performed (Panse and Sukhatme, 1985).

The formula is given below

$$t = \frac{m_1 - m_2}{s \times \sqrt{(1/n_1 + 1/n_2)}} \qquad \text{at } (n_1 + n_2 - 2) \text{ df}$$

where, m_1 and m_2 are two different means,

n_1 and n_2 are two different sample size

Regression analysis – Simple 2-variable polynomial and linear regression analyses were done using MS-Excel package to determine the relationship between (i) shoot growth rate and P-uptake rate and (ii) P-uptake rates at different soil P-levels.

Proximity matrix analysis – Proximity matrix analysis and dendrogram construction for determining the similarity relationship among the varieties based on measured traits were done by 'Single linkage' (Jhonson and Wichern, 2001) method using SPSS package.

15. Plan and design of experiments

15.1 Growth rate analysis of rice varieties in the nursery

Shoot and root growth rate analysis of the 8 rice varieties were performed with both alluvial and laterite soils during May -June in a side open nursery house, roof covered with transparent plastic sheet. Seedling plants of each variety were grown from surface sterilized pre-germinated seeds by direct seeding in large volume solarized soil.

In laterite soil where root growth rates were assessed, the plants were grown in 4.0 litre plastic root trainer pots with 5 kg soil, keeping 10 seedlings per pot in large number of replicates. The pots were routinely irrigated to keep soil moisture above field capacity. No other treatments were provided. Whole plant harvests were made at 25 and 50 days after seedling emergence from 3 replicate pots for each variety and root and shoot weights were determined after bulking of plants for each replicate. Data for the sake of convenience were analyzed by 1-factor CRD variance analysis and DMRT.

In alluvial soil, seedlings were grown in large cement vats taking 100 kg fertile alluvial garden soil in each vat. The soil was puddled with water and allowed to remain for 2-3 days before seeding. Eight varieties were grown as one variety in each vat with 3 replicates. These plants were grown in the open and

irrigated as needed. At 20 and 50 days after seedling emergence above ground shot mass of 10 plants each from 3 replicate pots were harvested and shoot dry weight determined. The data analysis was done as before.

15.2 Root morphology and root development study

Seedling plants were grown in open ended, black polythene tubes (6.5 cm. dia and 40 cm height) filled with 2 kg alluvial soil. The potting mixture was constituted with soil-sand-organic matter (5:1:1 by volume). Each tube contained a single direct seeded plant and for each variety 15 such tubes were maintained with adequate soil moisture for 35 days. At 15 days after plant emergence 10 seedlings were harvested by putting the tubes under running water and the whole root mass for each plant was sampled and put in fixative solution. Morpho-metric analysis of root development pattern was done by counting and measuring each branch root as seminal, 1^{st}, 2^{nd} and 3^{rd} order roots under low magnification stereomicroscope. For each variety data were recorded from a minimum of 10 plants. The data after processing as needed were analyzed by t-test. Gross root and shoot development data were recorded from the remaining 5 tubes at 35 days analyzed statistically to find out the differences between variety means.

15.3 Phosphorus demand- supply relations of the rice varieties

The experiment was conducted during April-May in the previously mentioned nursery house under ambient environment with laterilte soil. The rice plants were grown in 5 kg solarized laterite soil in 250 cm x 200 cm polythene. Prior to seed sowing the available P-content of the soil mixture was adjusted to 5, 10, 20, 40, 80, 120 ppm by the method described earlier. Each treatment pot contained five number of plants raised from pre-germinated seeds. Each variety was replicated in six pots for each soil phosphorus level. The experimental pots were arranged on a cemented floor allowing full light interception from all sides. Irrigation was given at a regular interval, keeping the moisture status at saturation. Data were recorded at 25 and 50 days after seedling emergence by whole plant harvest and measuring root and shoot weight and phosphorus concentration of roots and shoots from 3x5 plant replicates. The data were collected for shoot and root P-content relative to their mass – weight and

analyzed statistically to determine P-demand and supply according to the method described earlier.

15.4 AMF response or dependency of the rice varieties in the nursery

The experiment was conducted with solarized laterite soil in 5 liter plastic root trainer pots with 3 kg soil mixture per pot. Prior to pot filling the soil mixture was inoculated with 1-1.5 g fresh root based AMF inoculum giving 1.5 x 10^5 infective propagule density per g soil as determined by MPN analysis described earlier. The control non-inoculated set received equal amount of autoclaved root inoculum. Each soil pot was seeded with 8-10 seeds and immediately after emergence 5 seedlings of equal vigour were kept in each pot. Plants were irrigated as required to maintain the soil moisture level below field capacity. The experiment was conducted for 2 dates of measurement – 15 and 30 days after seedling emergence. For each variety 3 replications per treatment for each date were maintained. After whole plant harvest at the desired dates, root and shoot weight and P-concentration of roots and shoots were measured to determine the MIE % and estimation of shoot and root P-contents. The data were analyzed as needed to bring out the differences among the varieties.

15. 5 Histological study of AMF colonization

For histological examination of AMF colonization structures and intensity at the pre-penetration and post-penetration stages seedlings were grown in low volume sterilized soil in 250 ml thermocole cups keeping 3 seedlings per cup for 15 days. Sterilized soil was inoculated with pure root based AMF inoclum at the rate of 5 g inoculum per kg soil to raise the Infective inoculum density to approx. 3.5 X 10^5 propagules per g soil. Seedlings were raised from surface sterilized seeds in inoculated soil and whole plant harvests were made between 10-15 days of seedling growth. Roots taken from the harvested plants were preserved, processed, stained and examined for the AMF structures for required number of replicates.

15.6 Estimation of whole root peroxidase by colorimetry and gel electrphoresis of rice varieties

Seedling plants of rice were grown for the purpose of this experiment in similar manner in the same inoculated soil mixture as above (Histology of AMF colonization). The plants were grown in both inoculated and non - inoculated soil for 10 and 25 days and upon whole plant harvest the roots were processed and

analyzed for peroxidase by colorimetry and acrylamide gel electrophoresis by the methods described earlier, keeping adequate number of both plant and estimation replicates.

15.7 Post-infectional changes in root development pattern of rice varieties

This experiment was conducted with larger volume of similarly inoculated sterile laterite soil in long cylindrical tubes for 15 days. Seedlings were grown in both inoculated and non-inoculated soil from surface sterilized seeds as single plant in each tube with large number of replicates. After harvest, roots were separated, preserved and examined for root branch number and length following the methods described earlier. For each variety 3 X 10 plant root samples were examined and data analyzed as needed.

Chapter – IV Experimental Results

1. Physiological traits of rice genotypes in relation to AM responsiveness or dependency

Physiologically, the extent to which mycorrhizal infection influences plant performance (mycorrhizal responsiveness or dependency) is a function of the extent to which infection decreases plant phosphorus deficit by increasing the supply of phosphorus (Smith and Read, 1997). This is true for a given plant taxon under varying phosphorus availability, or for several taxa grown under the same phosphorus availability (Koide, 1991). In a given soil, variation among the plant taxa in phosphorus deficit at a given level of soil phosphorus, in as much as the same is genetically determined is expected to reflect the variation in mycorrhizal responsiveness or dependency and provide first order information about the genetic basis of mycorrhizal dependency at physiological level.

All other factors remaining constant, plant phosphorus deficit is a function of both demand for and supply of phosphorus, both being inherently determined by physiological, morphological and phenological traits of the plant. By 'phosphorus supply' is meant the actual rate of phosphorus uptake at a given time by the plant. The lowest rate of phosphorus uptake to give the maximum growth rate at the given time is its 'phosphorus demand'. When the supply of phosphorus is exceeded by the demand, a 'phosphorus deficit' results. When there is a positive phosphorus deficit, an increase in phosphorus supply (uptake), results in an increase in plant performance. Arbuscular mycorrhiza improves plant performance by reducing phosphorus deficit by increasing the supply.

By extending this physiological truism to the different genotypes of a plant taxon and looking at their inherent traits for phosphorus deficit in relation to mycorrhizal performance (responsiveness or dependency) we can approach to

understand the genetic basis of mycorrhizal responsiveness or dependency at first order level.

Experiments were carried out with eight selected rice varieties to determine the variation in their basic physiological traits that are expected to determine their mycorrhizal response or dependency.

1.1 Rate of growth of eight selected rice varieties during active growth phase in two different soils

Variations in plant phosphorus deficit in relation to mycorrhizal responsiveness of the different genotypes have to be understood in terms of variations in phosphorus demand of the varieties vis-à-vis variations in their phosphorus supply efficiency.

Among the many physiological factors, the rate of shoot growth is the major determinant of the rate of nutrient demand (Bouma, 1983; Fitter and Hay, 1987). This is true of phosphorus also and for the tissue phosphorus concentration to remain constant to support active growth, the rate of phosphorus uptake must be proportional to the rate of dry matter accumulation (Koide, 1991). Phosphorus uptake (plant P-content at a given time) is a function of root length density as this variable determines how far the phosphate ion must diffuse in soil for absorption to occur (Baldwin, 1975). Within a limit, the rate of phosphorus uptake of a plant is correlated with the rate of its root growth in a fixed volume of soil (root length density) (Newman and Andrews, 1973).

To study the relationship between phosphorus deficit of the rice varieties and their mycorrhizal responsiveness, variations in the rate of shoot and root growth of these varieties in different soils were studied first. The aim was to understand the variations in mycorrhizal responsiveness of the varieties in terms of measurable differences in the root and shoot growth phenotypes of the varieties.

The experiment was performed in the nursery under ambient environmental conditions during spring-summer season (March-June) with both laterite and alluvial

soils using the materials and following the procedure given in details in the Chapter – III (Materials and Methods). Surface disinfected, pre-germinated seeds were sown in solarized soil taken in large containers and dry weight of root and shoot mass of sample quantity of seedlings were measured at different dates after plant emergence from suitable plant and container replicates. Results are presented in Tables 1-3, Figures 1 and 2.

1.1.1 Shoot Growth

Table 1: Five-plant shoot dry weight (g) of eight varieties in two soils and per day per plant shoot growth rate between 20 / 25 and 50 days after emergence.

1.1 Laterite soil

Days	Shoot dry matter (g) of varieties, 5 plants' average							
	V1	V2	V3	V4	V5	V6	V7	V8
25	0.69	0.82	0.51	0.64	0.70	0.62	0.53	0.52
50	2.05	2.06	1.87	2.49	2.22	2.48	2.54	2.75
50-25	1.36	1.24	1.36	1.85	1.52	1.86	2.01	2.23
D*wt/dt*	0.054	0.050	0.054	0.070	0.061	0.074	0.080	0.089
Growth rate	Shoot dry matter accumulation, per day per plant (mg)							
per day	10.9^{cd}	9.9^{d}	10.9^{cd}	14.7^{abc}	12.1^{bcd}	14.8^{abc}	16.1^{ab}	17.7^{a}
S.E.m ±	1.26							
LSD $p_{0.05}$	3.85							

Values followed by the same letter are not significantly different by DMRT at $p_{0.05}$

1.2 Alluvial soil

Days	Shoot dry matter (g) of varieties, 5 plants' average							
	V1	V2	V3	V4	V5	V6	V7	V8
20	0.64	0.74	0.57	0.69	0.83	0.73	0.72	0.76
50	2.98	3.46	3.23	4.87	4.08	4.49	4.88	4.78
50-20	2.34	2.72	2.66	4.18	3.25	3.76	4.16	4.02
d*W/dt*	0.078	0.090	0.088	0.139	0.108	0.125	0.138	0.134
Growth rate	Dry matter accumulation per day per plant (mg)							
per day	15.3^{e}	17.7^{d}	17.0^{d}	27.8^{a}	21.6^{c}	25.0^{b}	27.4^{a}	26.8^{a}
S.E.m ±	0.50							
LSD $p_{0.05}$	1.53							

Values followed by the same letter are not significantly different by DMRT at $p_{0.05}$

Results of shoot growth rate analysis of the eight rice varieties originating from widely different sources and varying in their growth morphology showed predictable variations in their absolute shoot growth rate in both the soils (Table 1). Irrespective of the varieties, shoot growth rate was lower in the laterite soil than the alluvial soil but the inherent differences among the varieties in absolute shoot growth rate did not change with the change in the type and quality of soils. The laterite soil was poorer in nutrient content than the alluvial soil to give lower rate of shoot growth.

Based on these data, the eight different rice varieties were grouped into two major classes:

Slow growing or low growth rate varieties – Shoot dry matter accumulation below 15 and 25 mg per day, between 20 to 50 days, respectively in laterite and alluvial soils.

(V1) Black Gora, (V2) ARC - 12737, (V3) Yamuk and (V5) Dular

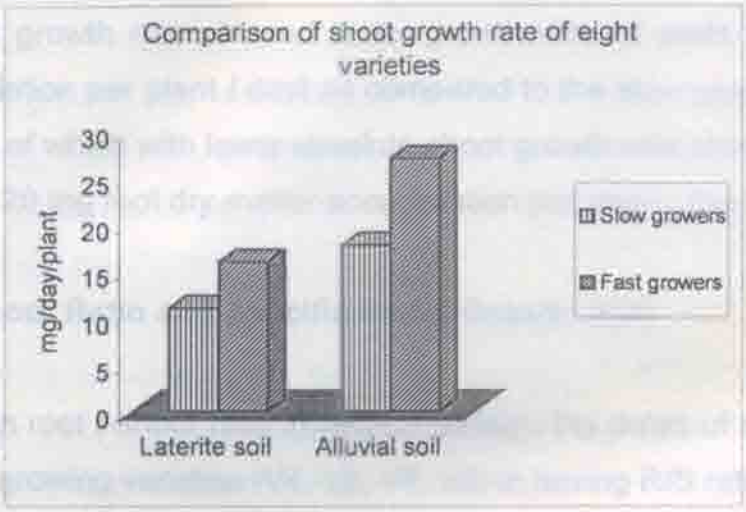
Fast growing or high growth rate varieties – Shoot dry matter accumulation above 15 and 25 mg per day, between 20 to 50 days, respectively in laterite and alluvial soils.

(V4) Jhingasail, (V6) TN - 1, (V7) MTU - 7029, (V8) IR – 64

Among the former group of varieties, growth rate of Dular (V5) was in the borderline of low and high, as in both the soils its growth rate was higher, although marginally, than the average growth rate of the other three varieties of the same group i.e. the slow growing varieties.

These data showed that in terms of absolute shoot growth rate, the traditional tall varieties and land race selections were slow growing than the improved varieties and the high yielding dwarf hybrids (Figure 1). But, this may not be universally true as growth rate of Jhingasail, a traditional variety of the Gangetic alluvial tract of erstwhile Bengal was equal to that of the modern day dwarf hybrids in both the soils.

Figure 1: Shoot growth rate (dry matter accumulation per day) of slow and fast growing rice varieties in laterite and alluvial soils



1.1.2 Root Growth

Table 2: Five plant root dry weight (g) and per plant per day root growth rate of eight rice varieties in laterite soil

Days	Root dry matter of varieties (g), 5 plants' average							
	V1	V2	V3	V4	V5	V6	V7	V8
25	1.59	1.55	1.38	1.03	1.32	0.98	0.86	0.83
50	4.48	4.32	4.25	2.57	4.58	2.64	2.48	2.88
50-25	2.89	2.77	2.87	1.76	3.26	1.66	1.62	2.05
d*W*/d*t*	0.12	0.11	0.11	0.06	0.13	0.07	0.06	0.08
Growth rate per day per	Dry matter accumulation per day per plant (mg)							
	24.0^{b}	22.3^{c}	21.9^{c}	12.1^{f}	25.8^{a}	14.3^{e}	11.9^{f}	16.0^{d}
S.E.m ±	0.29							
L.S.D *p* 0.05	0.89							

Root growth rate of the eight rice varieties (Table 2) when compared showed a contrasting picture. The fast growing varieties (V4, V6, V7, V8), all with higher absolute shoot growth rate showed lower growth rate of roots (< 20mg root dry matter accumulation per plant / day) as compared to the slow growing varieties (V1, V2, V3, V5), all of which with lower absolute shoot growth rate showed higher growth rate of roots (> 20 mg root dry matter accumulation per plant / day).

1.1.3 Root / Shoot Ratio and Specific Shoot Growth Rate

Based on root / shoot ratio (Table 3), at both the dates of measurement, the group of 4 fast growing varieties (V4, V6, V7, V8) in having R/S ratios lower than that of the group of 4 slow growing varieties (V1, V2, V3, V5), appeared to be physiologically more efficient in accumulating shoot dry matter per unit mass of roots than the latter group of 4 varieties. The difference in the shoot growth rate between the two variety groups became more pronounced at the later age as the observed difference in R / S ratio between the two groups widened at the later age. The average R/S ratios of the two variety groups, the slow and fast growing ones, were 2.05 and 1.60 at 25 days and 2.15 and 1.01 at 50 days respectively.

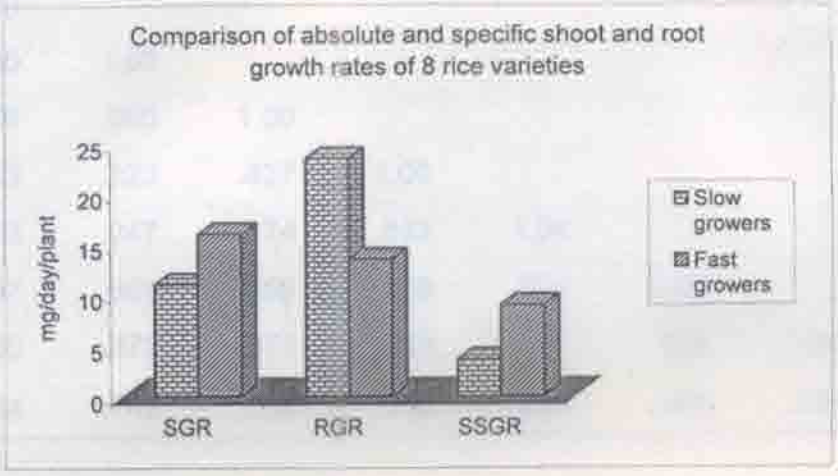
Table 3: Root / shoot ratios and specific shoot growth rates of 8 rice varieties in laterite soil at 25 and 50 days

Days	Root / shoot ratio							
	V1	V2	V3	V4	V5	V6	V7	V8
25	2.30	1.89	2.12	1.61	1.89	1.58	1.62	1.60
50	2.18	2.10	2.27	0.97	2.06	1.06	0.98	1.05
Specific shoot growth rate	Shoot dry matter accumulation (mg per day per plant) per unit root mass							
	$0.^{4c}$	0.44^{c}	0.50^{c}	1.22^{ab}	0.47^{c}	1.03^{b}	1.3^{a}	1.11^{ab}
S.E.m ±	0.091							
L.S.D *p* 0.05	0.277							

Specific shoot growth rate or the shoot growth rate per unit root mass of the varieties when compared (Table 3) revealed a pattern similar to that for absolute shoot growth rate. The fast growing varieties (V4, V6, V7, and V8) all having higher absolute shoot growth rate were higher in their specific shoot growth rate also (average 1.18 mg / day / plant). As compared to that, the varieties (V1, V2, V3, and V5) with lower absolute shoot growth rates had lower specific shoot growth rates (average 0.46 mg / day / plant).

These data showed that there were significant differences, among the 8 varieties in their absolute and specific shoot and root growth rates. The traditional varieties and land race selections which were higher in root growth rate than the modern HYVs and hybrid selections were all lower in both absolute and specific shoot growth rates than the latter group of varieties. Higher rate efficiency of root growth than shoot growth of the group of 4 traditional varieties was indicative of their higher nutrient uptake potential i.e. root supply efficiency than the latter group of modern and improved varieties. Similarly, higher specific and absolute shoot growth rate efficiency but lower root growth rate efficiency of the group of 4 modern HY varieties as compared to that of the group of 4 traditional varieties was a reflection of their lower nutrient uptake capacity or root supply efficiency relative to higher nutrient demand to satisfy their higher absolute shoot growth rate. These varieties in having a high shoot growth rate generated higher nutrient demand but lower root supply efficiency were expected to have a higher P-deficit in low phosphorus soils than the group of 4 traditional varieties which were expectedly higher in rate efficiency of supply but lower in demand for nutrients.

Figure 2: Comparison of absolute and specific root growth rates of 8 rice varieties in laterite soil between 25-50 days (dry matter accumulation per day per plant)



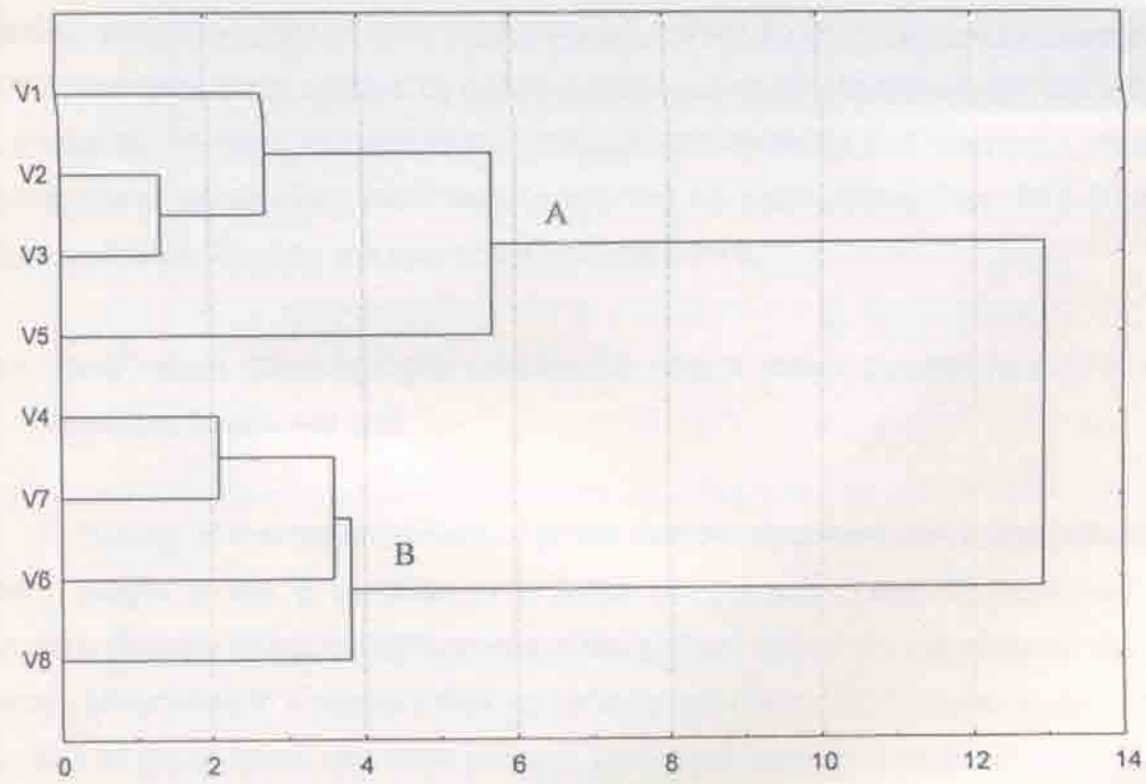
SGR- Shoot growth rate; RGR - Root growth rate;
SSGR- Specific shoot growth rate

The empirical classification of the 8 rice varieties based on shoot and root growth rates phenotype was then verified by proximity analysis for four parametric traits – shoot growth rates in laterite soil (Table 1.1), shoot growth rate in alluvial soil (Table 1.2), root growth in laterite soil (Table 2) and specific shoot growth rate in laterite soil (Table 3). The cumulative performance or proximity matrix was measured by single linkage method (Jhonson and Wichern, 2001) as Euclidean distance and dendrogram of the 8 varieties was constructed (Fig. 3) to cluster or group them on the basis of their measured phenotypic proximity or performance (Table 4).

Table 4: Proximity Matrix of 8 rice varieties based on cumulative performance of four traits

VARIETY	V1	V2	V3	V4	V5	V6	V7	V8
V1	1.00							
V2	.100	1.00						
V3	.078	.000	1.00					
V4	.963	.823	.827	1.00				
V5	.302	.247	.274	.832	1.00			
V6	.787	.659	.659	.128	.683	1.00		
V7	1.00	.872	.871	.043	.877	.138	1.00	
V8	.854	.742	.738	.210	.685	.141	.185	1.00

Fig 3: Dendogram of 8 rice varieties based on single Euclidean distances among the varieties for four growth rates traits



Phenotypic proximity analysis of the 8 varieties based on the four measured parametric traits confirmed the empirical grouping/clustering of the varieties into two distinct clusters /groups. Within the large cluster A belonged all the slow growing varieties (V 1,V2, V3 and V5) where V5 was placed at a higher distance from the rest three varieties. Within the large cluster B, the variety 8 was marginally more distanced than the rest three varieties (V4, V6 and V7).

The analysis confirmed that on the basis of growth related traits the varieties V1, V2, V3 and V5 (Cluster A) were more closely related among themselves than the varieties V4, V6, V7 and V8 (Cluster B) which were similarly closer among themselves.

2. Root development characters and P-supply efficiency of the eight rice varieties

Results of growth rate analysis of the 8 rice varieties in two soils revealed contrasting root and shoot growth patterns of the modern, high yielding and traditional, land race varieties. The modern high yielding varieties, with higher shoot growth rates than the traditional varieties and land race selections had lower root growth rates than the latter ones. This was an indication of higher demand but lower supply efficiency of nutrients of the modern varieties. In contrast, the traditional land race selections were lower in shoot demand but higher in root supply efficiency of nutrients. While differences in nutrient demand relations of the varieties were interpreted from the differences in their absolute and specific shoot growth rates, the differences in supply efficiency of nutrients among the varieties needed more detailed analysis of root development characters, as uptake or supply efficiency of nutrients is primarily a function of the morphology (branching pattern, length and density) and physiology of roots (Schachtman, 1998). Root development characters of the 8 varieties were studied by growing single plants in a fertile alluvial soil in PVC cylinders for 15 days as described in Chapter-III (Materials and Methods). Metrical characters of whole plant roots were measured for each variety from 10 x 3 single plant replicates. Results are presented in Tables 5 - 8.

2.1. Root mass development relative to shoot mass production of 8 rice varieties in alluvial soil

Results of the measurement of gross root development characters relative to shoot weight of the 8 varieties in a fertile alluvial soil (Table 5) confirmed the previous findings about the differences in the pattern of root growth between the two groups of varieties in a nutrient deficient laterite soil (Figure 2). Relative to equivalent amount of shoot mass, the slow growing traditional land race varieties (V1, V2, V3 and V5) produced a significantly higher amount of root mass, by weight and volume,

than the fast growing varieties (V4, V6, V7 and V8) (Table 5). Higher development of root mass per unit shoot mass production of the slow growing varieties confirmed their higher root development efficiency for nutrient uptake than the modern high yielding varieties in a nutrient rich soil also.

Table 5: Root development characters of eight varieties in alluvial soil at 35 days

Variety	Root Volume (cc)	Root weight (mg)	Shoot weight (mg)	Root /shoot ratio
Slow growing varieties				
V1	2.53±0.106	312±33.39	152±22.71	2.05
V2	3.04±0.209	378±22.87	187±29.87	2.02
V3	3.16±0.372	461±22.11	226±19.0	2.04
V5	2.78±0.191	306±24.02	176±10.54	1.74
Mean	**2.87±0.28**	**364±72.28**	**185±30.85**	**1.96±0.15**
Fast growing varieties				
V4	1.55±0.403	205±21.66	157±10.82	1.30
V6	1.62±0.360	212±26.0	171±22.54	1.24
V7	1.64±0.293	242±8.72	195±26.15	1.24
V8	1.56±0.190	210±12.53	170±7.55	1.24
Mean	**1.59±0.04**	**217±16.76**	**173±15.84**	**1.25±0.03**
Est. t	9.051**	3.962**	NS	9.283**
Table t	0. 05 *p* 2.447		0. 01 *p* 3.707	

2.2. Morphometric analysis of the root development pattern of the eight rice varieties

Nutrients, particularly phosphorus uptake by plants is a function of root length density as that determines the distance that the less mobile phosphate ions are to travel in soil to reach the root-soil interface for absorption to occur (Baldwin, 1975). Young rice seedlings produce, besides the coleoptile root, two other kinds of roots -

seminal roots which are thick and grow more or less vertically for anchorage and the lateral branch roots which are finer than the former and spread rather horizontally to the vertical axis and function in nutrient absorption. The seminal roots produce first order lateral branches from which successive order of finer branches are produced to form a network of fine lateral branches. These finer branches together with the hairs are the main absorptive roots of rice plants. Root branching pattern and the lateral branch root length density of the 8 rice varieties were determined by actual measurement to know the morphological basis of possible variations in nutrient (phosphorus) uptake efficiency among the fast and slow growing varieties. Results are presented in Tables 6 - 8 and Figure 4.

Results of the analysis showed gross differences in root developmental pattern of the 8 rice varieties, particularly for development of lateral branches (Table 6). Although average number of seminal roots produced at 15 days by a plant of the group of 4 modern HY varieties (V4, V6, V7 and V8) was higher (12.4 ± 2.7) than that of the traditional land race varieties (V1, V2, V3 and V5) (9.8± 2.1), total length traversed by the seminal roots per plant of the former group of varieties was lower than that of the later group of varieties (66 cm ± 16.2 against 75 cm ± 13.5). This was due to a significantly lower average length of the seminal roots of the HY varieties than that of the traditional varieties (Table 7).

The total number and total length of the lateral roots produced by a plant of any variety of the group of 4 traditional varieties, considering all the orders together was significantly higher than that of the modern high yielding varieties (Tables 6 and 8), giving a significantly higher lateral branch root length density (4.18 ± 0.62) than that of the modern HYVs (2.66 ± 0.49) (Table 8). By unit measurement, the number of first order roots per seminal root and per unit length of the seminal roots of the group of 4 traditional varieties was significantly higher (0.01*p*) than that of the traditional varieties. But the differences between the two groups of varieties in the number of the subsequent order lateral branches per unit number and length of the preceding order laterals were not mostly significant. Average length of any of the

orders of the lateral branch roots was also not different for the two groups of varieties.

Results of the above analysis showed that average length of the lateral branch roots being almost the same, the total number, hence, the total length traversed by the lateral branch roots, both on per plant and per seminal root basis was significantly higher in traditional land race varieties as compared to the modern HYVs (Figure 4). The significantly higher lateral branch root length density of the traditional varieties over that of the modern varieties (Table 8) further indicated higher nutrient uptake (or supply) efficiency of the former group of traditional, land race varieties as compared to that of the latter group of modern, HY varieties. These results were indirect confirmations of the contrasting feature of lower nutrient demand but higher supply efficiency of nutrients of the traditional varieties as compared to the higher nutrient demand but lower supply efficiency of nutrients of the modern HYVs, as discussed before.

Table 6: Metrical characters of lateral root branching pattern of the 8 rice varieties in alluvial soil at 15 days

Variety	Seminal roots		1st order roots		2nd order roots		3rd order roots	
	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)
V1	8.7±1.53	69.5±11.12	674±63.4	1719±161.5	882±68.5	1147±89.3	76±14.0	53±10.1
V2	12.7±1.15	95.4±13.70	967±90.8	2321±228.7	1088±78.5	1414±101.9	87±18.0	62±12.6
V3	8.0±1.15	67.0±10.71	658±88.7	1625±219.7	863±52.8	1036±62.4	64±18.6	44±12.6
V4	10.0±1.0	59.9±4.86	403±19.6	1096±53.4	573±28.7	802±40.9	46±12.7	34±9.1
V5	9.7±0.58	68.8±2.56	693±70.5	1871±175.1	1089±79.5	1198±86.5	78±15.0	55±11.0
V6	16.0±1.0	84.2±6.46	665±91.9	1529±226.3	669±8.3	803±9.8	49±18.4	34±12.9
V7	13.0±1.73	72.9±8.24	555±45.4	1276±111.5	575±26.6	805±37.2	44±13.7	32±10.1
V8	10.7±1.15	46.8±7.74	358±64.4	931±165.7	433±88.9	606±23.8	34±11.6	24±9..6
Mean of 4 slow growing varieties	9.8	75.2	748.0	1884.0	980.5	1198.7	76.2	53.5
Mean of 4 fast growing varieties	12.4	65.9	495.0	1208.0	562.5	754.0	45.7	31.0

Table7: Unit measurements of lateral branch roots of the 8 rice varieties at 15 days in alluvial soil

Variety	Seminal root average. Length (cm)	1st order roots			2nd order roots			3rd order roots		
		Per seminal root	Per unit length of seminal roots	Average length (cm)	Per 1st order root	Per unit length of 1st order roots	Average length (cm)	Per 2nd order root	Per unit length of 2nd order roots	Average length (cm)
V1	8.0	77.5	9.7	2.55	1.31	0.51	1.3	0.086	0.066	0.70
V2	7.5	76.1	10.1	2.40	1.12	0.47	1.3	0.080	0.061	0.71
V3	8.4	82.2	9.8	2.47	1.31	0.53	1.2	0.074	0.062	0.69
V4	6.0	40.3	6.7	2.72	1.42	0.52	1.4	0.080	0.057	0.73
V5	7.1	71.4	10.1	2.70	1.57	0.58	1.1	0.072	0.065	0.70
V6	5.3	41.6	7.9	2.30	1.01	0.44	1.2	0.073	0.061	0.70
V7	5.6	42.7	7.6	2.30	1.04	0.45	1.4	0.076	0.055	0.73
V8	4.4	33.5	7.6	2.60	1.21	0.46	1.4	0.078	0.056	0.70
Mean of 4 slow growing varieties	7.75±0.57	76.8±4.45	9.9±0.20	2.53±0.13	1.33±0.18	0.52±0.046	1.2±0.10	0.078±0.006	0.063±0.002	0.70±0.008
Mean of 4 fast growing varieties	5.30±0.68	40.0±4.13	7.4±0.52	2.48±0.21	1.17±0.18	0.47±0.039	1.3±0.10	0.076±0.003	0.057±0.003	0.72±0.015
Est. t	5.522**	12.13**	8.97**	NS	NS	NS	NS	NS	3.328*	NS
Table t	0. 05 p 2.447				0. 01p 3.707					

Table 8: **Lateral root development intensity and root length density of the 8 rice varieties in alluvial soil at 15 days**

Variety	Lateral root development				Lateral root length density cm/cc
	Number		Length (cm)		
	Per plant	Per seminal root	Per plant	Per seminal root	
Slow growing varieties					
V1	1622±81.7	186	2919±245.2	335	3.90
V2	2142±32.1	169	3797±126.7	299	5.06
V3	1585±203.2	198	2705±227.9	338	3.61
V5	1860±254.5	192	3124±198.7	322	4.16
Mean	**1802±257.2**	**186±12.5**	**3136±472. 5**	**323±17.75**	**4.18**± 0. 62
Fast growing varieties					
V4	1022±60.6	102	1932±103.0	193	2.58
V6	1383±346.1	86	2366±214.3	148	3.15
V7	1174±55.8	90	2113±128.5	162	2.82
V8	825±145.9	77	1561±161.4	146	2.08
Mean	**1101±236.1**	**89±10.37**	**1993±338. 6**	**162±21.70**	**2.66±0. 45**
Est. t	4.016**	11.944**	3.932*	18.058**	3.968*
Table t	0.05 *p* 2.447			0.01 *p* 3.707	

Figure 4: Comparison of lateral branch root development of the 8 rice varieties per plant and per seminal root

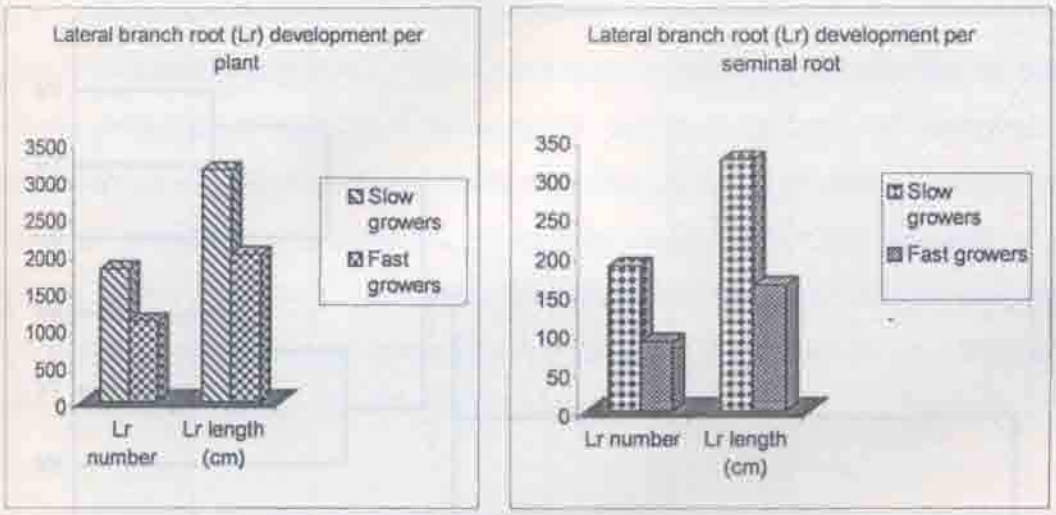
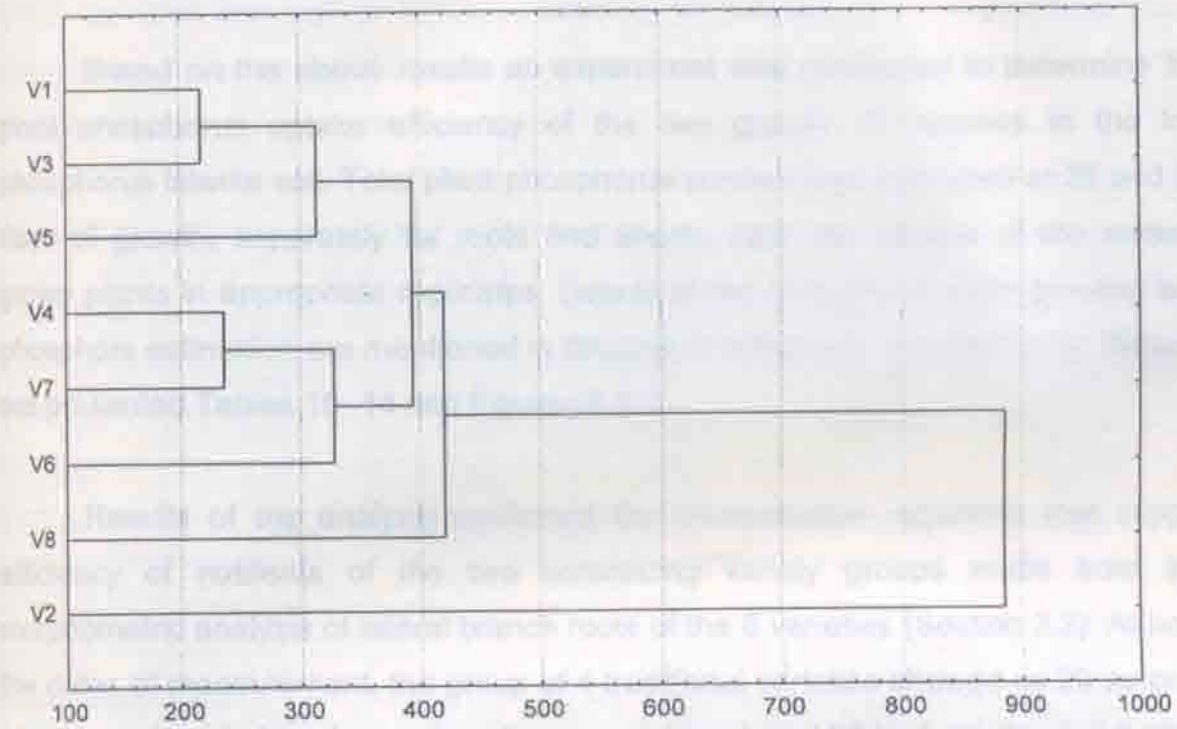


Table 9: Proximity matrix analysis of 8 rice varieties based on cumulative performance of 3 root development traits

VARIETY	V1	V2	V3	V4	V5	V6	V7	V8
V1	1.00							
V2	.379	1.00						
V3	.000	.457	1.00					
V4	.387	.831	.304	1.00				
V5	.040	.275	.117	.511	1.00			
V6	.159	.609	.073	.143	.280	1.00		
V7	.290	.737	.207	.008	.414	.046	1.00	
V8	.559	1.00	.476	.084	.683	.314	.180	1.00

Figure 5: Dendrogram of 8 rice varieties based on Euclidean distances among the varieties for 3 root development traits



Proximity matrix analysis of the 8 rice varieties based on cumulative performance of 3 lateral root development parameters (lateral root number / per plant, lateral root length / per plant and lateral root length density / per plant, Table-8) and dendrogram construction based on single Euclidean distances were then performed. The analysis revealed closer phenotypic proximity of root development pattern among 3 varieties each within respectively the slow (V1, V3 and V5) and fast (V4, V6 and V7) growing variety groups. Variety 2 placed at largest distance from the rest was also most separate and different from the rest of the varieties. Similarly, Variety 8 was more separate from its allies from the fast growing variety group or cluster and represented a separate identity not only from the more distanced slow growing varieties but also from the closer fast growing varieties. Separation of the two varieties (V2 and V8) from their phenotypic allies, however, was due to higher and lower primary trait values respectively from that of their allies. These did not contradict their empirical grouping these as low and high root length density varieties.

2.3. Phosphorus uptake efficiency of the 8 rice varieties in low nutrient laterite soil

Based on the above results an experiment was conducted to determine the plant phosphorus uptake efficiency of the two groups of varieties in the low phosphorus laterite soil. Total plant phosphorus content was estimated at 25 and 50 days of growth, separately for roots and shoots after the harvest of the nursery grown plants in appropriate replicates. Details of the methods of plant growing and phosphors estimation are mentioned in Chapter III (Materials and Methods). Results are presented Tables 10 -14 and Figures 6 & 7.

Results of the analysis confirmed the interpretation regarding root supply efficiency of nutrients of the two contrasting variety groups made from the morphometric analysis of lateral branch roots of the 8 varieties (Section 2.2). At both the dates of measurement, the group of 4 traditional varieties showed ca.20 percent higher uptake of phosphorus than the group 4 modern HYVs, from the soil having extremely low level of available phosphorus (Tables 11 and 13). Characteristically, in the traditional varieties proportional partition of total plant phosphorus content between roots and shoots was 13 - 19 % higher in the roots over shoots (Table 11 & 13). The same was exactly reverse in case of the modern HYVs where apportioning of total plant phosphorus was 13-19 % higher in the shoots over the roots. At 25 days, 68 % of the 20% excess plant phosphorus of the traditional varieties over that of the modern varieties was concentrated in roots and 32% in shoots. At 50 days, the whole of 19 % excess phosphorus of the traditional varieties over the modern varieties was concentrated in roots. This showed that the modern HYVs, in spite of having a lesser efficiency of phosphorus uptake, due essentially to lower lateral branch root length density, concentrated proportionately higher amount of phosphorus in shoots than in roots. In contrast, the traditional varieties having higher P- uptake efficiency, concentrated more amount of phosphorus in roots than in shoots. This would suggest that the modern varieties with faster growth rate and having a higher phosphorus demand were physiologically more efficient to

concentrate more amount of phosphorus in shoots (to satisfy the demand for higher growth rate). The traditional varieties with slower shoot growth rate and having a lower demand but higher uptake efficiency of phosphorus concentrated more phosphorus in the roots. This was an indirect indication of higher P-demand of the modern, fast growing varieties.

Table 10: Plant P-uptake at basal soil P-level in laterite soil at 25 days

Varieties	P-concentration (mg/g)		P-content (mg / plant)		Total plant P (mg /plant)
	Root	Shoot	Root	Shoot	
V1	0.99	1.23	0.315	0.170	0.485
V2	0.99	1.36	0.307	0.223	0.530
V3	1.15	1.74	0.317	0.177	0.494
V4	1.16	1.40	0.240	0.185	0.425
V5	1.04	1.59	0.274	0.223	0.497
V6	0.94	2.05	0.184	0.254	0.438
V7	1.06	2.16	0.182	0.229	0.411
V8	1.02	2.13	0.169	0.221	0.390

Average of 5x3 replicate analysis

Table 11: Comparison of phosphorus uptake features of the 8 rice varieties at 25 days

Mean of varieties	Phosphorus content (mg per plant)				
	Total / plant (mg)	Root		Shoot	
		Amount (mg)	% of total	Amount (mg)	% of total
(A) V1, V2, V3, V5	0.501±0.019	0.303 ± 0.02	60.5	0.198 ± 0.02	39.5
(B) V4, V6, V7, V8	0.416±0.020	0.194 ±0.03	46.6	0.222 ± 0.02	53.4
% difference (A over B)	+ 20.4		+ 13.9		-13.9
Est. t	6.162**	6.046**	-	NS	-
Table t	0.05 p 2.447		0.01 p 3.707		

Table 12: **Plant P-uptake at basal soil P-level in laterite soil at 50 days**

Varieties	P-concentration (mg/g)		P-content (mg / plant)		Total P (mg /plant)
	Root	Shoot	Root	Shoot	
V1	0.89	0.95	0.797	0.389	1.186
V2	0.90	0.98	0.778	0.404	1.182
V3	0.89	1.23	0.756	0.460	1.216
V4	0.90	0.97	0.463	0.469	0.932
V5	0.90	1.10	0.824	0.488	1.312
V6	0.86	1.25	0.454	0.620	1.074
V7	0.90	1.07	0.446	0.543	0.989
V8	0.86	1.12	0.495	0.616	1.111

Based on 5x3 replicate analysis

Table 13: **Comparison of phosphorus uptake efficiency of the 8 rice varieties at 50 days**

Mean of varieties	Phosphorus content (mg / plant)				
	Total / plant (mg)	Root		Shoot	
		Amount (mg)	% of total	Amount (mg)	% of total
(A) V1, V2, V3, V5	1.224 ± 0.06	0.789 ± 0.03	64.5	0.435 ± 0.05	35.5
(B) V4, V6, V7, V8	1.026 ± 0.08	0.464± 0.02	45.2	0.562 ± 0.07	54.8
% difference (A over B)	+19.3		+19.3		- 19.3
Est. t	3.960**	18.027**	-	2.953*	-
Table t	0.005 p	2.447	0.001 p	3.707	

Table 14: **P-uptake efficiency or specific P-content of the 8 rice varieties at low soil P-level**

Variety	Specific plant P-content mg x 10^{-3}		Specific shoot P-content mg x 10^{-3}	
	25 days	50 days	25 days	50 days
V1	1.525	1.324	0.535	0.434
V2	1.710	1.368	0.719	0.467
V3	1.790	1.430	0.641	0.541
V4	2.063	1.813	0.898	0.912
V5	1.882	1.432	0.845	0.533
V6	2.235	2.034	1.296	1.174
V7	2.390	1.994	1.331	1.095
V8	2.350	1.929	1.331	1.069
Mean of 4 slow growing varieties	1.727±0.152	1.388±0.052	0.685±0.132	0.494±0.052
Mean of 4 fast growing varieties	2.259±0.146	1.942±0.096	1.214±0.211	1.062±0.110
Est. t	5.048**	10.148**	4.251**	9.320**
Table t	0.05*p* 2.447		0.01*p* 3.707	

Based on above data, P-uptake efficiency or specific plant and shoot P-contents of the 8 rice varieties at 25 and 50 days were analysed (Table 14; Figures 6 & 7). Results of the analysis (Table14) revealed interesting features which further confirmed the previous interpretations. While the group of 4 slow growing traditional varieties had higher total plant P-content than the group of 4 modern varieties, the relation was reversed when specific plant P-contents were considered. At both the dates, specific plant P-contents were higher in modern varieties than traditional varieties. In other words the slow growing traditional varieties with higher root development (or absorptive) efficiency had higher, plant P-uptake efficiency than the modern varieties from the deficient soil. But their specific P-contents in were lower than that of the modern varieties. Total shoot P-contents of the modern varieties was higher than that of traditional varieties, and the relation became more significant when specific shoot P-contents were considered. Specific shoot P-contents of the

modern varieties were significantly higher than that of the traditional varieties. This again show that the modern HYVs having a higher shoot P-demand to satisfy their high absolute shoot growth rate made a higher partition of the total P in shoots than in roots, although due to the lower biomass production their total plant P- contents may be lower than that of the traditional varieties. Root supply efficiency of P being higher in traditional varieties, their P- partition to shoots was lower due to the lower P-demand than that of the modern varieties.

Fig. 6: Comparison of total and specific plant and shoot P- contents at 25 days; (a) total P, (b) specific P

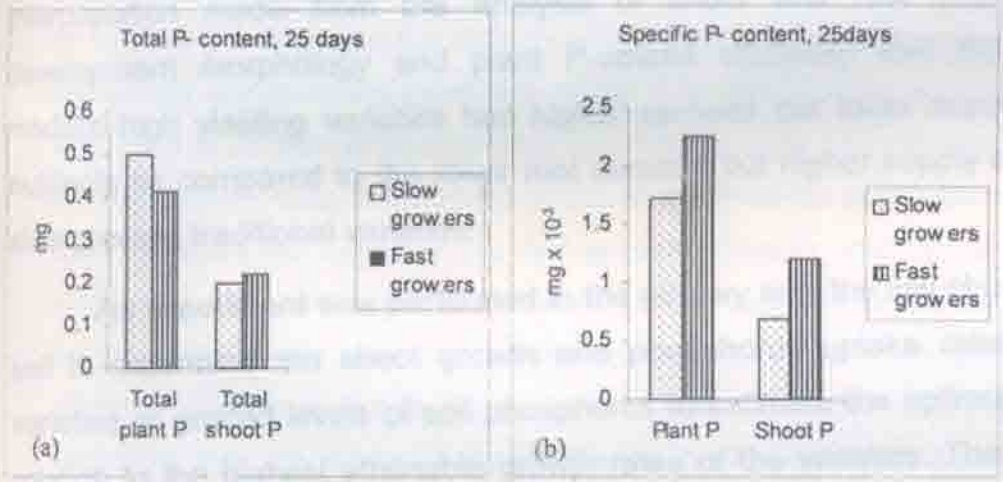
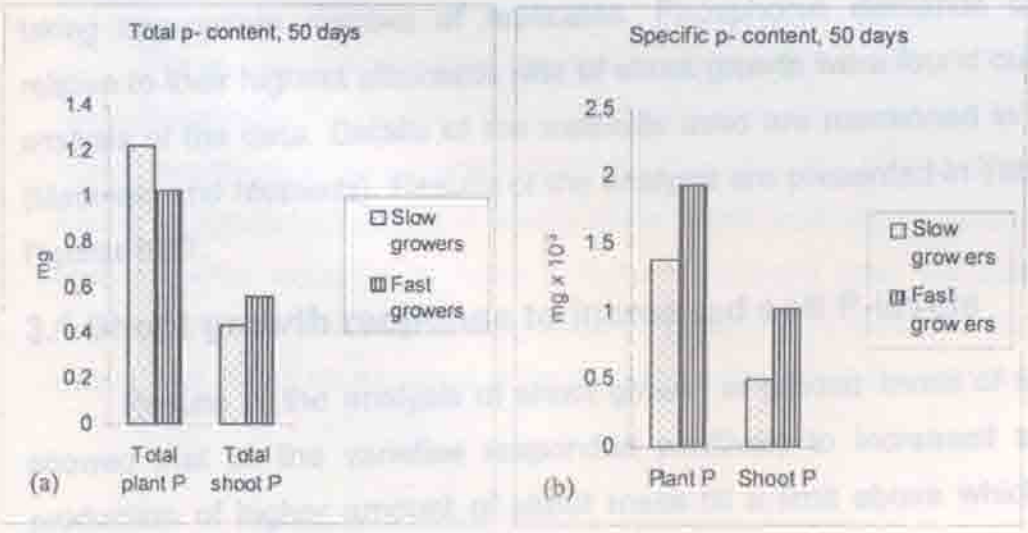


Fig. 7: Comparison of total and specific plant and shoot P- contents at 50 days; (a) total P, (b) specific P



3. Phosphorus demand relations of the eight rice varieties relative to their optimum shoot growth rate

Results of the analysis of shoot growth rate of the 8 rice varieties in two different soils indicated that the varieties might differ in their demand for nutrients (especially, phosphorus) as was reflected from the variations in their absolute and specific shoot growth rates at the phosphorus levels of average soils (Tables 1 and 3). Actual demands for phosphorus to support potential maximum growth rate of the varieties were further determined to obtain conclusive evidence in support of the interpretation made from the analysis of shoot and root growth rates, root development morphology and plant P-uptake efficiency that the fast growing, modern high yielding varieties had higher demand but lower supply efficiency of nutrients as compared to the lower root demand but higher supply efficiency of the slow growing traditional varieties.

An experiment was performed in the nursery with the low phosphorus laterite soil to determine the shoot growth and phosphorus uptake rates of the 8 rice varieties at graded levels of soil phosphorus to estimate the optimum P-demand in relation to the highest attainable growth rates of the varieties. The 8 rice varieties were grown in laterite soil by adjusting the available soil phosphorus at 5 levels between 5-120 ppm. Shoot mass development and shoot P-concentrations of the varieties were measured and shoot P-contents were estimated at 25 and 50 days taking appropriate number of replicates. Phosphorus demands of the varieties relative to their highest attainable rate of shoot growth were found out by regression analysis of the data. Details of the methods used are mentioned in the Chapter III (Materials and Methods). Results of the analysis are presented in Tables 15 -19 and Figures 8-17.

3.1 Shoot growth response to increased soil P-levels

Results of the analysis of shoot growth at graded levels of soil phosphorus showed that all the varieties responded positively to increased soil P-levels by production of higher amount of shoot mass till a limit above which the response started declining (Table 15; Figures 8 and 9). In other words, there was a clear trend

of diminishing return of P-responsive growth of the varieties above a limit of maximum soil P. The polynomial nature of the shoot growth response curves of the slow and fast growing variety groups to increasing soil P levels, at both 25 and 50 days, were grossly similar (Figures 8 & 9). But, there were some characteristic differences between the response patterns of the two variety groups at both the plant ages also. The rate of increase in shoot growth at lower P- levels (till 12-15 ppm soil P) was identical for both the variety groups. Similarly, the rate of decline in shoot growth at higher P levels (above 50 ppm) was also identical for the two variety groups. But at both the plant ages, the limit of soil P above which the diminishing response of shoot growth set in was higher in case of the fast growing than the slow growing varieties. The difference between the two variety groups in the upper limit of soil P for the onset of declining response was more at 25 than at 50 days plant age. For the slow growing varieties, the fall in shoot growth rate at increased levels of soil P at 25 days was nearer to 20 ppm, whereas the same for the fast growing varieties was farther to 20 ppm (Figure 8). The magnitude of increased growth response to increased P-levels being higher for the modern high yielding varieties, at both the plant ages, the extent of decline in growth above the critical limit of soil P was higher for the traditional than the modern varieties (Figure 9). The difference between the two variety groups in the magnitude of positive response to increased soil P-levels was wider at early than late plant age. Figure 10 presents the shoot growth curves of the varieties in response to increased levels of available soil phosphorus individually.

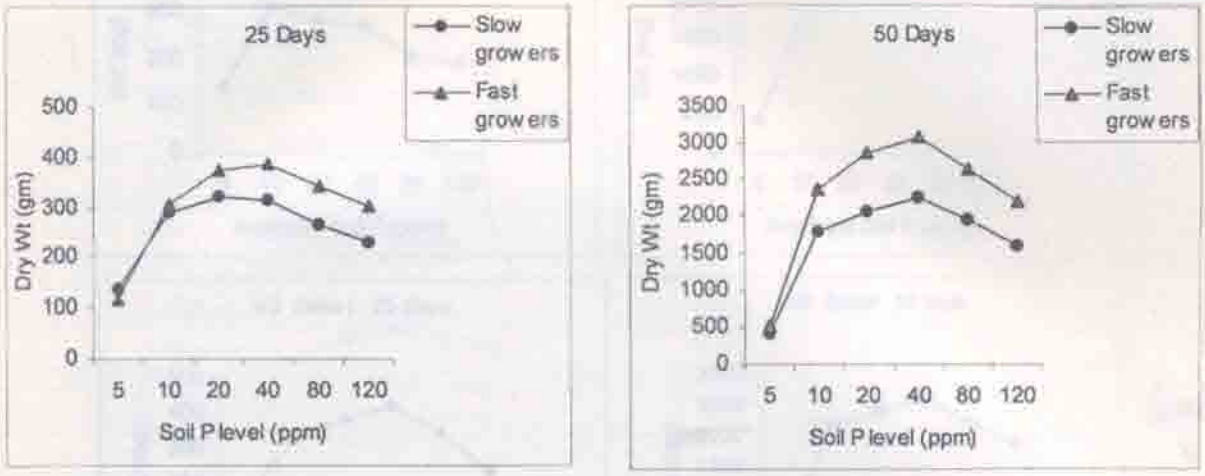
These data supported the previous interpretation about the difference in P-demand between the two variety groups. The fast growing varieties in showing a higher magnitude of shoot growth response to increased levels of soil P and also having a higher limit of soil P-level beyond which their shoot growth response declined were expected to have a higher P-demand than the slow growing varieties which showed lower magnitude of shoot growth response and also had a lower soil P-limit for the onset of the declining response of shoot growth.

Table 15: Per plant shoot dry weight at 25 and 50 days at different levels of soil phosphorus in laterite soil

Variety	Shoot weight (mg)											
	25 days						50 days					
	P0	P1	P2	P3	P4	P5	P0	P1	P2	P3	P4	P5
V1	138	308	280	264	204	188	410	1750	2042	2120	1672	1324
V2	164	326	384	425	343	237	412	2150	2416	2602	2218	1878
V3	102	230	256	224	198	164	374	1296	1584	1788	1576	1104
V4	128	288	424	396	352	308	498	2444	2739	3018	2766	2446
V5	140	294	368	348	320	286	444	1984	2243	2573	2355	2136
V6	124	298	340	366	344	320	496	2082	2545	2848	2560	2292
V7	106	326	388	412	348	300	508	2276	2939	3162	2556	1996
V8	104	318	356	378	324	290	550	2680	3205	3326	2728	2032
Mean of four slow growing varieties (V1, V2, V3, V5)	136 ±17.51	289 ±143.76	322 ±63.46	315 ±89.57	266 ±75.97	231 ±54.16	410 ±28.61	1795 ±370.93	2071 ±358.90	2271 ±390.24	1955 ±388.54	1610 ±478.31
Mean of four fast growing varieties (V4, V6, V7, V8)	115 ±12.26	307 ±17.54	377 ±37.15	388 ±20.20	342 ±12.44	304 ±12.69	513 ±25.22	2370 ±253.87	2857 ±282.31	3088 ±203.81	2652 ±110.23	2191 ±214.89
Magnitude of increased response over P0	Slow growing varieties	2.12	2.37	2.32	1.95	1.70	-	4.3	5.05	5.54	4.77	3.93
	Fast growing varieties	2.67	3.28	3.37	2.97	2.64	-	4.62	5.57	6.02	5.17	4.27

P0: 5 ppm, P1: 10 ppm, P2: 20ppm, P3: 40 ppm, P4: 80ppm, P5: 120 ppm

Figure 8: Shoot growth response of 4 slow and 4 fast growing varieties to increased levels of soil phosphorus at 25 and 50 days



Scales on X/Y axis are different.

Figure 9: Magnitude of increased shoot growth response of the slow growing and fast growing rice varieties to increased levels of soil phosphorus at 25 and 50 days

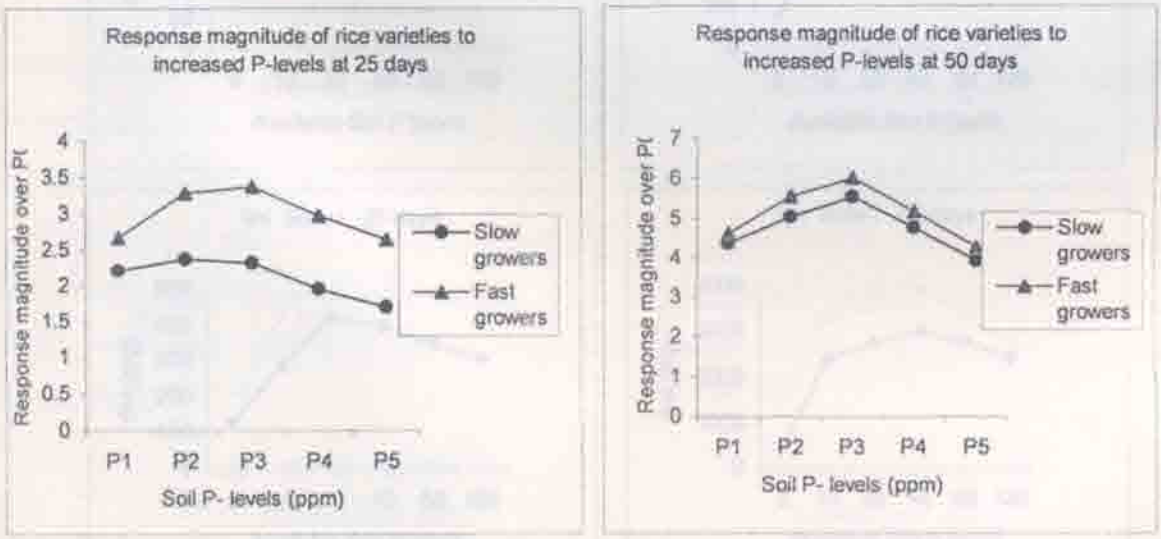
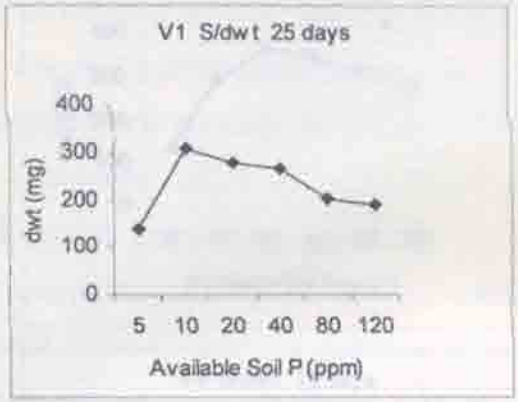
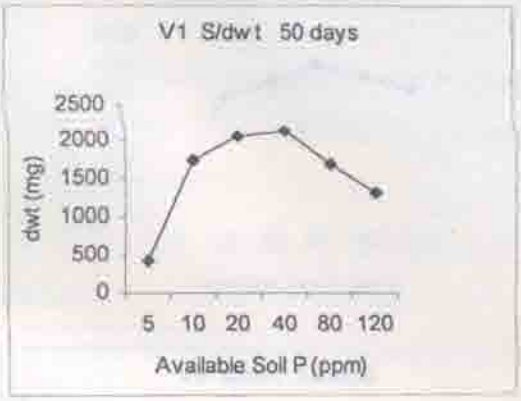
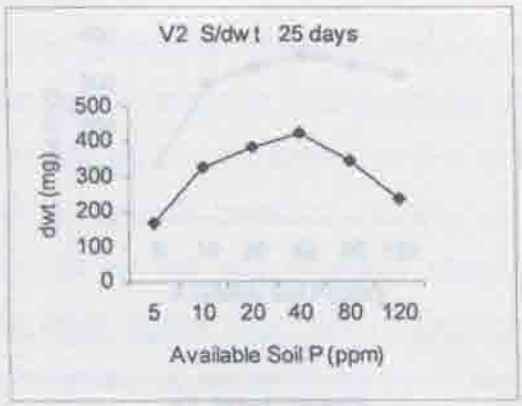
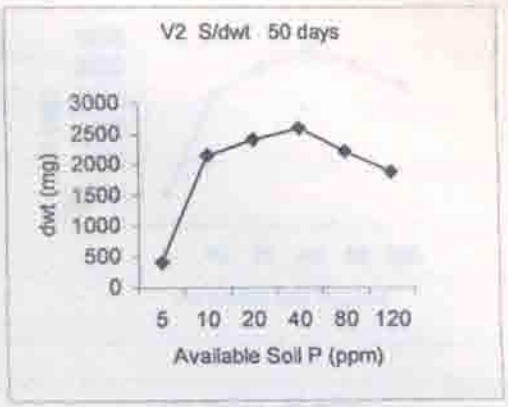


Figure 10: Shoot growth response curves of the eight rice varieties at varying levels of available phosphorus in soil at 25 and 50 days (S/dwt = Shoot dry weight)

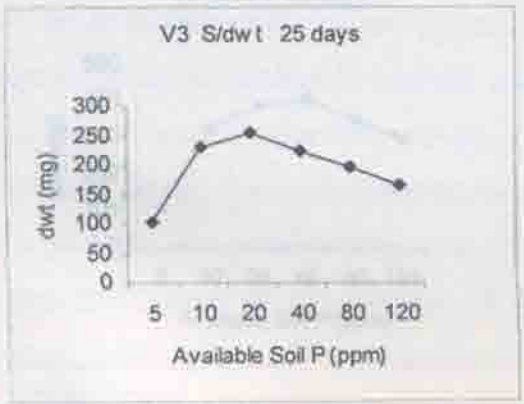


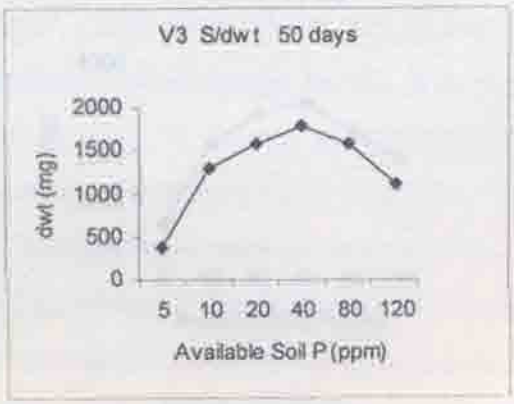


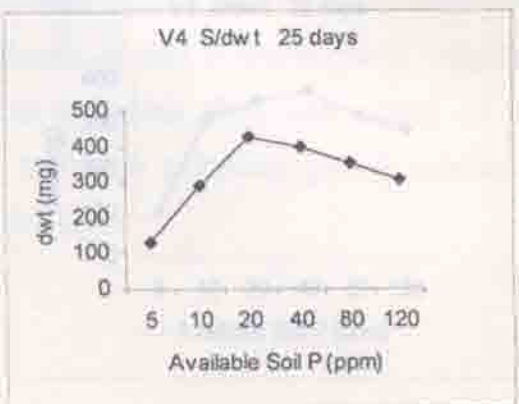


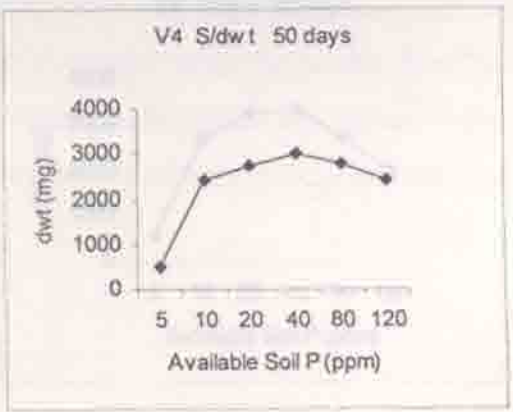


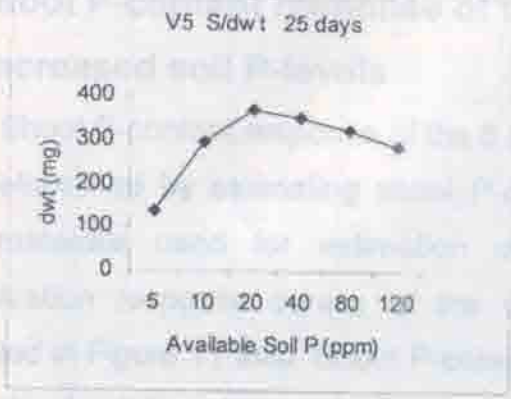
Scales
Y,
Y

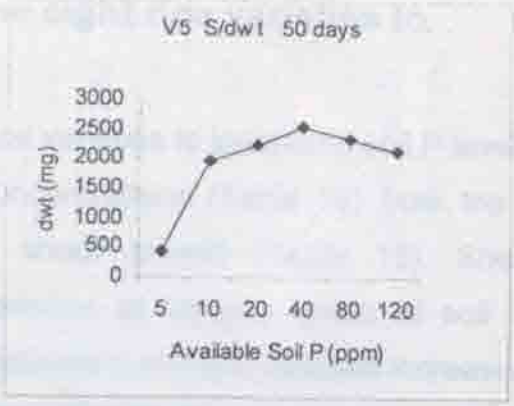


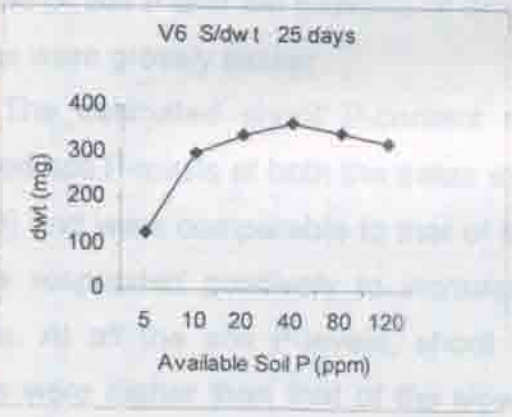


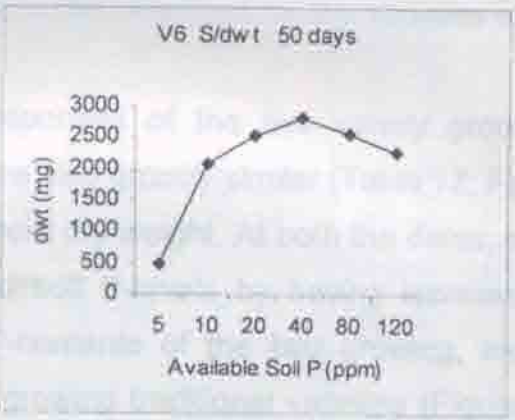


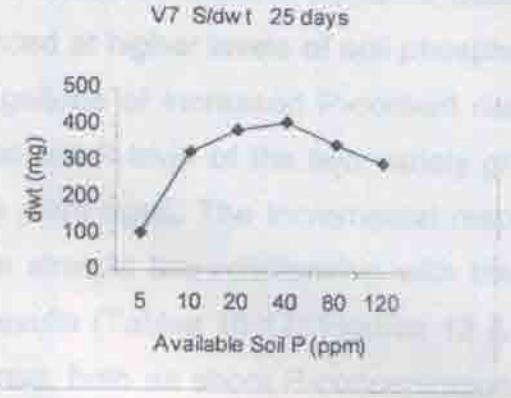


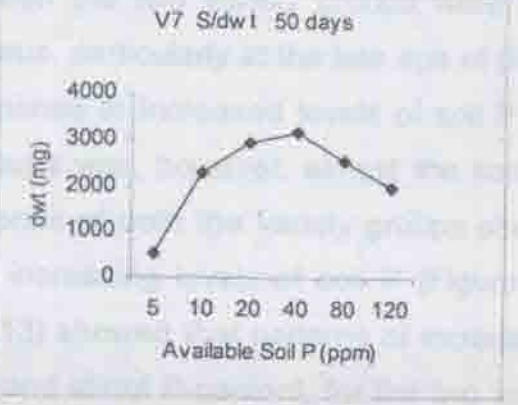

V5 S/dwt 25 days
dwt (mg)
400
300
200
100
0
5 10 20 40 80 120
Available Soil P (ppm)

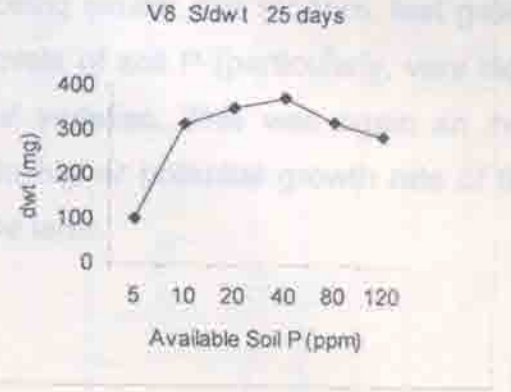

V5 S/dwt 50 days
dwt (mg)
3000
2500
2000
1500
1000
500
0
5 10 20 40 80 120
Available Soil P (ppm)

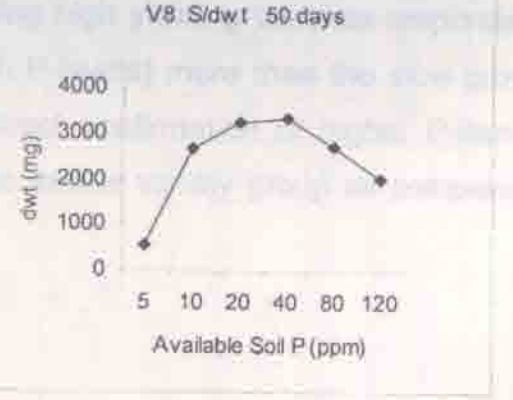

V6 S/dwt 25 days
dwt (mg)
400
300
200
100
0
5 10 20 40 80 120
Available Soil P (ppm)


V6 S/dwt 50 days
dwt (mg)
3000
2500
2000
1500
1000
500
0
5 10 20 40 80 120
Available Soil P (ppm)


V7 S/dwt 25 days
dwt (mg)
500
400
300
200
100
0
5 10 20 40 80 120
Available Soil P (ppm)


V7 S/dwt 50 days
dwt (mg)
4000
3000
2000
1000
0
5 10 20 40 80 120
Available Soil P (ppm)


V8 S/dwt 25 days
dwt (mg)
400
300
200
100
0
5 10 20 40 80 120
Available Soil P (ppm)


V8 S/dwt 50 days
dwt (mg)
4000
3000
2000
1000
0
5 10 20 40 80 120
Available Soil P (ppm)

3.2 Shoot P-content response of the eight rice varieties to increased soil P-levels

Shoot P-content response of the 8 rice varieties to increased soil P-levels was then determined by estimating shoot P-concentrations (Table 16) from the same plant materials used for estimation of shoot growth (Table 15). Shoot P-concentration response curves of the varieties at varying levels of soil P are presented in Figure 11 also. Shoot P-concentrations of the 8 varieties increased with the levels of soil P and the patterns of incremental response for the varieties at both the ages were grossly similar.

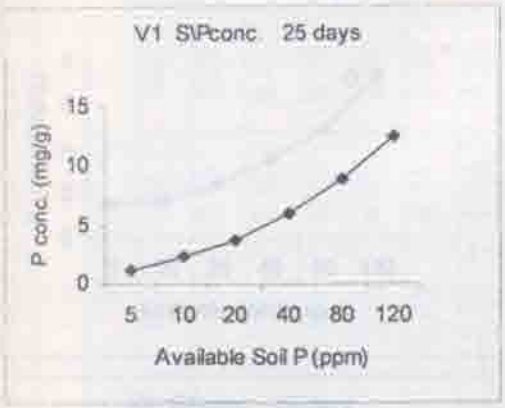
The estimated shoot P-content responses of the two variety groups to increased soil P-levels at both the dates were also grossly similar (Table 17; Figures 12 & 13) and were comparable to that of shoot dry weight. At both the dates, all the varieties responded positively to increased soil P-levels by having increased P-contents. At all the soil P-levels, shoot P-contents of the fast growing, modern varieties were higher than that of the slow growing traditional varieties (Figure 12). The differences in shoot P-contents between the two variety groups were more pronounced at higher levels of soil phosphorus, particularly at the late age of plants. The magnitude of increased P-content response to increased levels of soil P over the basal soil P-level of the two variety groups was, however, almost the same at both the plant ages. The incremental response of both the variety groups showed almost a straight line relationship with the increasing levels of soil P (Figure 13). These results (Tables 16-17; Figures 12 & 13) showed that patterns of incremental P-response, both as shoot P-concentration and shoot P-content, for the two variety groups being similar, the modern, fast growing high yielding varieties responded to higher levels of soil P (particularly, very high P-levels) more than the slow growing traditional varieties. This was again an indirect confirmation of higher P-demand relative to higher potential growth rate of the former variety group as compared to that of the later.

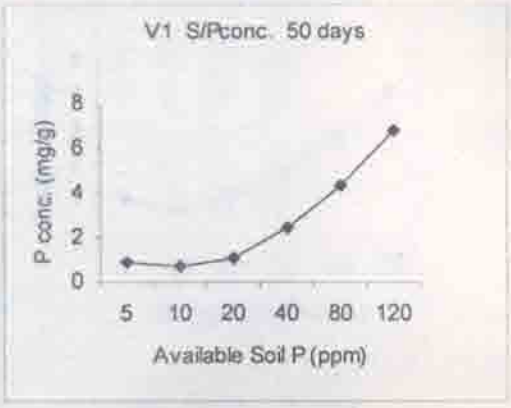
Table 16: Shoot P-concentrations of the eight varieties at 25 and 50 days at varying soil P-levels

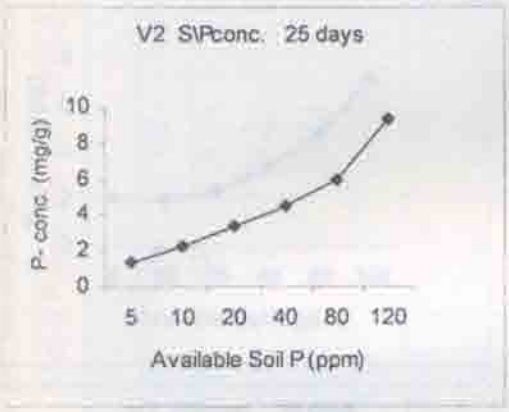
Variety	Shoot P – conc. (mg/g)											
	25 days						50 days					
	P0	P1	P2	P3	P4	P5	P0	P1	P2	P3	P4	P5
V1	1.23	2.40	3.70	6.05	8.83	12.43	0.95	0.70	1.09	2.42	4.32	6.81
V2	1.36	2.28	3.32	4.53	5.92	9.34	0.98	0.68	1.02	1.96	2.89	5.12
V3	1.73	2.11	2.46	4.12	6.96	11.20	1.23	0.95	1.26	2.31	3.84	7.35
V4	1.40	2.06	2.65	3.42	4.45	5.89	0.97	0.55	0.84	1.64	3.05	4.52
V5	1.59	1.70	2.50	3.59	4.78	7.34	1.10	0.75	1.14	1.84	2.83	4.07
V6	2.05	1.98	2.32	3.40	4.78	7.21	1.25	0.78	1.31	1.98	3.21	4.47
V7	2.16	2.01	2.46	3.07	5.22	9.45	1.07	0.64	1.21	1.90	3.62	5.79
V8	2.13	2.04	2.70	3.74	5.88	8.21	1.12	0.66	1.13	1.88	3.38	5.34
Mean of four slow growing varieties (V1, V2, V3, V5)	1.48 ±0.225	2.12 ±0.306	2.99 ±0.615	4.57 ±1.06	6.62 ±1.72	10.08 ±2.22	1.06 ±0.128	0.77 ±0.123	1.13 ±0.101	2.13 ±0.276	3.47 ±0.731	5.64 ±1.681
Mean of four fast growing varieties (V4, V6, V7, V8)	1.93 ±0.360	2.02 ±0.035	2.53 ±0.175	3.41 ±0.274	5.08 ±0.618	7.69 ±1.510	1.10 ±0.116	0.66 ±0.095	1.12 ±0.202	1.85 ±0.146	3.31 ±0.244	5.01 ±0.644

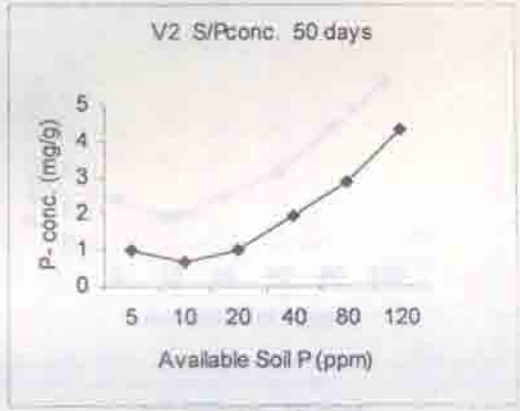
Average 5x3 determinations

Figure 11: Shoot P-concentration response of the eight rice varieties at varying levels of soil phosphorus at 25 and 50 days.

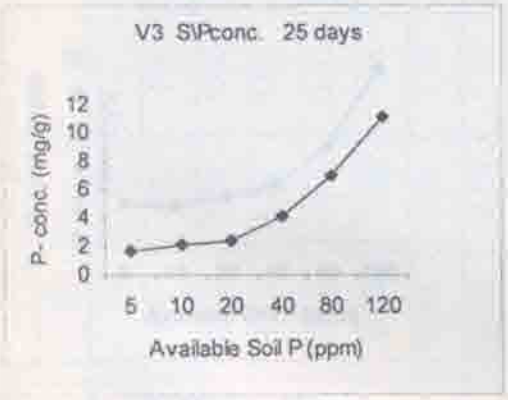


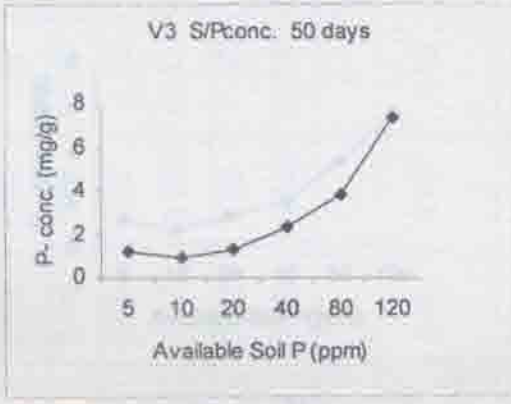


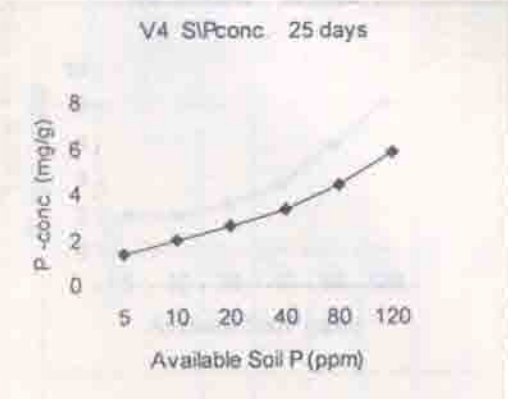


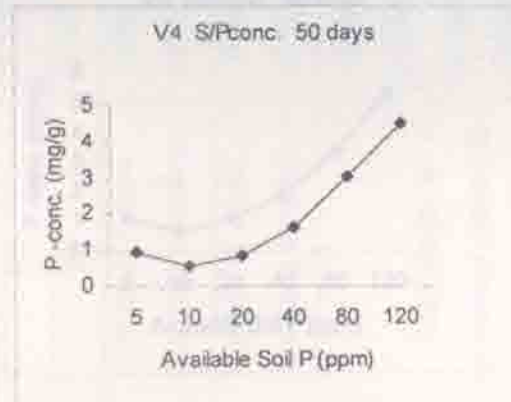


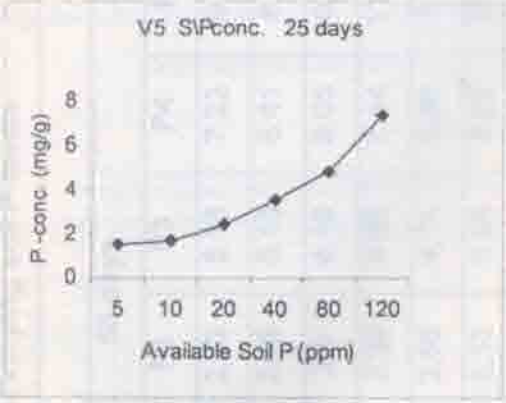
Soils x, y are dissimilar

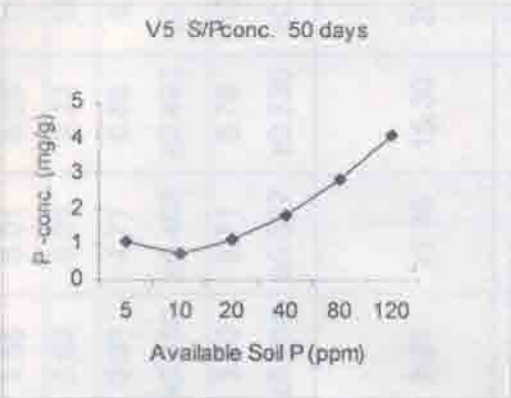


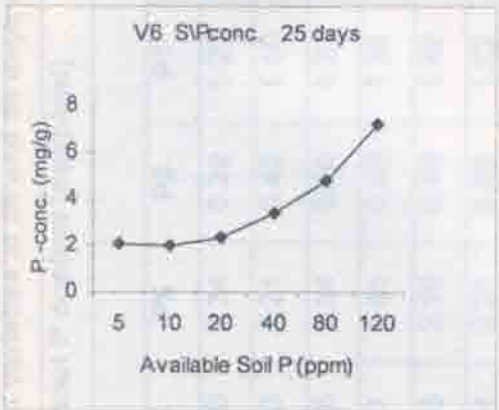


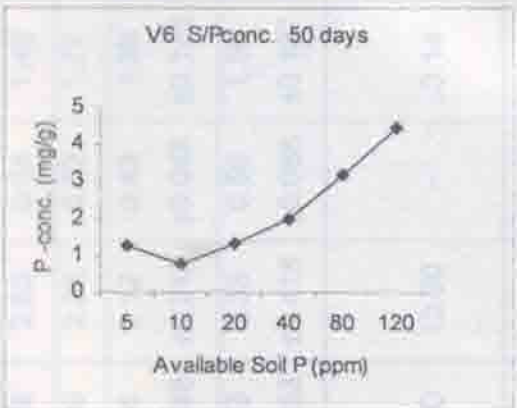


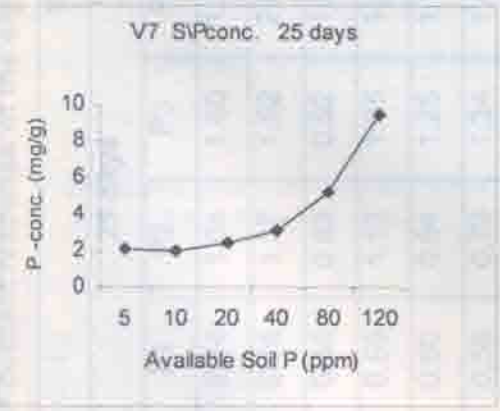


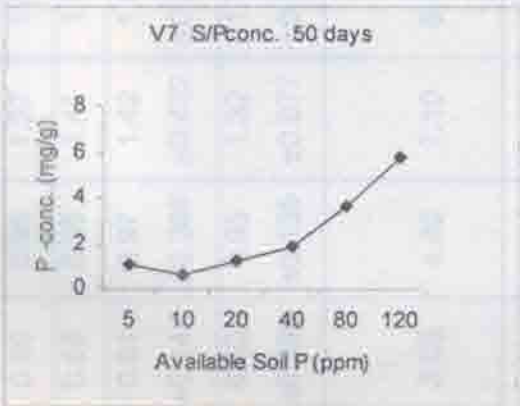

V5 S\Pconc. 25 days
P -conc. (mg/g)
0
2
4
6
8
5 10 20 40 80 120
Available Soil P (ppm)

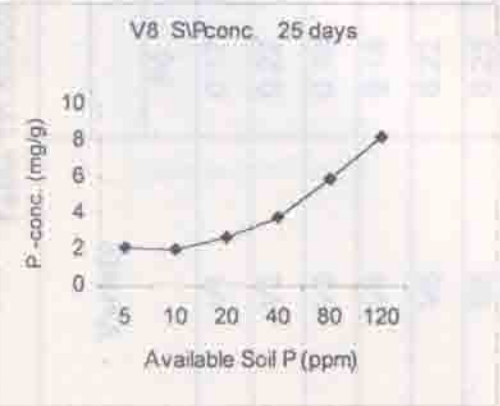

V5 S/Pconc. 50 days
P -conc. (mg/g)
0
1
2
3
4
5
5 10 20 40 80 120
Available Soil P (ppm)


V6 S\Pconc. 25 days
P -conc. (mg/g)
0
2
4
6
8
5 10 20 40 80 120
Available Soil P (ppm)


V6 S/Pconc. 50 days
P -conc. (mg/g)
0
1
2
3
4
5
5 10 20 40 80 120
Available Soil P (ppm)


V7 S\Pconc. 25 days
P -conc. (mg/g)
0
2
4
6
8
10
5 10 20 40 80 120
Available Soil P (ppm)


V7 S/Pconc. 50 days
P -conc. (mg/g)
0
2
4
6
8
5 10 20 40 80 120
Available Soil P (ppm)


V8 S\Pconc. 25 days
P -conc. (mg/g)
0
2
4
6
8
10
5 10 20 40 80 120
Available Soil P (ppm)

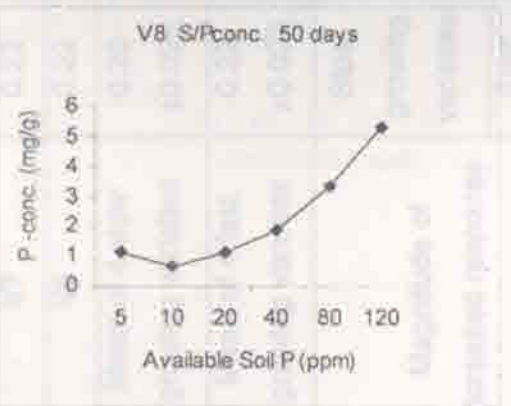
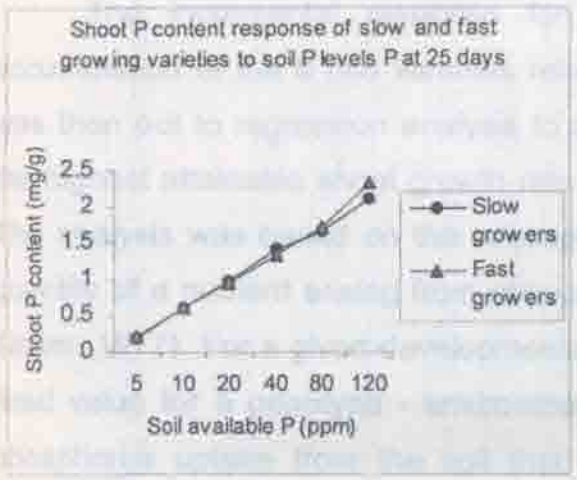

V8 S/Pconc. 50 days
P -conc. (mg/g)
0
1
2
3
4
5
6
5 10 20 40 80 120
Available Soil P (ppm)

Table 17: Shoot P-content response of the eight varieties at 25 and 50 days at varying soil P-levels

Variety		Shoot P content (mg/plant)											
		25 days						50 days					
		P0	P1	P2	P3	P4	P5	P0	P1	P2	P3	P4	P5
V1		0.17	0.74	1.04	1.60	1.80	2.34	0.39	1.22	2.23	5.13	7.22	9.02
V2		0.22	0.74	1.27	1.92	2.03	2.21	0.40	1.46	2.46	5.10	6.41	8.13
V3		0.18	0.48	0.63	0.92	1.38	1.84	0.46	1.23	2.0	4.13	6.05	8.11
V4		0.18	0.59	1.12	1.35	1.57	1.81	0.48	1.34	2.30	4.95	8.44	11.06
V5		0.22	0.50	0.94	1.25	1.53	2.10	0.49	1.49	2.56	4.74	6.66	8.70
V6		0.25	0.59	0.79	1.24	1.64	2.31	0.62	1.62	3.33	5.64	8.22	10.24
V7		0.23	0.66	0.95	1.27	1.82	2.83	0.54	1.46	3.56	6.01	9.25	11.56
V8		0.22	0.65	0.96	1.41	1.90	2.38	0.62	1.77	3.62	6.25	9.22	10.85
Mean of 4 slow growing varieties		0.20 ±0.026	0.61 ±0.144	0.97 ±0.265	1.42 ±0.432	1.68 ±0.288	2.12 ±0.212	0.43 ±0.048	1.35 ±0.145	2.31 ±0.250	4.77 ±0.465	6.58 ±0.492	8.49 ±0.447
Mean of 4 fast growing varieties		0.22 ±0.029	0.62 ±0.038	0.95 ±0.135	1.32 ±0.077	1.73 ±0.153	2.33 ±0.418	0.56 ±0.068	1.55 ±0.187	3.20 ±0.614	5.71 ±0.567	8.78 ±0.530	10.93 ±0.546
Magnitude of increased response over P0	Slow growing varieties	3.05	4.80	7.10	8.40	10.60		-	3.14	5.37	11.10	15.30	20.60
	Fast growing varieties	2.80	4.32	6.0	7.86	10.59		-	2.77	5.71	10.2	15.7	19.5

Figure 12: Shoot P-content response means of 4 slow and 4 fast growing varieties at increased levels of soil P at 25 and 50 days (Based on Tables 15,16 and 17)



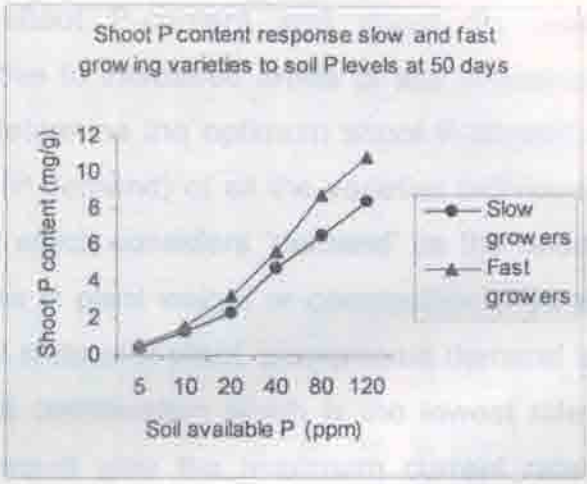
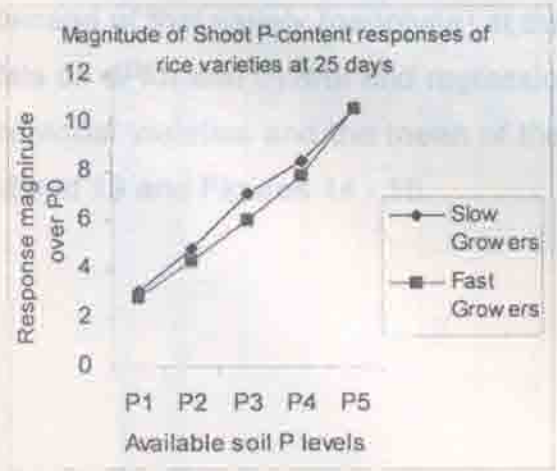
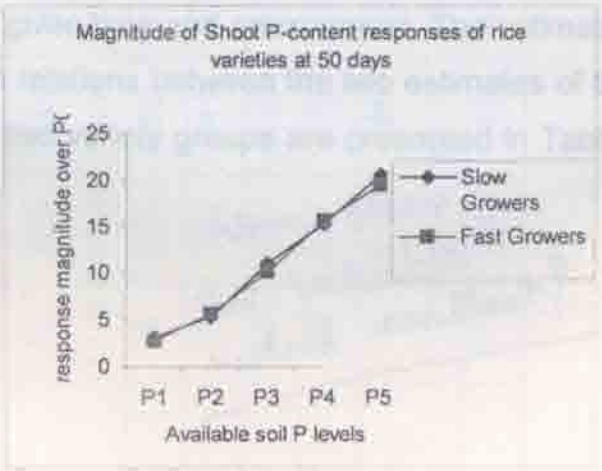


Figure 13: Response magnitude of shoot P-content of slow and fast growing variety groups to increased soil P-levels at 25 and 50 days





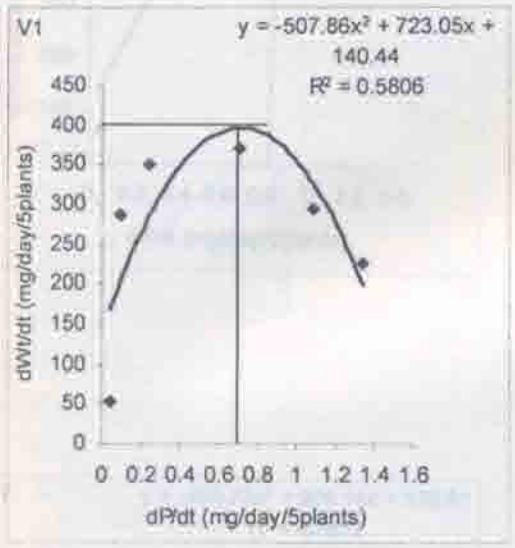
3.3 Phosphorus demand and supply estimates of the eight rice varieties in laterite soil relative to highest attainable shoot growth rate between 25 and 50 days

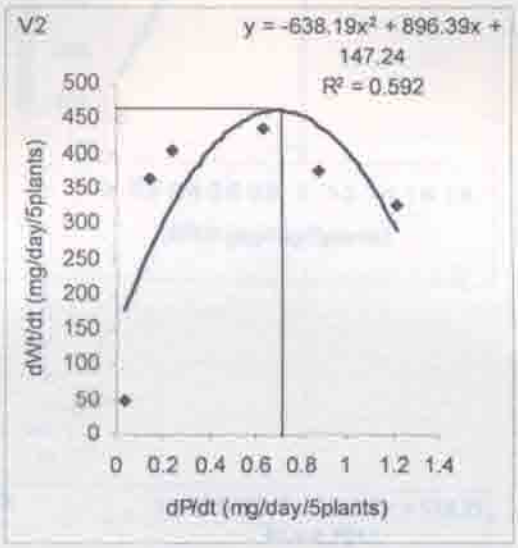
The incremental response for shoot P-content and shoot dry matter accumulation of the 8 rice varieties relative to increased levels of soil phosphorus was then put to regression analysis to determine the optimum shoot P-content for the highest attainable shoot growth rate (P-demand) of all the varieties individually. The analysis was based on the concept which considers 'demand' as the needed quantity of a nutrient arising from changes in plant weight or composition (Nye and Tinker, 1977). For a given developmental state of a plant, phosphorus demand is a fixed value for a genotype - environment combination which is the lowest rate of phosphorus uptake from the soil that would give the maximum current rate of growth, all other factors held optimum (Koide, 1991). The genotype - environment specific potential P-demand can be estimated by determining the regression relationship between the changes in the rate of P-uptake (d*P*/d*t*) and the corresponding changes in the rate of shoot growth (d*Wt*/d*t*), by varying the soil P-supply. The lowest rate of P-uptake needed to satisfy the maximum potential growth rate of a variety (genotype) at a given period of time can be taken as the potential P-demand of that variety (genotype) at the given time and environment. The estimated data on d*P*/d*t* and d*Wt*/d*t* and regression relations between the two estimates of the individual varieties and the mean of the two variety groups are presented in Tables 18 and 19 and Figures 14 - 15.

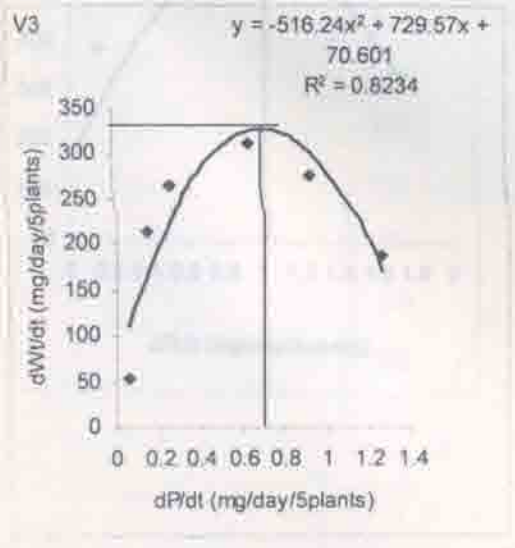
Table 18: Rate of shoot growth (d*Wt*/d*t*) and rate of increase in P-content (d*P*/d*t*) of the eight rice varieties between 25 and 50 days at varying soil P-levels (based on Tables 15 and 17)

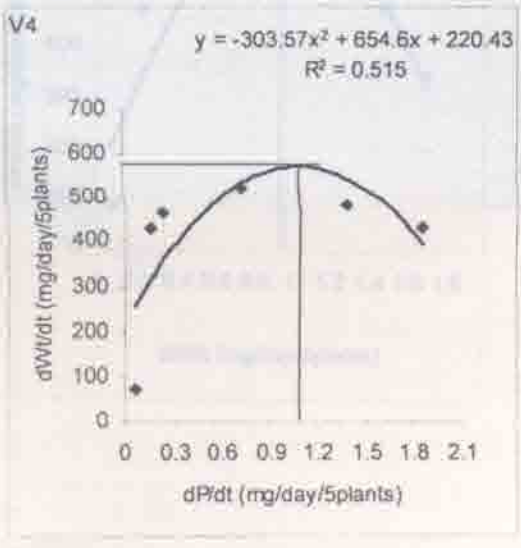
Variety	d *Wt* / d *t*						d *P* / d *t*					
	mg / day x 5						mg / day x 5					
	P0	P1	P2	P3	P4	P5	P0	P1	P2	P3	P4	P5
V1	54	288	352	371	294	227	0.044	0.097	0.238	0.707	1.084	1.336
V2	50	365	406	435	375	328	0.036	0.144	0.238	0.635	0.876	1.220
V3	54	213	266	313	276	188	0.057	0.149	0.273	0.641	0.934	1.256
Mean	**53** ±2.31	**289** ±76.0	**341** ±70.61	**373** ±61.02	**315** ±52.73	**248** ±72.25	**0.046** ±0.01	**0.130** ±0.029	**0.250** ±0.20	**0.661** ±0.04	**0.965** ±0.107	**1.271** ±0.059
V4	74	431	463	524	483	428	0.061	0.151	0.233	0.722	1.374	1.848
V6	74	357	441	496	443	394	0.073	0.208	0.508	0.880	1.317	1.588
V7	80	390	510	550	442	339	0.063	0.154	0.519	0.949	1.486	1.744
V8	89	472	570	590	481	348	0.079	0.222	0.529	0.968	1.460	1.694
Mean	**79** ±7.09	**412** ±49.90	**496** ±57.11	**540** ±39.97	**462** ±22.82	**377** ±41.53	**0.069** ±0.0085	**0.184** ±0.036	**0.447** ±0.143	**0.880** ±0.112	**1.409** ±0.078	**1.718** ±0.108
V5	61	338	375	445	407	370	0.053	0.198	0.325	0.699	1.026	1.320

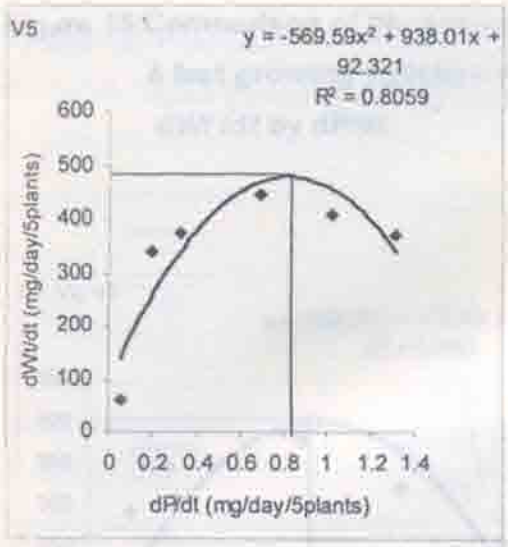
Figure 14: Relation between per day increase in shoot growth (d*Wt*/d*t*) and per day increase of shoot P-content (d*P*/ d*t*) of the eight rice varieties relative to varying levels of soil phosphorus in laterite soil: Polynomial trend lines between d*Wt*/d*t* and d*P*/d*t*.

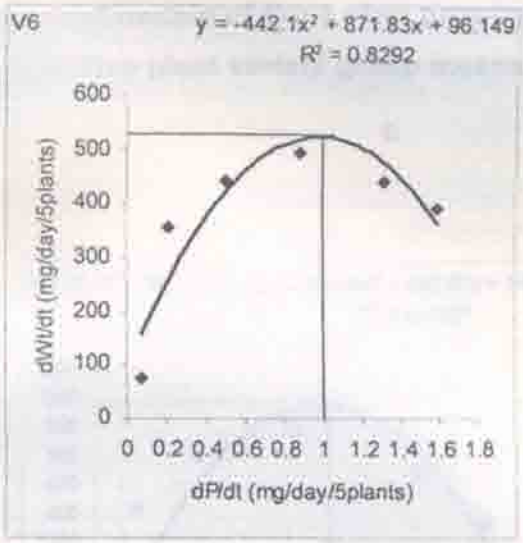


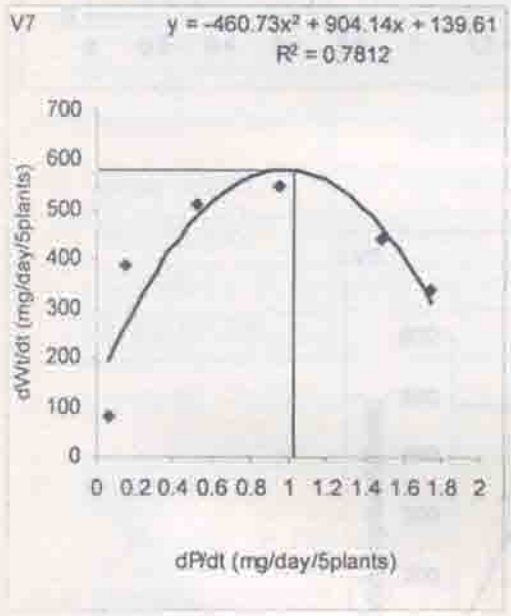







V5
y = -569.59x² + 938.01x + 92.321
R² = 0.8059
dWt/dt (mg/day/5plants)
dP/dt (mg/day/5plants)


V6
y = -442.1x² + 871.83x + 96.149
R² = 0.8292
dWt/dt (mg/day/5plants)
dP/dt (mg/day/5plants)


V7
y = -460.73x² + 904.14x + 139.61
R² = 0.7812
dWt/dt (mg/day/5plants)
dP/dt (mg/day/5plants)

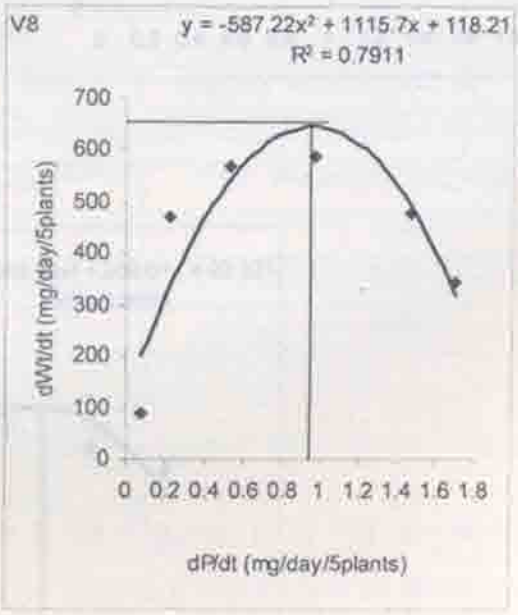
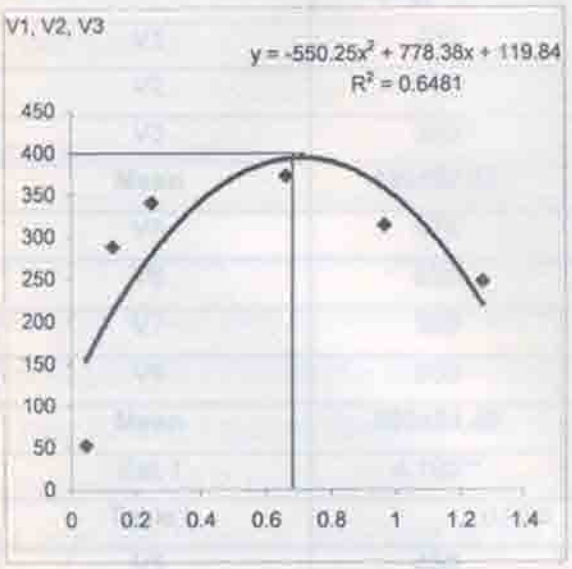
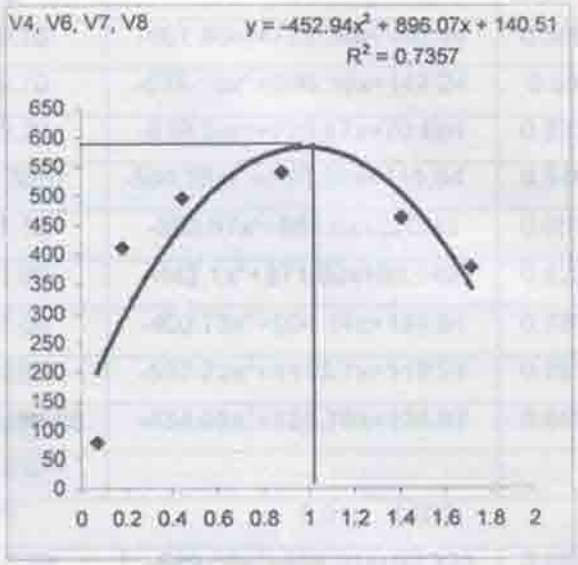

V8
y = -587.22x² + 1115.7x + 118.21
R² = 0.7911
dWt/dt (mg/day/5plants)
dP/dt (mg/day/5plants)

Figure 15.Comparison of phosphorus demand relationship of the 4 slow growing and 4 fast growing varieties in laterite soil: Five plant variety group means of d*Wt* /d*t* by d*P*/d*t*





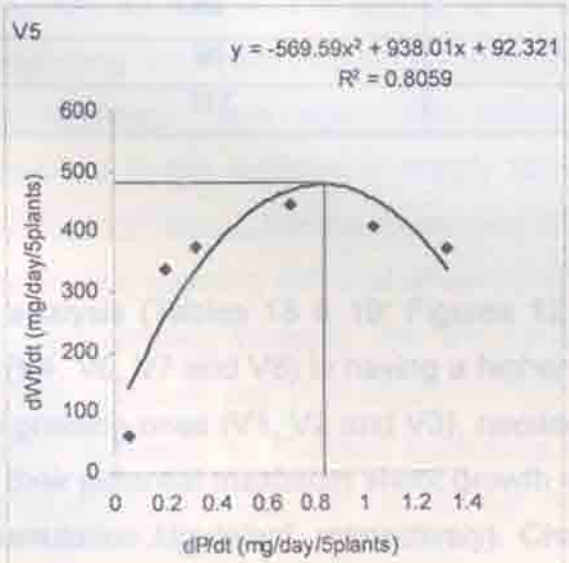


Table 19: **Potential P-demand of the 8 rice varieties and the P-demand means of the slow growing and fast growing variety groups (based on Figures 10 & 11)**

Variety	d*Wt*/d *the* (mg) max	d*P*/d*t* (mg) opt	Polynomial equation	
			Y=	R^2
V1	400	0.70	$-507.86x^2+723.05x+140.44$	0.5806
V2	465	0.70	$-638.19x^2+896.39x+147.24$	0.592
V3	330	0.70	$-516.24x^2+729.57x+70.601$	0.8234
Mean	**398±67.51**	**0.70±0**	**$-550.25x^2+778.38x+119.84$**	**0.6481**
V4	575	1.10	$-303.57x^2+654.6x+220.43$	0.6515
V6	525	1.00	$-442.1x^2+871.83x+96.149$	0.8292
V7	590	1.00	$-460.73x^2+904.14x+139.61$	0.7812
V8	650	0.95	$-587.22x^2+1115.7x+118.21$	0.7911
Mean	**585±51.48**	**1.01±0.06**	**$-434.05x^2+725.79x+126.97$**	**0.6676**
Est. t	4.195**	8.832**		
Table t	0.05*p* 2.571		0.01*p* 4.032	
V5	480	0.82	$-569.59x^2+938.01x+92.321$	0.8059
Variety means	Per day per plant growth rate and P-demand between 25-50days			
	Growth rate (mg) Max		P-demand (mg) Opt	
V1, V2, V3	80		0.14	
V5	96		0.16	
V4, V6, V7, V8	117		0.20	

P – Demand

Results of the analysis (Tables 18 & 19; Figures 12 & 13) showed that the fast growing varieties (V4, V6, V7 and V8) in having a higher rate of shoot growth as compared to the slow growing ones (V1, V2 and V3), needed a higher rate of shoot P-turnover to support their potential maximum shoot growth rate (117 mg and 80 mg shoot dry weight accumulation /day/plant, respectively). Characteristically, both the potential maximum growth rate (dWt/dt_{max}) and the lowest rate of P-uptake or current supply (dP/dt_{opt}) needed to achieve the highest potential growth rate of the fast growing modern varieties were significantly higher than that of the slow growing traditional varieties. The traditional variety V5 which behaved as an intermediate

between the two variety groups for the growth related traits showed intermediate P-demand optimum for potential maximum growth rate which was also in between values of the two variety groups. This confirmed the interpretation made earlier from all other analyses that the modern HYVs have a higher P-demand to support their higher rates of shoot growth than the traditional varieties which have a lower rate of shoot growth.

Phosphorus deficit

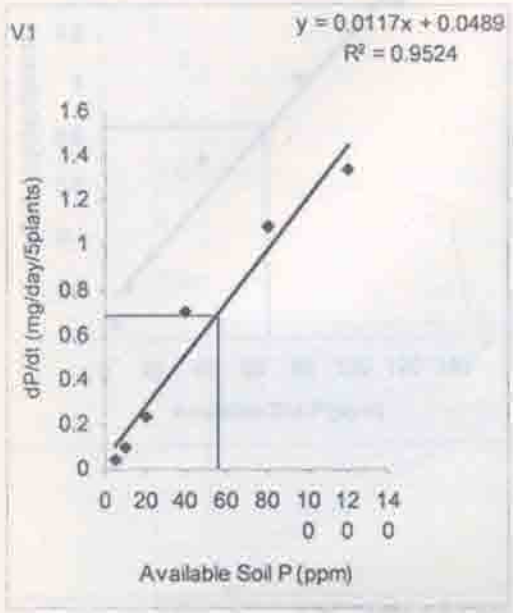
Phosphorus deficit values of the eight rice varieties relative to their optimum P-demand against maximum potential growth rate were then determined by regression analysis of the data on changes in the rate of shoot P turnover (shoot p-supply) at varying levels of available soil phosphorus. Results of the analysis are presented in Table 20 and Figures 16and 17. As previously mentioned, shoot P-turnover rates at increasing soil P levels of the modern high yielding varieties were higher, particularly at high soil P-levels, than that of the slow growing traditional varieties. The variety group trend means of changes in P-supply rates (dP/dt) showed identical straight line relationship with the changes in soil P-levels (Figure 17), showing R^2 values near to 1 (0.9591 and 0.964, for modern and traditional varieties respectively). But, the slope of the P-supply trend line of the modern HYVs (Figure 17) was higher than that of the traditional varieties. Here again the variety V5 behaved as an intermediate. With reference to the optimum P-supply rate (dP/dt_{opt}) needed for highest potential growth rate (dWt/dt_{max}) or the P-demand of the two variety groups, the P-deficit of the modern fast growing HYVs at all levels of soil P was higher than that of the traditional slow growing varieties and the variety V5 belonged to an intermediate position. The optimum soil P at which the optimum P-supply rate for maximum growth rate (P-demand) or P-deficit balancing of the two contrasting variety groups and the variety V5 was attained was almost the same.

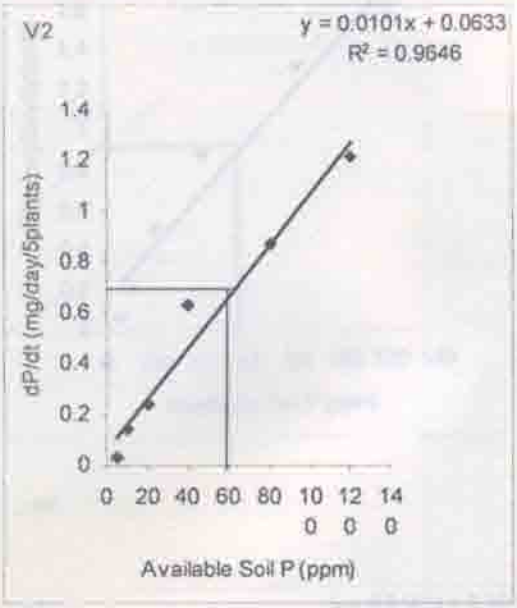
Results of these analyses were interpreted to mean that if the two variety groups were put in a soil phosphorus level below the optimum (i.e. the levels which their maximum potential growth rates and optimum P-supply rates are achieved), the

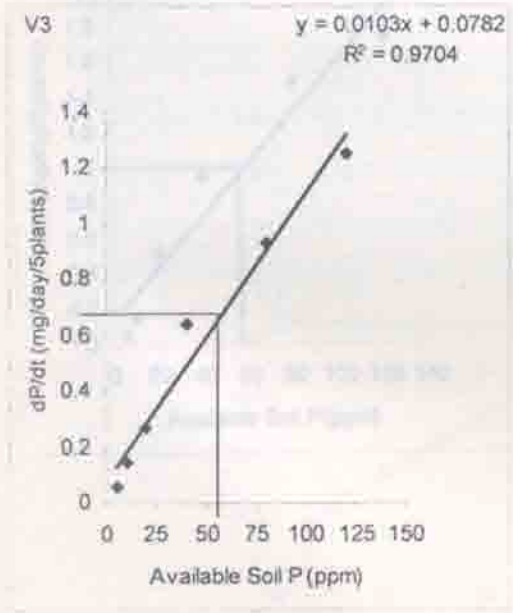
fast growing, modern high yielding varieties will have a higher P-deficit than the slow growing traditional varieties and they will be affected more in terms of loss of their achievable P-responsive shoot growth rate or the yield of shoot biomass at the given period of time. The traditional V5 will behave as an intermediate.

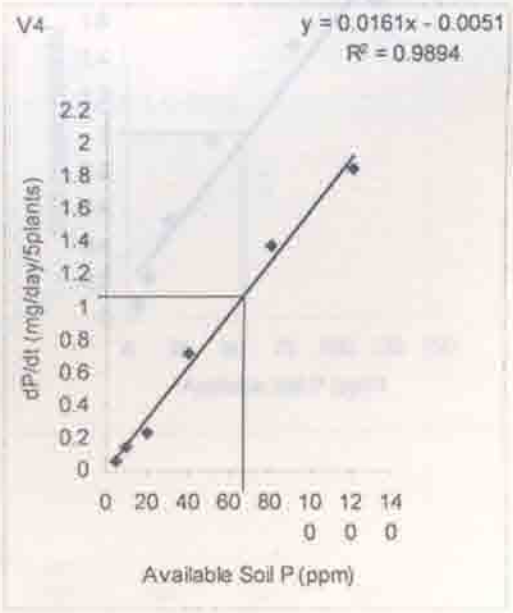
Results of the analysis of the physiological traits of the rice genotypes in relation to AM dependency in the low nutrient soil (Sections 1 – 3) thus showed that the selected modern HYVs have higher demand but lower supply efficiency of phosphorus as compared to lower demand but higher supplyefficiency of most traditional varieties and land race selections. Thus, the modern HYVs are expected to suffer from higher phosphorus deficit in a deficient soil more than most of the traditional varieties and land race selections.

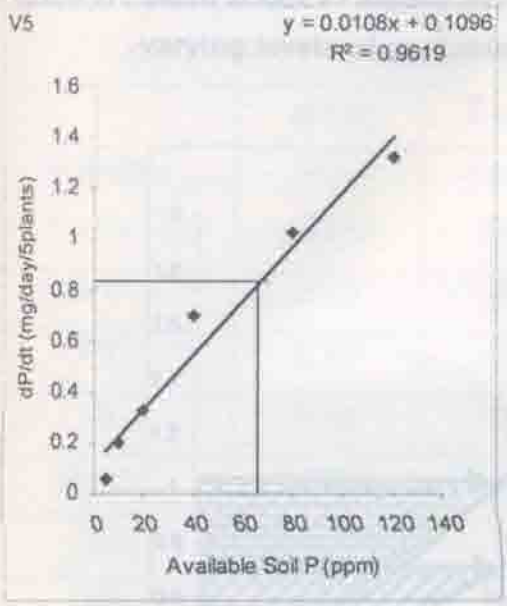
Figure 16: Trend lines of shoot P-supply rates at varying levels of soil P of the eight rice varieties in laterite soil during 25 – 50 days

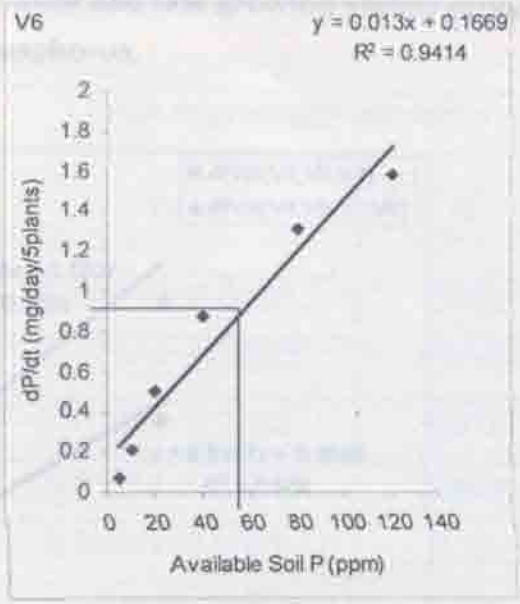


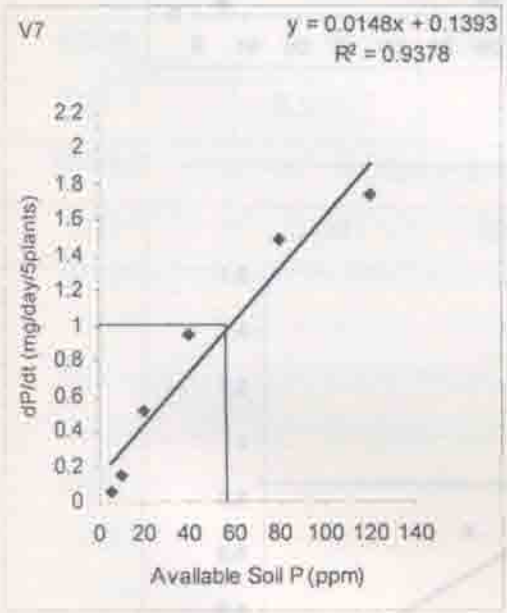







V5
y = 0.0108x + 0.1096
R² = 0.9619
dP/dt (mg/day/5plants)
Available Soil P (ppm)


V6
y = 0.013x + 0.1669
R² = 0.9414
dP/dt (mg/day/5plants)
Available Soil P (ppm)


V7
y = 0.0148x + 0.1393
R² = 0.9378
dP/dt (mg/day/5plants)
Available Soil P (ppm)

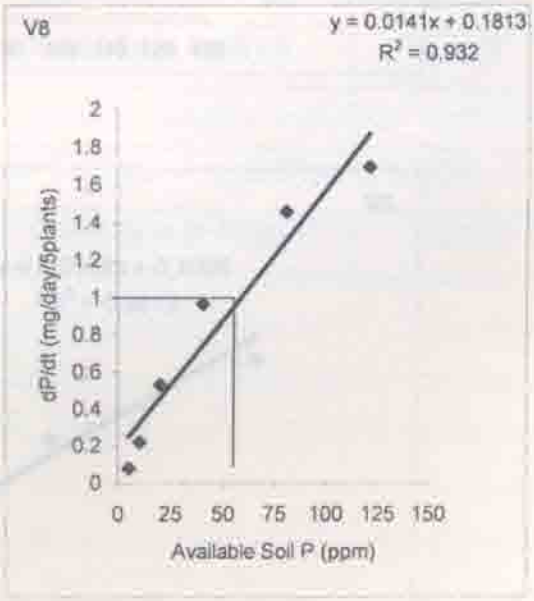
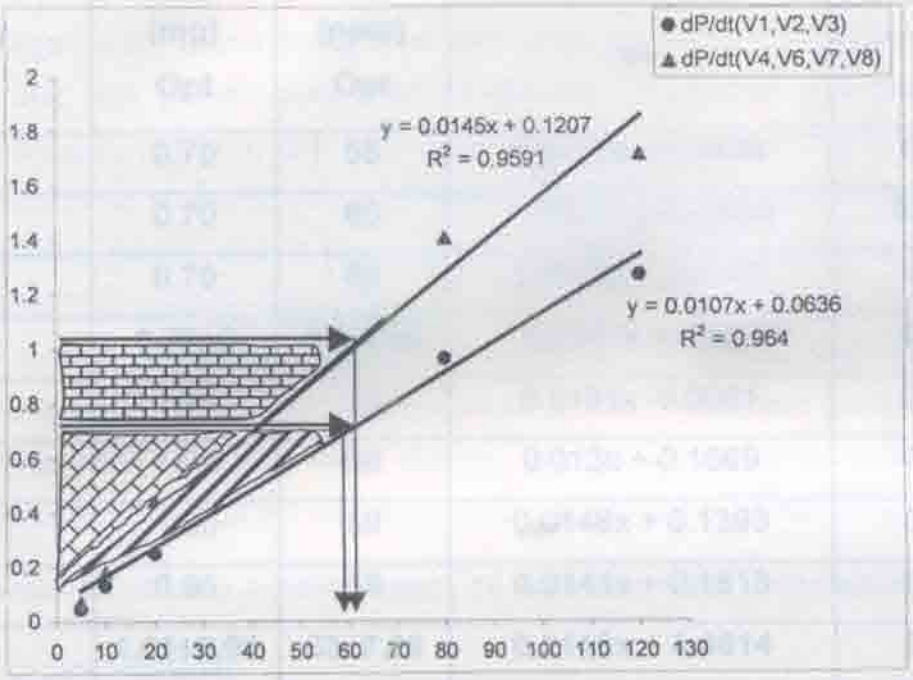

V8
y = 0.0141x + 0.1813
R² = 0.932
dP/dt (mg/day/5plants)
Available Soil P (ppm)

Figure 17: Mean shoot P- supply trend of the slow and fast growing variety groups at varying levels of available soil phosphorus.



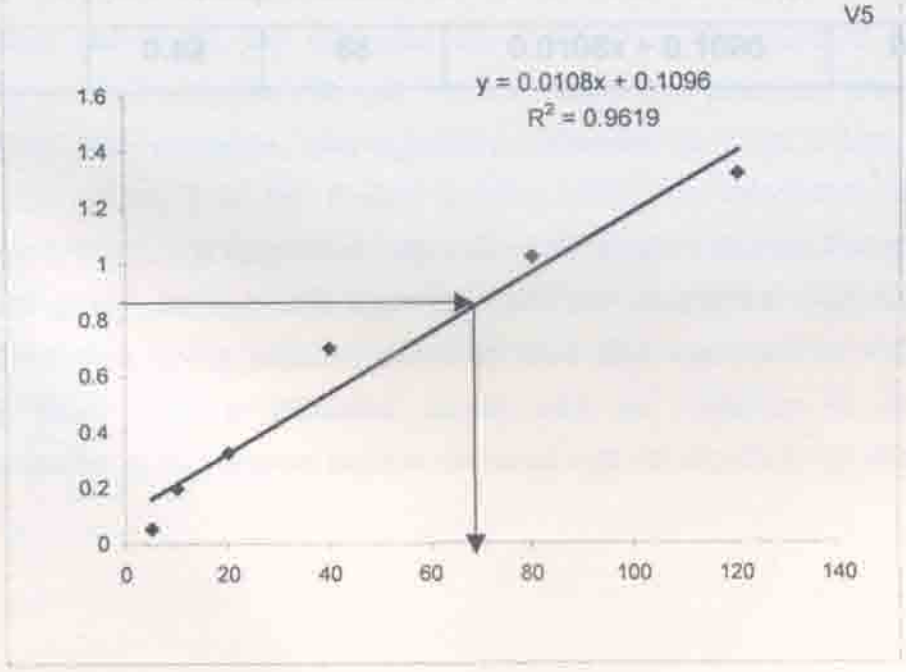


Table 20: Optimum shoot P supply rate for maximum shoot growth rate and optimum soil P-values of the 8right rice varieties.

Variety	*dP/dt* (mg) Opt	Soil P (ppm) Opt	Linear regression equation	
			Y=	R^2
V1	0.70	55	0.0117x + 0.0489	0.952
V2	0.70	65	0.0101x +0.0633	0.9646
V3	0.70	60	0.0103x + 0.0782	0.970
Mean	**0.70±0**	**58±2.89**	**0.0107x + 0.0636**	**0.964**
V4	1.10	70	0.0161x -0.0051	0.989
V6	1.00	66	0.013x + 0.1669	0.941
V7	1.00	59	0.0148x + 0.1393	0.938
V8	0.95	58	0.0141x + 0.1813	0.932
Mean	**1.01±0.06**	**63±7.26**	**0.0119x + 0.0614**	**0.979**
Esti. t	8.832**	NS		
Table t	0.05*p* 2.571		0.01*p* 4.032	
V5	0.82	68	0.0108x + 0.1096	0.9619

4. Dependency or response relations of the eight rice varieties to AMF inoculation in laterite soil

Against the above background information about the physiological traits – shoot and root growth rates, root development characters and phosphorus demand-supply relations, AMF inoculation responses of the eight rice varieties in the low nutrient laterite soil were then determined. The rice varieties were grown in solarized laterite soil in the nursery with or without AMF inoculation as described in Chapter III (Materials and methods). Plant growth (dry matter yield) and P-content, separately for root and shoot, at 15 and 30 days after seed germination was determined and responses to AMF inoculation were estimated as 'mycorrhiza inoculation effect' (MIE%). Results are presented in Tables 21-36. Response pattern of two selected varieties are shown in plates 1 and 2.

4.1 Response at early seedling stage, 15 days after seed germination

Shoot growth response

At 15 days after seed germination, the rice varieties responded differently to AMF presence in the inoculated soil as compared to the plants in the non-inoculated soil (Table 21). Whereas the shoot dry matter yield of the 4 fast growing, modern varieties (V4, V6, V7, V8) responded positively (MIE mean + 14.0%) to AMF presence, with significant difference ($p < 0.01$) from the non-inoculated plants, 3 of the 4 slow growing land race selections / traditional varieties (V1, V2, V3) responded negatively (MIE mean - 12.2%). The differences in shoot growth between the inoculated and non-inoculated plant sets of the above varieties, giving negative response were also significant ($p < 0.05$). The variety Dular (V5), a traditional variety was an exception to the above categorization as its marginal positive response was not significant ($p < 0.05$).

Table 21: Shoot growth responses of the 8 rice varieties to AMF inoculation at early seedling stage, 15 days after seed germination in low nutrient laterite soil

Shoot dry weight (mg) per 5 plants								
Treatment	Variety							
	V 1	V2	V3	V5	V4	V6	V7	V8
AM-	278± 7.2	276±13.4	244± 11.8	210 ± 7.5	233± 8.1	231± 10.5	204±7.6	240±8.7
AM+	247± 9.8	243± 9.5	221± 8.6	218 ±11.5	266± 8.9	269± 10.4	237±8.9	285±9.5
Est. t	4.416*	3.480*	NS	NS	4.751**	4.454*	4.885**	6.342**
Table t	0.05 *p* 2.776				0.01*p* 4.604			
MIE%	**- 12.5**	**- 13.6**	**- 10.4**	**3.7**	**12.4**	**14.1**	**13.9**	**15.8**
Mean MIE % of varieties	3 slow growing varieties (V1, V2, V3)			V5	4 fast growing varieties (V4, V6, V7. V8)			
	- 12.2			**+ 3.7**	**+ 14.0**			

Root growth response

Root growth measured as root mass dry matter of the 8 rice varieties responded similarly as shoot growth to AMF inoculation. Whereas the root mass yield of the 4 fast growing varieties (V4, V6, V7, V8) showed significant positive response (p <0.05) to AMF presence (Mean MIE 24.2%), the 3 traditional varieties / land race selections (V1, V2, V3) showed marginal negative response (Mean MIE -4.2%). The response of the traditional variety Dular (V5) was only marginally positive as in case of shoot growth. The responses of the 4 slow growing traditional varieties including V5 were not significant (p <0.05).

Table 22: Root growth responses of the 8 rice varieties to AMF inoculation at early seedling stage, 15 days after seed germination in low nutrient laterite soil

Root dry weight (mg) / 5 plants								
Treatment	Variety							
	V 1	V2	V3	V5	V4	V6	V7	V8
AM-	235±14.0	225±15.0	215±6.6	202±14.0	167±13.8	176±22.9	145±24.5	183±19.0
AM+	228±15.7	216±7.0	204±7.9	215±9.8	209±9.8	236±20.2	194±17.1	248±18.6
Est. t	**NS**	**NS**	**NS**	**NS**	**4.299***	**12.481****	**2.841***	**4.235***
Table t	0.05 *p* 2.776				0.01 *p* 4.604			
MIE%	**- 3.0**	**- 4.2**	**- 5.4**	**6.0**	**20.0**	**25.4**	**25.2**	**26.2**
MIE % of Varieties	3 slow growing varieties (V1, V2, V3)			V5	4 fast growing varieties (V4, V6, V7. V8)			
	- 4.2			**+ 6.0**	**+ 24.2**			

Root / shoot ratio response

Root / shoot ratios of the traditional varieties/land race selections were significantly higher (as observed previously also) than that of the fast growing modern varieties. Irrespective of their evolutionary status, the root /shoot ratios of the two variety groups changed positively due to inoculation, the incremental change being more pronounced and significant in case of the modern fast growing varieties than the traditional varieties (13% vs. 7%). Varieties responding negatively (V1, V2, V3) both for root and shoot development had root/shoot ratios higher than 0.8; varieties responding positively (V4, V6, V7, V8) had ratios lower than 0.8; V5 having very high root / shoot ratio did not show any significant (+ve / -ve) response, neither its root shoot ratio changed with inoculation.

Table 23: Root / shoot ratios of the 8 rice varieties in laterite soil under AMF inoculation

Treatment	V 1	V 2	V 3	V 5	V4	V 6	V 7	V 8
AM -	0.84	0.81	0.88	0.96	0.72	0.76	0.71	0.76
AM +	0.92	0.89	0.92	0.99	0.78	0.88	0.82	0.87
Mean of varieties	Slow growing varieties (V1, V2, V3, V5)				Fast growing varieties (V, V6, V7, V8)			
	Mycorrhiza -		Mycorrhiza +		Mycorrhiza -		Mycorrhiza +	
	0.87		0.93		0.74		0.84	
% change	7.0				13.0			

Results of the above analysis showed that the rice genotypes varying in their evolutionary status and differing in their physiological traits, like growth rate, phosphorus demand-supply etc. varied in their arbuscular mycorrhizal response even at a very early stage of seedling growth. The modern HYVs and those varieties which have higher potential shoot growth rate, low root/shoot ratios and a higher P- deficit in low phosphorus soil as compared to the traditional varieties / land race selections responded positively to AMF inoculation. The latter group of varieties having lower shoot growth rate, high root/shoot ratios and lower P-deficit at the same soil responded negatively. The most characteristic change in growth response of the varieties due to inoculation even at the very early stage was the increase in R/S ratio, where the magnitude of the change was significantly higher in the positively responding modern HYVs than the negatively responding traditional varieties. This was in spite of the fact that in terms of absolute response, root growth of the traditional varieties was affected negatively.

P-acquisition response

P-acquisition responses of the varieties to AMF inoculation were then determined by measuring phosphorus concentrations of shoots and roots separately. P-content (uptake) responses of the varieties were estimated from the measured values of dry matter yield (Tables 21 & 22) and P-concentration values of the same shoot and root samples (Tables 24 & 25).

Table 24: Shoot phosphorus concentrations (mg /g) of the 8 rice varieties at 15 days in laterite soil under AMF inoculation

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM -	0.76±0.04	0.70±0.07	0.83±0.08	0.87±0.03	0.44±0.09	0.31±0.06	0.29±0.07	0.41±0.04
AM+	1.04±0.06	0.92±0.06	1.12±0.10	1.25±0.07	1.11±0.11	0.78±0.03	0.92±0.03	1.06±0.10
Est. t	6.727**	4.134*	3.923*	8.644**	8.167**	12.138**	14.331**	10.455**
Table t	0.05 *p* 2.776				0.01 *p* 4.604			
MIE%	26.9	23.9	25.9	30.4	60.4	60.3	68.5	61.3
Mean	**26.8**				**62.6**			

Table 25: Root phosphorus concentrations (mg /g) of the 8 rice varieties at 15 days in laterite soil under AMF inoculation

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM –	0.54±0.06	0.61±0.05	0.76±0.01	0.56±0.04	0.56±0.05	0.50±0.03	0.52±0.02	0.71±0.02
AM+	0.82±0.02	0.93±0.03	1.08±0.05	0.82±0.06	1.28±0.05	0.83±0.06	1.26±0.04	1.26±0.02
Est. t	7.670**	9.507**	10.872**	6.246**	17.640**	8.522**	28.666**	33.687**
Table t	0.05 *p* 2.776				0.01 *p* 4.604			
MIE %	34.1	34.4	29.6	31.7	56.2	39.7	58.7	43.6
Mean	**32.4**				**49.5**			

Shoot phosphorus concentration data (Table 24) showed that the 4 slow growing traditional varieties (V1, V2, V3 and V5) which responded negatively or only marginally to AMF presence for shoot growth, all had higher shoot P-concentrations than the modern, fast growing positively AM responding varieties (V4, V6, V7, V8). For both the group of varieties, AMF inoculation resulted in highly significant ($p < 0.01$) increases in shoot P-concentrations where the magnitude of increase was higher in the modern fast growing varieties than the traditional varieties (mean magnitude of incremental response 2.73 times and 1.33 times respectively). The traditional variety V5 which showed only marginally positive growth response showed MIE% for P-concentration equal to that of the other traditional varieties. It was apparent that the rice varieties irrespective of their significant differences in growth response to AMF inoculation, ranging from negative to positive, gained in shoot P-concentrations even at this early stage due to possible AMF infection. It was characteristic that the high P-concentration gaining varieties responded or benefited for shoot growth more than the low P-concentration gaining varieties.

The inherent differences in root P-concentrations between the varieties (Table 25) were not very significant but, as in case of shoot, all the varieties gained significantly ($p < 0.01$) in root P-concentrations also. There was not much variation in the magnitudes of root P-concentration gains among the varieties, but the trend of higher increase in positively responding varieties, as in case shoot, was also present in root.

Estimates of shoot phosphorus contents of the 8 rice varieties (Table 26) revealed a predictable pattern. Slow growing traditional varieties had higher shoot P-contents than the fast growing modern varieties. Under AMF inoculation, shoot P-contents of all the varieties showed increase. But the incremental response was highly significant ($p < 0.01$) in all the 4 fast growing modern varieties and only 2 of the 4slow growing traditional varieties, including the variety V5 which showed non-significant shoot growth response to AMF. The incremental response was non-significant ($p < 0.05$) in the other two traditional varieties. The magnitude of

incremental response of the variety V5 in spite of being significant was intermediate between the negatively and positively responding variety groups. This showed that corresponding to their higher shoot growth response, the modern, fast growing, high P-demanding varieties had higher P-uptake response to AMF inoculation than the non-responding or negatively responding traditional varieties which had a lower and less significant P-uptake response.

Root P-content responses (Table 27) to AMF inoculation of the varieties showed a comparable pattern to that of their shoot P-content responses. The highly significant incremental responses of the modern varieties were higher than that of the traditional varieties but the difference between the two variety groups in the magnitude of response was less than that for shoot P-content response.

Based on the above data whole plant growth response and P-acquisition responses of the varieties were computed by taking the shoot and root responses into consideration. The computed data are presented in Table 28.

Table 26: Shoot Phosphorus content (mg / 5 plants) of the 8 rice varieties at 15 days in laterite soil under AMF inoculation

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM -	0.211 ± 0.02	0.193 ± 0.03	0.202 ± 0.06	0.183 ± 0.06	0.102 ± 0.008	0.072 ± 0.003	0.059 ± 0.005	0.098 ± 0.008
AM +	0.257 ± 0.02	0.224 ± 0.04	0.247 ± 0.04	0.272 ± 0.014	0.295 ± 0.021	0.210 ± 0.015	0.218 ± 0.012	0.302 ± 0.020
Est. t	2.817*	NS	NS	14.879**	15.629**	21.189**	16.407**	17.85**
Table t	0.05 *p* 2.776				0.01*p* 4.604			
MIE %	17.9	13.8	18.2`	32.7	65.4	65.7	72.9	67.5
Mean	**16.6**			**32.7**	**67.9**			

Table 27: Root Phosphorus content (mg / 5 plants) of the 8 rice varieties at 15 days in laterite soil under AMF inoculation

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM -	0.127 ± 0.007	0.137 ± 0.009	0.163 ± 0.005	0.113 ± 0.008	0.093 ± 0.015	0.088 ± 0.012	0.075 ± 0.012	0.130 ± 0.011
AM +	0.187 ± 0.013	0.201 ± 0.006	0.220 ± 0.008	0.176 ± 0.008	0.267 ± 0.013	0.196 ± 0.016	0.244 ± 0.021	0.312 ± 0.023
Est. t	7.040**	10.250**	10.467**	9.647**	15.186**	9.355**	12.105**	12.367**
Table t	0.05 *p* 2.776				0.01*p* 4.604			
MIE %	32.1	31.8	25.9	35.8	65.2	55.1	69.3	58.3
Mean	**31.4**				**62.0**			

Table 28: **Comparison of plant growth and P-acquisition responses of the rice varieties to AMF inoculation at 15 days**

Treatment	Dry matter content of varieties (mg/5plants)							
	V1	V2	V3	V5	V4	V6	V7	V8
	Whole plant dry matter (mg /5 plants)							
AM -	513	501	459	412	400	407	349	423
AM +	475	459	425	433	475	478	431	533
MIE %	- 7.4	- 9.1	- 8. 0	4.8	15.8	14.8	19.0	20.6
Mean MIE% of varieties	**- 8.1**			**4.8**	**17.5**			
	Whole plant P-content (mg /5 plants)							
AM -	0.338	0. 330	0.365	0.296	0.195	0.160	0.134	0.228
AM +	0.444	0.425	0.467	0.448	0.562	0.406	0.462	0.614
MIE %	23.9	22.3	21.8	33.9	65.3	60.6	71.0	62.9
Mean MIE% of varieties	**25.5**			**33.9**	**65.0**			
	Proportional partition of incremental P (%) between							
Root	56.6	67.4	55.9	41.5	47.4	43.9	51.5	47.2
Shoot	43.4	32.6	44.1	58.5	52.6	56.1	48.5	52.8
Mean of varieties	**V1, V2, V3**				**V4, V6, V7, V8**			
Root	60			41.5	47.5			
Shoot	40			58.5	52.5			

Results of the analysis of whole plant growth and P-acquisition responses of the rice varieties at very early growth stage (Table 28) showed that the modern, fast growing high P-demanding varieties responded to AMF inoculation for both the traits with a higher magnitude than the traditional, slow growing low P-demanding varieties. The traditional varieties, in spite of gaining in P-acquisition, did not show corresponding gains in plant growth, rather some of these varieties showed a negative response to possible AMF infection. Another important point that emerged from the analysis was the lack of parallelism between growth and P-acquisition responses due to AMF infection of the varieties which indicated that the whole of incremental phosphorus acquired by the plant due to AMF might not be utilized or needed for growth at such early stage. Proportional partition of the incremental phosphorus gained by the plants between root and shoot of the varieties when

analyzed (Table 28) showed a characteristic difference between the two variety groups. For the low P-demanding, slow growing traditional varieties incremental P under AMF infection partitioned in 60:40 proportions between root and shoot. The same changed to nearly 50:50 proportions in the high P-demanding, fast growing modern varieties. Although the varieties where P- gain was more in shoot than root responded for growth with a higher magnitude, the same might not be a rule as the traditional variety Dular where the shoot P-gain was highest among all the varieties did not show a comparable high growth response.

4.2 Response at late seedling stage, 30 days after seed germination

Shoot growth response

At later stage of seedling growth, 30 days after seed germination, the shoot growth response pattern of the traditional varieties/ land race selections to AMF presence showed change from that of the early seedling stage (Table 29). The 3 varieties which responded negatively at 15 days now showed marginal positive responses to AMF presence. The positive responses of these varieties were not significant ($p < 0.05$). Corresponding to that, the 4 fast growing modern varieties showed a higher magnitude of positive response than that shown by them at the early seedling stage. The differences in the magnitude of positive responses of the two variety groups was significant ($p < 0.01$). The traditional variety Dular (V5) which showed insignificant positive response at the early seedling stage now showed a higher magnitude of positive response, almost equal to that of the modern varieties. These data showed that there was an age dependent trend of increase in mycorrhizal response of the rice varieties. Irrespective of the varieties, the magnitude of shoot growth response to AMF infection changed positively with increase in plant age. The varieties which responded negatively at the early seedling stage gained in shoot growth to show a positive response at later stage. The varieties which responded positively at early seedling stage further gained in shoot growth and responded positively with a higher magnitude at later stage.

Table 29: **Mycorrhiza inoculation shoot growth response at 30 days at in laterite soil**

Treatment	Shoot dry weight (mg / 5 plants)							
	Variety							
	V 1	V2	V3	V5	V4	V6	V7	V8
AM-	458± 50.9	478±18.6	348± 24.4	354± 32.7	415± 29.5	426±15.0	367± 32.4	388± 24.1
AM+	502± 21.4	548± 45.5	408± 23.6	456± 20.3	652± 17.3	600± 28.9	585± 22.6	556± 33.1
Est. t	**NS**	**NS**	**3.062***	**4.591***	**12.006****	**9.258****	**9.560****	**7.108****
Table t	0.05 *p* 2.776				0.01 *p* 4.604			
MIE%	**8.8**	**12.8**	**14.7**	**22.4**	**26.3**	**29.0**	**30.8**	**30.2**
Mean MIE % of varieties	V1, V2, V3			V5	V4, V6, V7, V8			
	+12.1			**+ 22.4**	**+29.1**			

Root growth response

Root growth responses (Table 30) of the two variety groups to AMF presence in soil at this stage of seedling growth showed corresponding changes also. Root growth of the varieties belonging to both the groups responded positively to AMF presence where the response trend was similar to that of shoot growth. The traditional varieties, including V5, which showed a non-significant negative response to AMF presence at 15 days, now responded positively with a significant difference form the non-inoculated plants ($p < 0.05 - 0.01$). The magnitude of positive response of the fast growing modern varieties increased significantly from that observed at 15 days. Apparently, there was a strong promotion of root growth due to AMF infection in both the variety groups and there was a correspondingness between the increased responses of root and shoot growth due to AMF infection.

Table 30: Mycorrhiza inoculation root growth response at 30 days at in laterite soil

Root dry weight (mg /5 plants)								
Treatment	Variety							
	V 1	V2	V3	V5	V4	V6	V7	V8
M-	351± 24.9	369± 7.5	413± 23.1	349± 32.1	278±17.8	295± 25.5	253± 22.1	272± 22.1
M+	458± 35.6	504±11.5	616± 24.6	533± 37.0	469±15.4	520± 33.8	539± 34.5	468± 6.1
Est. t	4.267*	17.034**	10.421**	6.507**	14.058**	9.206**	12.093**	14.811**
Table t	0.05 *p* 2. 776				0.01 *p* 4. 604			
MIE%	**23.4**	**26.8**	**32.9**	**34.5**	**40.7**	**43.3**	**53.1**	**41.9**
Mean MIE % of varieties	V1, V2, V3, V5				V4. V6. V7. V8			
	+29. 4				**+44. 7**			

Root/shoot ratio Response

Table 31: Root / shoot ratios of the varieties under AMF inoculation at 30 days

Treatment	V 1	V 2	V 3	V 5	V 4	V 6	V 7	V 8
M -	0.77	0.77	1.19	0.98	0.67	0.74	0.78	0.72
M +	0.91	0.92	1.51	1.17	0.72	0.87	0.92	0.83
Mean of varieties	Traditional varieties				Modern varieties			
	Mycorrhiza -		Mycorrhiza +		Mycorrhiza -		Mycorrhiza -	
	0.93		1.13		0.73		0.83	
% change	**21.5**				**13.7**			

Root / shoot ratios of the varieties (Table 31) showed positive responses to AMF presence where the pattern of response between the two variety groups changed from that observed at the early, 15 days growth stage. The slow growing traditional varieties had higher root / shoot ratios as compared to the fast growing modern varieties. For both the variety groups, root / shoot ratios increased significantly due to AMF infection, but the magnitude of change was higher for the

traditional varieties (MIE 21. 5%) as compared to the modern varieties (MIE 13. 7%). This was reverse to what was observed at the early seedling stage. This showed that under AMF infection magnitude of increase in root growth as compared to that of shoot growth was higher in case of the traditional varieties than the modern varieties.

P-acquisition response

Incremental P-acquisition responses of the 8 rice varieties at 30 days were determined by measuring root and shoot P-concentrations (Tables 32 and 33) and estimating the P-contents from dry matter yield (Tables 29 and 30) and corresponding P-concentration values of the same samp

Shoot P-concentrations (Table 32) of the rice varieties showed significant ($p <$ 0.01) increases due to AMF presence in the low phosphorus laterite soil where the magnitude of increase was higher in the fast growing modern varieties than the slow growing traditional varieties (MIE % 59.1% vs. 40.8%). The traditional variety Dular (V5) behaved as an exception as its incremental P-concentration response was comparable to that of the fast growing varieties. Corresponding to that, root P-concentrations of the varieties also showed significant increases where the magnitude of increase in the two variety groups was almost the same (Table 33).

Table 32: Shoot phosphorus concentrations (mg /g) of the 8 rice varieties under AMF inoculation at 30 days in laetrile soil

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM –	0.66±0.04	0.58±0.03	0.54±0.05	0.38±0.07	0.35±0.05	0.38±0.02	0.41±0.04	0.50±0.03
AM +	1.16±0.04	0.93±0.03	0.93±0.07	1.04±0.07	1.00±0.04	0.89±0.05	0.94±0.04	1.23±0.04
Est. t	15.312**	14.292**	7.854**	11.550**	17.586**	16.407**	16.231**	25.293**
Table t	0.05*p* 2.776				0.01*p* 4.604			
MIE %	43.1	37.6	41.9	65.0	63.5	57.3	56.4	59.3
Mean MIE % of varieties	V1, V2, V3			V5	V4, V6, V7, V8			
	40.8			**65.0**	**59.1**			

Table 33: Root phosphorus concentrations (mg /g) of the 8 rice varieties under AMF inoculation at 30 days in laetrile soil

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM –	0.39±0.02	0.34±0.04	0.38±0.03	0.38±0.03	0.44±0.06	0.41±0.05	0.43±0.08	0.44±0.05
AM +	0.70±0.06	0.67±0.03	0.61±0.03	0.75±0.05	0.64±0.04	0.73±0.03	0.75±0.03	0.75±0.02
Est. t	8.491**	11.434**	9.392**	10.993**	4.805**	9.507**	6.488**	9.973**
Table t	0.05 *p* 2.776				0.01 *p* 4.604			
MIE %	44.3	49.2	37.7	49.3	31.2	43.8	42.7	41.3
Mean MIE % of varieties	Traditional varieties				Modern varieties			
	42.8				**39.7**			

Plant uptake of phosphorus (P- content / plant) separately for shoot (Table 34) and root (Table 35) showed highly significant increases ($p < 0.01$) due to AMF infection at 30 days with a predictable relation. The magnitude of increase in shoot P-content in the fast growing modern varieties was 1.5 times higher than that of the slow growing traditional varieties. The traditional variety Dular (V5) behaved as an exception as its P-content response was equivalent to that of the fast growing modern varieties. The root P-content responses of the two variety groups behaved similarly as shoot P-contents, except that the magnitude of increases in the two groups, including the variety V5 was almost the same.

Results of these analyses showed that commensurate with the increased responses of shoot and root growth under AMF presence at 30 days, from that at 15 days, there were comparable increases in P-acquisition responses of the varieties. The incremental responses for both growth and P-acquisition were higher in the fast growing, modern high P-demanding varieties than the slow growing, traditional, low P-demanding varieties. The traditional variety, Dular, with a relatively higher P-demand than the other varieties of the group, behaved as an exception as its P-acquisition response was comparable more to that of the modern varieties than the traditional varieties.

Table 34: Shoot Phosphorus contents (mg / 5 plants) of the 8 rice varieties under AMF inoculation at 30 days in laetrile soil

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM -	0.302± 0.034	0.277± 0.016	0.188± 0.024	0.134± 0.012	0.145± 0.011	0.162± 0.015	0.150± 0.013	0.195± 0.012
AM +	0.582± 0.024	0.510± 0.042	0.379± 0.022	0. 474± 0.021	0.652± 0.017	0.534± 0.025	0.550± 0.021	0.684± 0.041
Est. t	11. 656**	8.981**	10.163**	24.353**	43.378**	22.105**	28.057**	19.830**
Table t	0.05*p* 2. 776				0.01*p* 4. 604			
MIE %	**48.1**	**45. 7**	**50. 4**	**71. 7**	**77. 7**	**69. 7**	**72. 7**	**71. 5**
Mean MIE %of varieties	V1, V2, V3			V5	V4, V6, V7, V8			
	48. 0			**71.7**	**72. 6**			

Table 35: Root Phosphorus contents (mg / 5 plants) of the 8 rice varieties under AMF inoculation at 30 days in laetrile soil

Treatment	V1	V2	V3	V5	V4	V6	V7	V8
AM -	0.137± 0.009	0.125± 0.006	0.157± 0.009	133± 0.012	0.122± 0.008	0.121± 0.012	0.109± 0.009	0.120± 0.009
AM +	0.321± 0.024	0.338± 0.007	0.377± 0.015	400± 0.028	0.300± 0.016	0.380± 0.018	0.404± 0.026	0.351± 0.004
Est. t	12.436**	40.024**	21.788**	15.184**	17.238**	20.741**	18.575**	40.633**
Table t	0.05*p* 2. 776				0.01*p* 4. 604			
MIE %	**57.2**	**63.0**	**58.2**	**66.7**	**59.3**	**68.1**	**73.0**	**65.8**
Mean MIE% of varieties	V1, V2, V3				V4, V6, V7, V8			
	59.5			**66.7**	**66.5**			

The plant growth and P-content responses of the varieties due to AMF infection were then compared (Table 36). Conclusions drawn from the computed data were similar to that made for the 15 day old plants earlier. The modern fast growing varieties which had higher P-demand and showed higher growth responses to AMF, showed higher plant P - uptake responses also. The varieties which had lower P-demand and showed lower growth responses to AMF, showed lower P-

uptake responses also. The variety Dular in spite of showing equal P- uptake response as the former varieties did not show equivalent growth response to that of the former varieties. Conclusions regarding proportional partition of the incremental phosphorus due to AMF infection between root and shoot of the varieties were also the same as before. For all the variety groups proportional P-gain in shoot showed increase from that of the 15-day old plants, where the high P-demanding modern varieties had the highest proportion of incremental P-gain in shoot. The traditional variety, Dular, in spite having equivalent incremental shoot P-gain to the other traditional varieties responded for plant more than the later varieties.

The differential behaviour of the varieties with respect to their AMF inoculation responses for plant growth and P-uptake at both 15 and 30 days are compared in Figures 18 -19.

Table 36: Comparison of plant growth and P-acquisition responses of the rice varieties to AMF inoculation at 30 days

Treatment	Whole plant dry matter content of varieties (mg/5 plants)							
	V1	V2	V3	V5	V4	V6	V7	V8
	Whole plant dry matter (mg /5 plants)							
AM -	809	847	761	703	693	721	620	660
AM +	960	1052	1024	989	1121	1120	1124	1024
MIE %	15.7	19.5	25.7	28.9	38.2	35.6	44.8	35.5
Mean MIE% of varieties	**20.3**			**28.9**	**38.5**			
	Whole plant P-content (mg /5 plants)							
AM -	0.439	0.402	0.345	0.267	0.267	0.283	0.259	0.315
AM +	0.902	0.848	0.755	0.874	0.952	0.914	0.954	1.035
MIE %	51.3	52.6	54.3	69.4	71.9	69.0	72.8	69.6
Mean MIE% of varieties	**52.7**			**69.4**	**70.8**			
	Proportional partition of incremental P (%) between							
Root	39.5	47.8	53.4	44.0	26.0	41.1	42.5	32.1
Shoot	60.5	52.2	46.6	56.0	74.0	58.9	57.5	67.9
Mean of varieties	**V1, V2, V3**			**V5**	**V4, V6, V7, V8**			
Root	46.9			44.0	35.4			
Shoot	53.1			56.0	64.6			

Figure 18: Shoot growth responses of the 8 rice varieties to soil inoculation of AMF at 15 and 20 days after seed germination

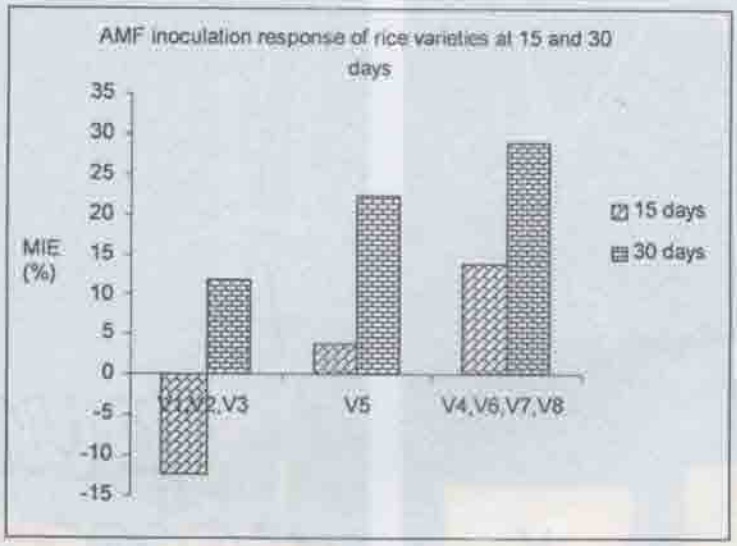
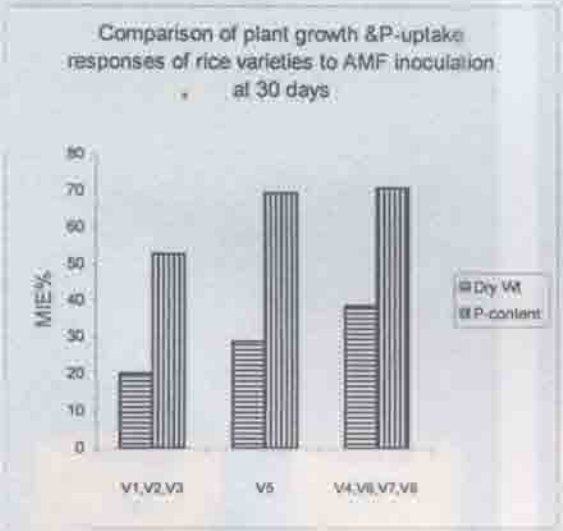


Figure 19: Comparison between whole plant growth and P-uptake responses of the 8 rice varieties at 15 and 30 days after seed germination



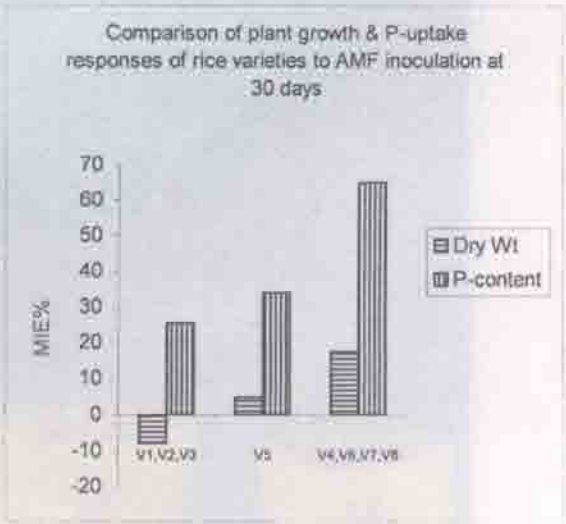


Plate 1: AMF inoculation response of traditional land race varieties Black Gora (V1) & Yamuk (V3) and modern HYVs MTU 7029 (V7) & IR 64 (V8) at 15 days.

Plate 2: AMF inoculation response of traditional land race varieties Black Gora (V1) & Yamuk (V3) and modern HYVs MTU 7029 (V7) & IR 64 (V8) at 30 days.

5. Histological study of arbuscular mycorrhizal development of differently responding rice varieties at early seedling stage

Results of the previous experiments on AMF response related morphological and physiological traits, growth and P-acquisition responses due to AM infection of the 8 varieties revealed significant inherent differences among them. Based on their AMF responses, some varieties belonging to land race selections and old traditional accessions behaved as non-responsive or low responsive to AMF infection by soil inoculation. Some other varieties belonging mostly to modern high yielding hybrid selections behaved as high responsive to AMF infection. The varieties showing comparatively high AMF response had fast shoot growth rates, low root/shoot ratios, high P-demand and low root P-supply efficiencies. The varieties with low AMF response had low shoot growth rates, high root/shoot ratios, low P-demand and high root P-supply efficiencies. One traditional variety, in spite of having high P-demand behaved as less responsive to AMF than the former group of high P-demanding varieties.

Selected varieties from the low (early negative) and high responsive variety groups were then studied for structural features of AM development in roots by inoculation. Histological examination of the roots of the varieties was made at various periods between 10-15 days of seed germination in inoculated soil. The purpose of the study was to see whether the differently responding varieties had any differences in the root colonization pattern starting from pre-colonization root-fungus interactions to full development of mycorrhiza.

5.1 General histological features of AM development in roots

Structural development of arbuscular mycorhiza by the 5 recognized AMF genera (*Glomus, Gigaspora, Acaulospora, Scutellospora and Entrophospora*) in all hosts follow a common pattern at the pre-penetration phase. Infection is initiated by germinating spores, hyphal pieces, and pre-infected root pieces. Germinated spores form one or a few germ tubes which in the vicinity of roots soon develop as

branched hyphae and move towards roots to establish contact. Old hyphal pieces, infected root pieces etc. that are randomly distributed in soil may get attached with a passing lateral root and may renew or give rise to new hyphal growth. These hyphae (*surface colonizing hypha*), originating either from spores or hyphae or pre-infected root pieces branch, proliferate, curl or form tufts on root surface or in close proximity of the roots. These hyphae, either directly or by producing lateral finer branches (*infective hypha*) establish contact with roots at multiple points. At such points of contact swollen knob or hemispherical appresoria are formed by these hyphae. The appresoria in turn produce penetration hyphae to penetrate the intact epidermis which first proliferate inside the host epidermis hypodermis and then the cortex.

The penetration hyphae more commonly enter the host through the space in between two epidermal cells or sometimes by penetrating the wall of an epidermal cell. In both the cases, especially former, one or a few cells of the epidermis or the hypodermis or even the outer cortex may be colonized by intracellular hyphae as source point of further proliferation. From there on two morphological types of intraradical development take place. In the first and more common type, the hypahe grow rapidly and extensively through intercellular spaces of the upper cortex, often running length wise, parallel to the cortical cells, to ultimately reach and ramify in the inner cortex. The intercellular hyphae penetrate a series of cortical cells, mostly in the inner cortex with lateral branch hyphae which form much branched terminal arbuscules in the penetrated cells. Cortical colonization of this type is predominantly intercellular. In the other type, intraradical proliferation is rather slow and intracellular with cell to cell penetration by branch hyphae in the epidermis, hypodermis and cortex. In the cortical cells there is extensive formation of intracellular hyphal coils with or without a few less developed intercalary arbuscules. Intraradical colonization of this type is wholly intracellular. These two types are designated as 'arum type' and 'paris type' AMs of based on plant species from which first described (Smirh and Smith, 1997). The basis of morphological variation between the two types is not well known but host factors controlling the differential morphogenesis is hinted. Basic structural details of these two types are shown schematically by Figure 20.

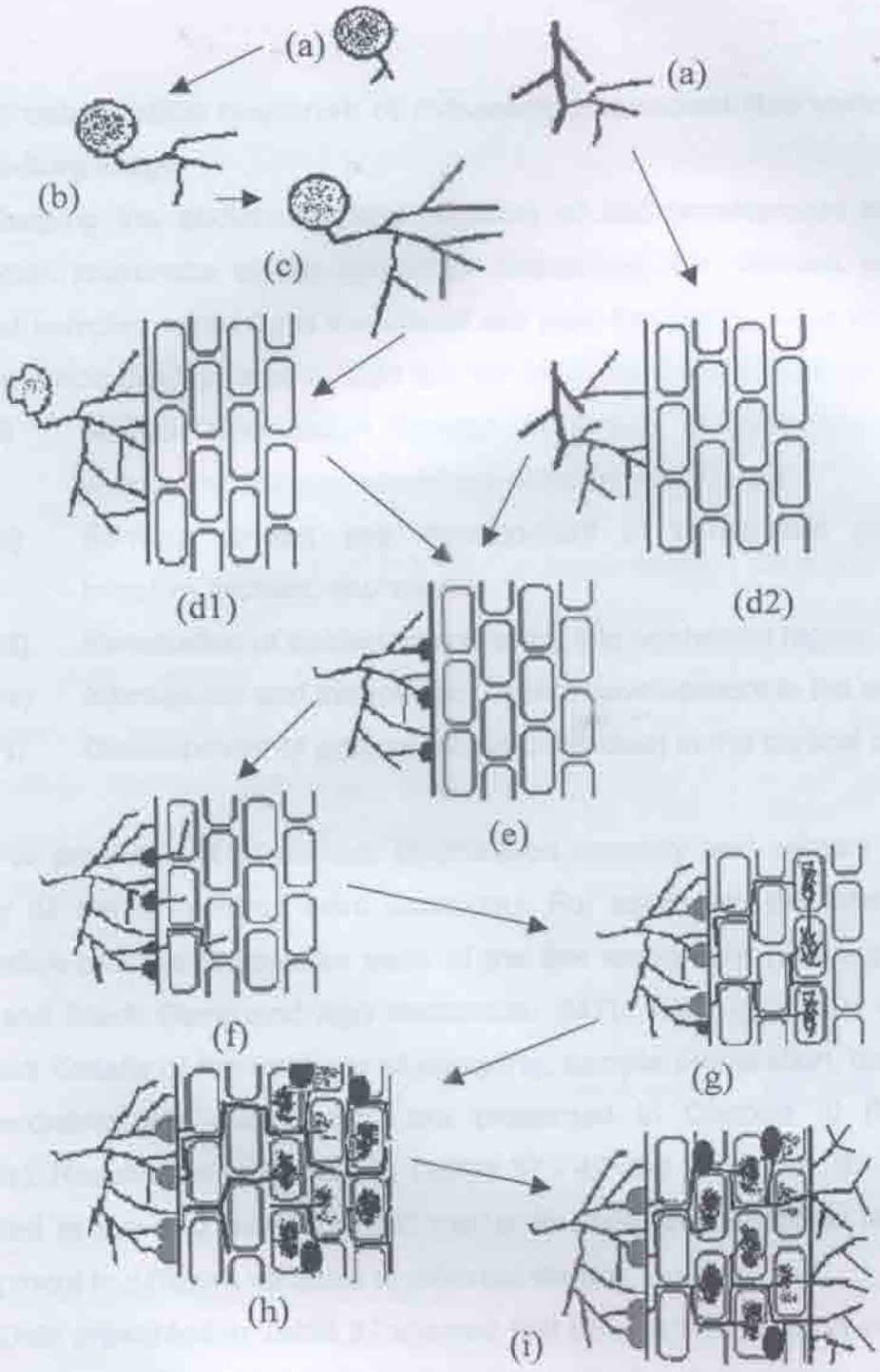


Figure 20: (a-c) stages of spore germination and hypha growth; (d) surface colonizing hyphae making root contact (d1) from spore, (d2) from hyphal piece; (e) appresorium formation on root surface; (f) penetration of epidermis (g) hyphal spread by intracellular coils and intercellular hyphal run, arbuscules and vesicles in cortical cells; (i) extramatrical hyphae development

5.2 AMF colonization response of differently dependent rice varieties at early seedling stage

Keeping the above structural features of AM development in mind, AMF colonization responses of the differently responding rice varieties was assessed from root samples taken from inoculated soil pots For the purpose of assessment, the colonization process was divided into the following 5 distinct stages.

(i) Surface colonization through production of colonizing hyphae from spores, hyphal pieces and pre-infected root pieces

(ii) Surface contact and development of penetration points through infective hyphae, appresoria

(iii) Penetration of epidermis and entry into epidermal region

(iv) Intercellular and intracellular hyphal development in the cortex

(v) Development of arbuscules (and vesicles) in the cortical cells

For assessment of surface colonization intensity and primary root infection intensity all the 8 varieties were examined. For assessing the later parts of the colonization process 2 varieties each of the low responsive (Black gora and ARC 12737 and Black Gora) and high responsive (MTU 7029 and TN1) varieties were examined. Details of the methods of sampling, sample preparation, observation and data recording and photography are presented in Chapter III (Materials and Methods). Results are presented in Tables 37 - 40 and Plates No. 3 - 20 have been appended at the end, after the text matter to show the structural features of AM development in different varieties at different stages.

Data presented in Table 37 showed that between 10-15 days pre-penetration and post-penetration colonization intensities of the two variety groups varied significantly in terms of root pieces showing the designated pre-penetration and post-penetration structures of colonization. There was evidence that intensity of both pre- and post-penetration colonization was higher in the high responding varieties than the low or non-responsive varieties. This showed that at the given period of time when initial colonization was being attempted the differently AM responding varieties might differ in the rate at which both surface and internal colonization were

being established. It would seem that the process of colonization in the non- or low responsive varieties was slower than that of the high responsive varieties. However, so far as the structural features of the colonization were concerned there were no differences between the varieties.

Table 37: AMF colonization intensity – pre- and post infection responses of the rice varieties between 10 and 15 days after seed germination in inoculated soil

Features	4 low or non-responsive varieties	4 high responsive varieties	Est-t
Percent root pieces showing pre-penetration structures – surface colonization hyphae, infective hyphae, penetration hyphae, and post penetration intraradical hyphae, appresoria etc	54.4 ± 8.6	79.1±11.2	4.321**
Percent root pieces with both pre-penetration surface and post-penetration internal structures – inter- and intracellular hyphae, hyphal coils and arbuscules and vesicles	34.3 ±4.7	63.7 ± 9.3	7.274**
Percent root pieces with surface colonization & penetration structures but without any post-penetration internal structures	20.1 ± 2.8	15.4 ± 3.6	NS

Based on 3 x 100 – 1.5 cm root pieces of the 8 varieties sampled from 3 random quadrants of whole root mass (details in Chapter III); Table t at 3 df - 0.05 p 3.182, 0.01p 5.841

Table 38: Pre-penetration AM structures on root surface of differently dependent rice varieties between 10 and 15 days after seed germination in inoculated soil

Variety	Number of surface colonization sites		Average length of surface colonizing hypha		No. of penetration points from surface colonization			
	per mm root length	Per mm^2 root surface	Per colonization site (mm)	Per mm root length (mm)	Per colonization site	Per mm hyphal length	Per mm root length	Per mm^2 root surface
Mean of two negatively responding varieties	0.1 ± 0.03	0.076 ± 0.007	7.0 ± 0.95	0.67 ± 0.05	5.6 ± 1.20	0.76 ± 0.03	0.51 ± 0.03	0.38 ± 0.02
Mean of two positively responding varieties	0.2 ± 0.05	0.180 ± 0.047	35.2 ± 0.043	6.1 ± 0.082	9.4 ± 1.84	0.78 ± 0.04	1.86 ± 0.61	1.02 ± 0.32
Est. $t_{0.05}$	5.59**	8.61**	91.38 **	18.40**	5.59**	8.13**	9.43**	8.42**
Table t at 5 df	0.05 p 2.571				0.01 p 4.032			

Based on 3 x 100 - 1.5 cm root pieces sampled from 3 random quadrants of the whole root mass

Upon assessment of the intensity of pre- and post penetration colonization of the varieties, as above, differences in the structural details of pre-penetration colonization between the two variety groups, if any, were assessed from two varieties each of the two groups. Results are presented in Table 38. Data showed that based on the three distinct features of pre-penetration development – number of surface colonization sites per unit root surface area (representing an individual spore or a hyphal or a pre-infected root piece associated with the sampled root), length of such hyphae per unit root length and colonization site, and the number of

penetration attempts (through penetration hyphae) per unit surface colonization site and surface area of the root, the high and low AM responding varieties had significant differences. At the given period of time the intensity of pre-penetration surface colonization through root associated hyphal development prior to appresorium formation was significantly higher in the high responding varieties than the low (or early non-) responding varieties. These details confirmed that the progress of surface colonization for establishing mycorrhiza in the non- or low responding varieties was comparatively slow than that in the high responding varieties.

These results were then followed by assessment the possible differences in actual penetration intensity of the varieties by counting the number of appresoria formed by surface colonizing and penetration hyphae per unit surface colonized root surface area and per unit length of surface colonizing hypha. The estimates (Table 39) showed that at the given point of time appresorium formation intensity on the two high responding varieties was significantly higher than that on the low responding varieties. Slower progress of appresorium formation on the root surface of the low AM responding varieties was again indicated by the results.

Table 39: AMF penetration response of differentially AM responsive varieties at 10 – 15 days after seed germination in inoculated soil

Features	2 low responsive varieties	2 high responsive varieties	Est. t
Percent root pieces showing appresorium development on surface from penetration hypha	32.2 ± 3.2	44.5 ± 4.5	7.14**
Mean number of appresoria per mm ^{2} root surface showing surface hyphal colonization	11.4 ± 1.5	21.8 ± 1.9	13.68**
Mean number of appresoria per mm length of surface attached hypha	0. 8 ± 0.04	1.3 ± 0.08	18.63**

Based on 3 x 100 – 1.5 cm root pieces sampled from 3 random quadrants of whole roots (Chapter III)

Table t at 5 df - 0.05 p 2.571, 0.01 p 4.032

Variations, if there were any between the two variety groups in internal colonization response – intra- and intercellular hypha development and arbuscule formation were then assessed. These estimates (Table 40) also showed that internal colonization intensity as judged by score values for intercellular, intracellular and arbuscule development in the cortex was significantly higher in high responding varieties than the low responding varieties.

All these estimates of pre- and post penetration colonization of the host varieties by the AMF fungi used in the study pointed out differences in the intensities in host-fungus interactions between the two differently responding variety groups at the early seedling stage, not for any qualitative aspects of pre- and post-penetration colonization structures but for the intensity of both pre- and post- penetration colonization. There were no apparent differences in host response between the variety groups also. The observed difference was interpreted as difference in the rapidity with which colonization was established in the two variety groups, the high responding varieties allowing faster and earlier establishment of mycorrhiza than the low or non-responsive varieties.

Table 40: Post-penetration AMF development response of differentially AM responsive varieties at 10-15 days of seed germination in inoculated soil

Features (by 0-4 point scale)	Non-responsive varieties	Responsive varieties	Est. t
Relative score of intercellular hypha development in upper cortex of the colonized root pieces	2.2±0.06	3.4±0.08	38.33**
Relative score of intracellular hypha including coils development of the colonized root pieces	1.4± 0.03	2.7±0.07	58.14**
Relative score of arbuscule and / or vesicles development in inner cortex of colonized root pieces	1.2± 0.04	2.3±0.06	49.19**

Based on 3 x 100 - 1.5 cm root pieces sampled from 3 random quadrants of whole root (Chapter III);

Table t at 5 df - 0.05 p 2.571, 0.01 p 4.032

6. Changes in whole root peroxidase activity of rice roots due to AM infection

In response to microbial attack, plants elaborate an array of inducible defense reactions many of which involve the transcriptional activation of the corresponding defense genes. These include the genes that encode enzymes involved in

(i) the phenylpropanoid pathway – synthesis of lignin and phytoalexins

(ii) PR-proteins

(iii) Cell wall hydrolases

(iv) Reinforcement of plant cell walls, such as hydroxyproline reach glycoproteins (HRGP)

During initial stages of AM colonization, only weak and transient increases in gene expression for cell wall hydrolases, HRGP, and those involved in phenylpropanoid pathway are observed (Bonfante and Perroto, 1995; Gianinazzi-Pearson *et al.*, 1996). Production of reactive O_2 intermediates through an oxidative burst is the hallmark of plant's defense responses, especially through the phenylpropanoid pathway. Peroxidases participate in a variety of defense mechanisms (Moershbaher, 1992) in which H_2O_2 is often supplied by the oxidative burst. The cell wall appears to be the site of defense related peroxidase polymerization reactions, such as lignification, suberization and cross linking of structural cello wall proteins (Lamb and Dixon, 1997).

The differently AM responsive rice varieties showed differences in the relative rapidity with which colonization was established at the pre- and post- penetration phases of host-fungus interaction at the initial stage. Root colonization of the varieties which showed early negative response was relatively slow as compared to that of the high responding varieties. Whether there is any difference in the expression of the transitory defense response that is normally observed in compatible host – AM fungus interactions, between the two differently AM responding variety groups at the early stage was studied next. One recent reference

has shown of phenylalanaine ammonia lyase (PAL) gene expression in rice roots colonized by the AM fungus, *Glomus mosseae* (Blilou et al., 2000). Considering peroxidase as the primary, non-specific enzyme that participates in initial defense response reactions, whole root peroxidase activity of the differently AM responsive rice varieties was studied at the early stage of AM interaction. Differences in whole root peroxidase activity of the varieties following AM infection by inoculation were studied by (i) colorimetric estimation of whole root peroxidase, and (ii) densitometry following acrylamide gel electrophoresis of perxodiase isozymes. Changes in the peroxidase isozyme profile of the varieties were also followed by gel electrophoresis. Rice varieties were grown with challenge inoculation of root based AM inoculum in small volume sterilized laterite soil and the whole root peroxidase activity was measured from crude protein extracts by colorimetry and gel electrophoresis. Details of the methods of plant growing, extraction and estimation of enzyme and polyacrylamide gel electrophoresis of isozymes are given in Chapter III (Materials and Methods).

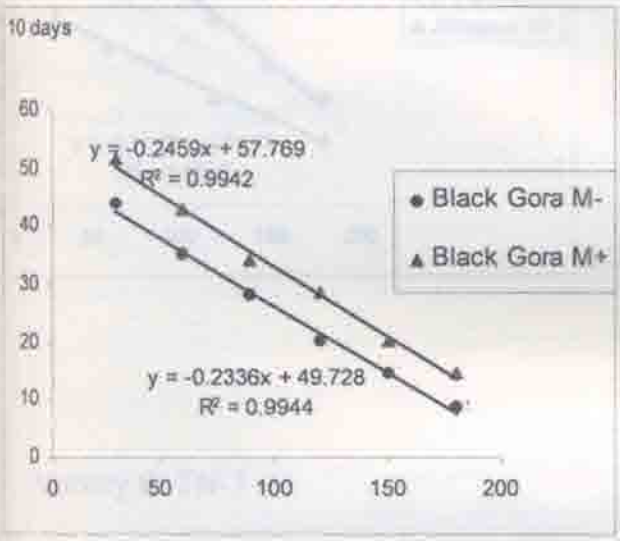
6.1 Changes in whole root peroxidase activity of the differentially AM responsive rice varieties due to AM infection at early stage

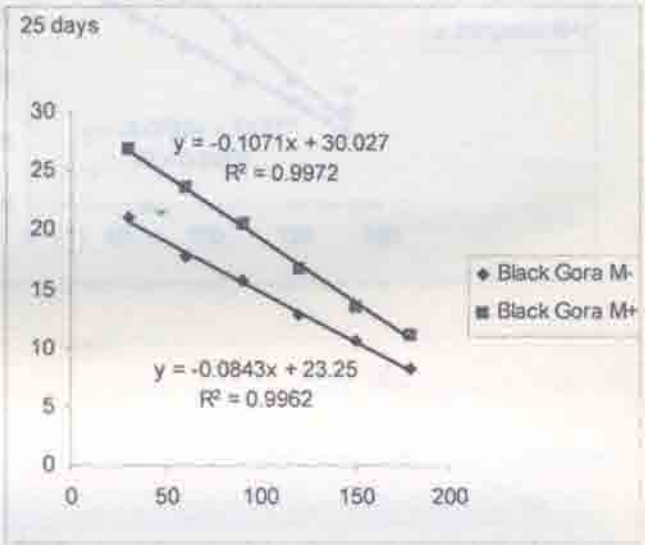
Whole root peroxidase activity was estimated by colorimetric measurement of enzymatic dehydrogenation of O-dianisidine in presence of H_2O_2. Five selected rice varieties (2 non-responsive varieties – Black Gora and ARC- 12737 and 3 responsive varieties – Jhingasail, TN1 and MTU-7029) were grown in small volume sterile soil with or without AM inoculation for 25 days. Quantitative estimation of peroxidase activity was done from whole root protein extracts after harvest of plants at 10 and 25 days of seed germination. Results of the analysis are presented in Figures 21-22 and Table 41.

Figure 21: Whole root peroxidase activity of rice varieties at 10 and 25 days under AM inoculation and no-inoculation

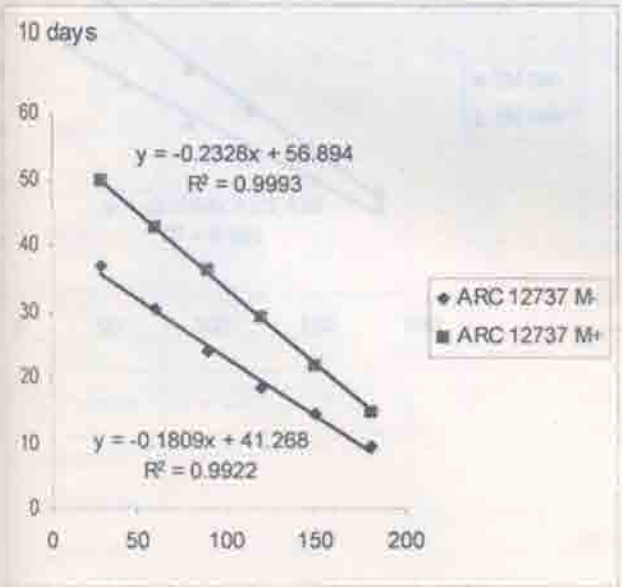
(y -axis: change in OD X 10^3 per microgram crude protein; x-axis: time interval in sec over 180 sec)

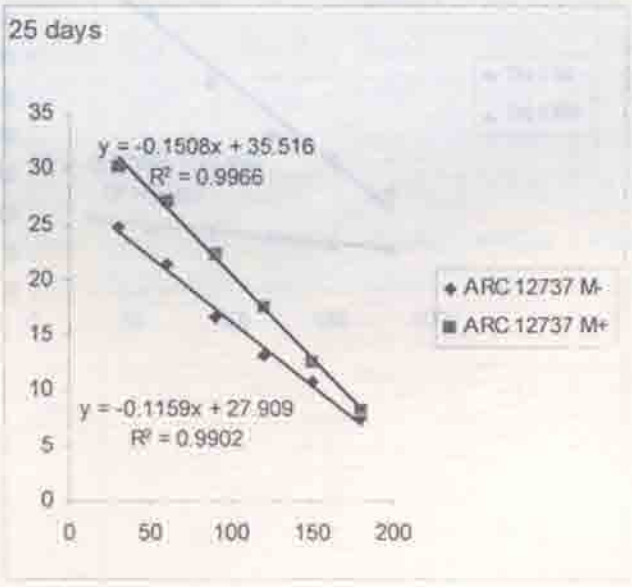
Variety 1: Black Gora



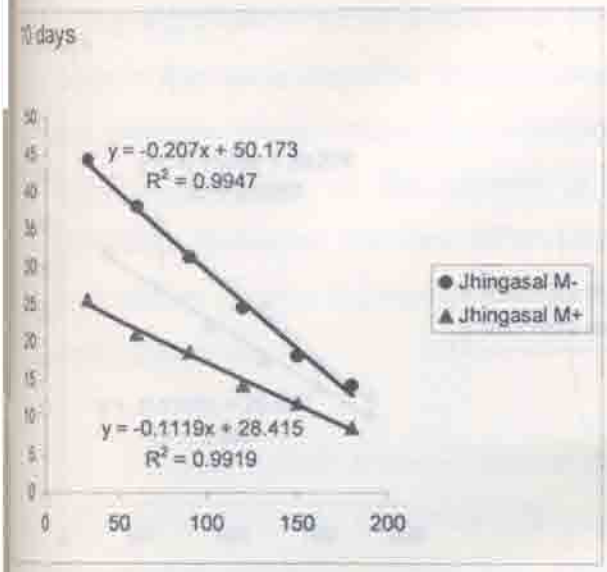


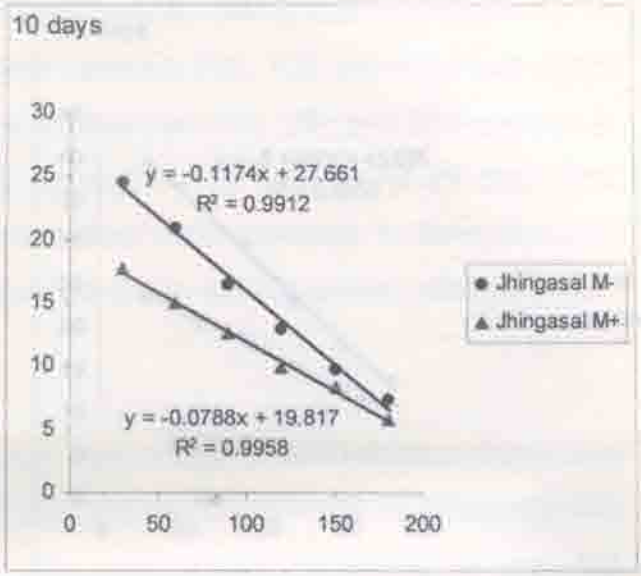
Variety 2: ARC-12737



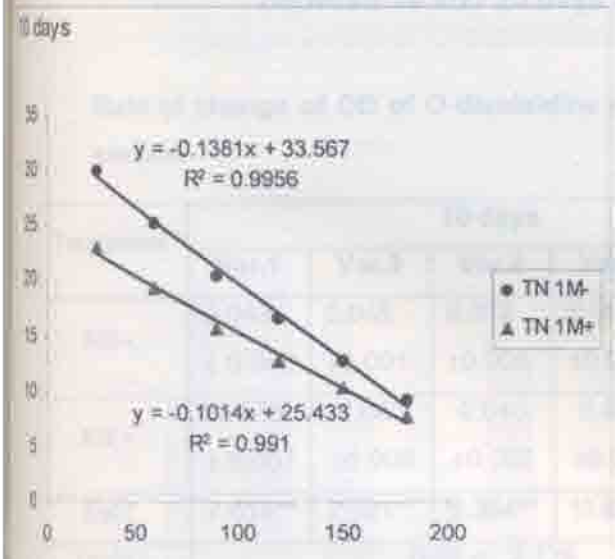


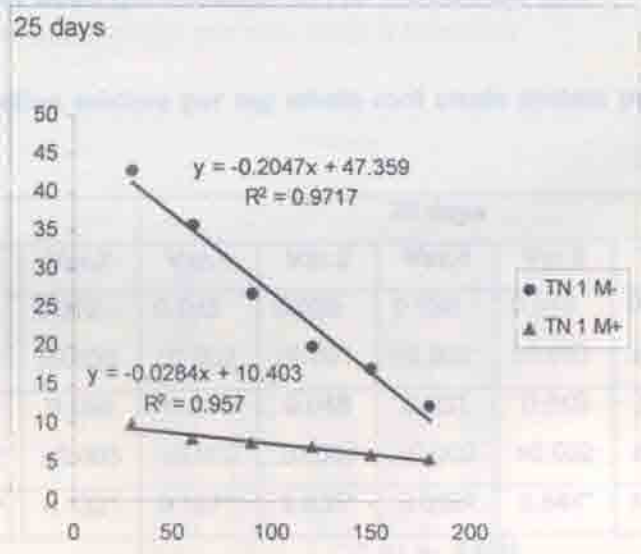
Variety 4: Jhingasail





Variety 6: TN-1





Variety 7: MTU-7029

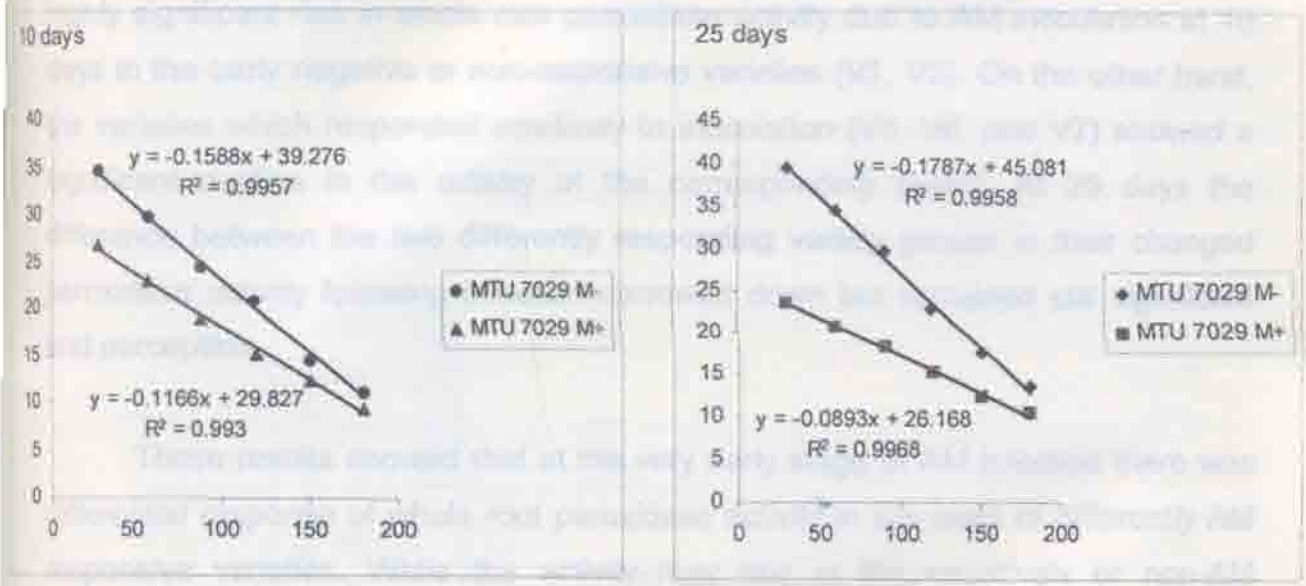


Table 41: Whole root peroxidase activity of 5 differently responsive rice varieties between 10 and 25 days after seed germination in AM inoculated soil

Rate of change of OD of O-dianisidine reaction mixture per mg whole root crude protein per second

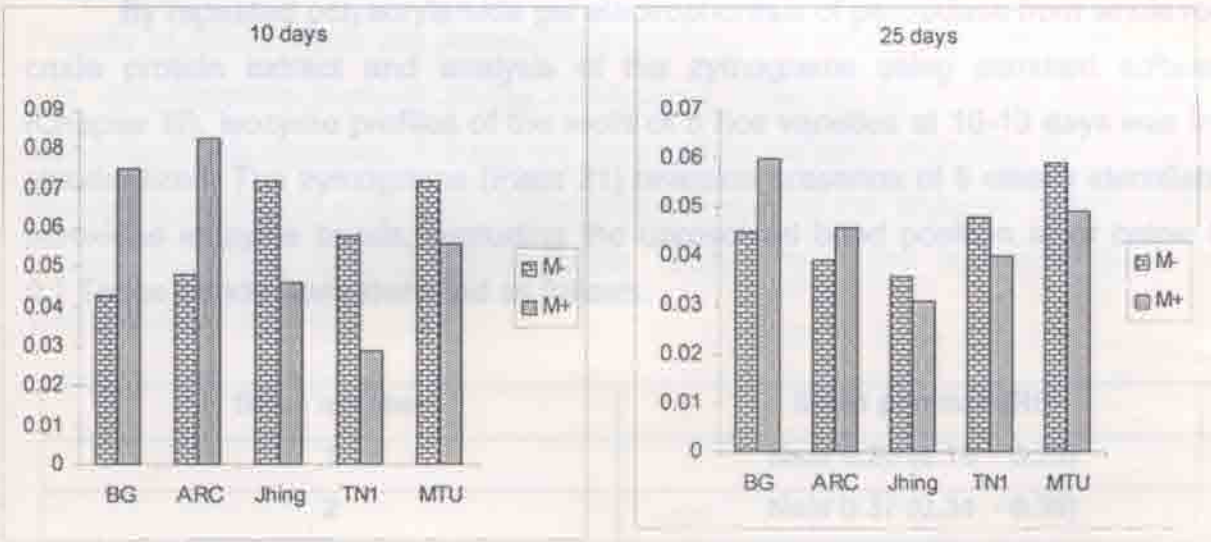
Treatment	10 days					25 days				
	Var.1	Var.2	Var.4	Var.6	Var.7	Var.1	Var.2	Var.4	Var.6	Var.7
AM -	0.043 ± 0.002	0.048 ±0.001	0.072 ±0.005	0.058 ±0.003	0.072 ±0.006	0.045 ±0.002	0.039 ±0.001	0.036 ±0.002	0.048 ±0.003	0.059 ±0.003
AM +	0.075 ± 0.007	0.083 ±0.008	0.046 ±0.002	0.029 ±0.002	0.056 ±0.003	0.060 ±0.002	0.046 ±0.003	0.031 ±0.002	0.040 ±0.002	0.049 ±0.002
Est t	7.615**	7.521**	8.364**	13.934**	4.132*	9.187**	3.835*	3.062*	3.844*	4.805**
Table t	0.05 *p* 2.776					0.01 *p* 4.604				
% change	+ 74	+ 73	-36	- 50	-22	+ 33	+ 18	- 14	- 17	- 17
Mean of differently responsive varieties	+73.5		-36.0			+25.5		-16.0		

Based on 3 x 3 root sample analyses of each variety

Results of the analysis (Table 41, Figures 21 & 22) showed that there was a highly significant rise in whole root peroxidase activity due to AM inoculation at 10 days in the early negative or non-responsive varieties (V1, V2). On the other hand, the varieties which responded positively to inoculation (V4, V6, and V7) showed a significant decline in the activity at the corresponding period. At 25 days the difference between the two differently responding variety groups in their changed peroxidase activity following infection narrowed down but remained still significant and perceptible.

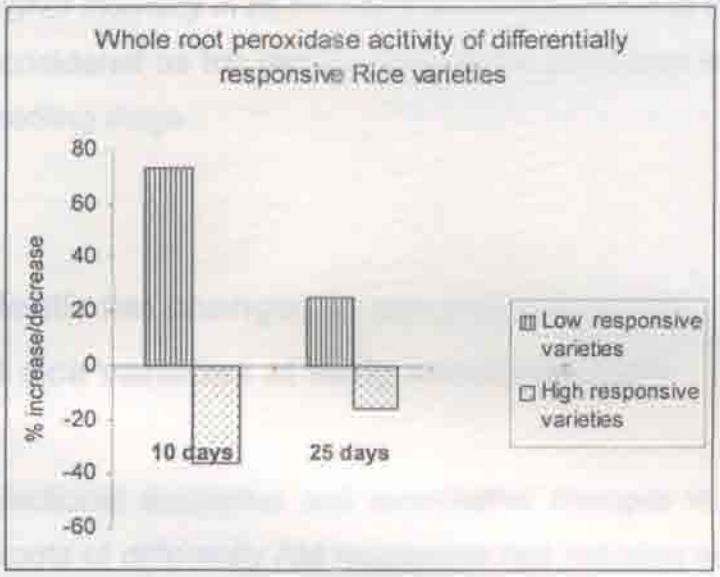
These results showed that at the very early stage of AM infection there was differential response of whole root peroxidase activity in the roots of differently AM responsive varieties. While the activity may rise in the negatively or non-AM responding varieties, the same may decline in positively responding varieties. With progress of colonization the magnitude of both negative and positive changes might diminish.

Figure21: Changes in whole root peroxidase activity due to AM infection: Rate of change in OD of O-dianisidine per mg protein per sec over 3 minutes



(y axis: Rate of change in OD per mg protein per sec)

Figure 22: Comparison of changes in whole peroxidase activity of the differently responsive rice varieties following infection at 15 and 25 days.



6.2 Peroxidase isozyme profile of rice roots at early seedling stage

By repeated polyacrylamide gel electrophoresis of peroxidase from whole root crude protein extract and analysis of the zymograms using standard software (Chapter III), isozyme profiles of the roots of 8 rice varieties at 10-13 days was first standardized. The zymograms (Plate 21) revealed presence of 5 clearly identifiable peroxidae isozyme bands, excluding the unresolved band position at or below Rf 0.1.These bands were identified as follows:

Band number	Band position (Rf)
1	Near 0.20 (0.18 – 0.23)
2	Near 0.37 (0.34 - 0.39)
3	Near 0.60 (0.56 – 0.60)
4	Near 0.65 (0. 61 – 0.68)
5	Near 0.85 (0.81 – 0.88)

The variety Dular (V5) had shown one more exceptional band at position Rf 0.29. Out of these 5 or 6 bands, the bands number 3 and 4 were present in significantly higher intensity in all the varieties than the rest of the bands. These two bands were considered as the most predominant peroxidase isozymes of rice roots at the early seedling stage

6.3 Post-infectional changes in peroxidase isozyne profile of the roots of rice varieties at early seedling stage

Post-infectional qualitative and quantitative changes in peroxidase isozyme profile of the roots of differently AM responsive rice varieties were then analyzed at early seedling stage. The rice varieties were grown in small volume sterile laterite soil with or without inoculum under controlled environment for 10-13 days. Soluble protein extracted from whole root of harvested plants was analyzed for peroxidase isozyme profile by standard methods of acrylamide gel electrophoresis (Chapter III). Results of the analysis are presented as peroxidase zymogram diagrams together with densitometric analysis of the zymograms of seven varieties (3 negatively or non-responding varieties, 4 positively responding varieties including the variety V5 where the response was comparatively low. Result of the analysis is presented in Plates 22-29 and corresponding Tables. Summary of the analyzed results are presented in Table 42.

Table 42: Analysis of the changes in whole root peroxidase isozymes profile by gel electrophoresis (Based on densitometric analysis of zymograms shown in Plates 23-29)

Variety	Treatment	Peroxidase enzyme (activity units / mg crude soluble root protein)						
		Total	Band 1	Band 2	Band 3	Band 4	Bands 3+4	Band 5
Black Gora (V1)	AM -	**12.4**	0.52	2.04	4.08	2.84	**6.92**	2.92
	AM +	**20.4**	1.28	2.08	4.86	8.59	**13.45**	3.59
ARC 12737 (V2)	AM -	**37.6**	0.49	2.23	27.02	6.49	**33.51**	1.38
	AM +	**40.8**	0.29	0.75	28.21	9.77	**37.98**	1.78
Yamuk (V3)	AM -	**8.4**	0.38	0.80	1.96	3.22	**5.18**	2.04
	AM +	**11.6**	0.64	1.05	2.90	4.38	**7.28**	2.63
Dular (V5)	AM -	15.0	0.82 + 0.46	0.87	4.08	6.57	10.65	2.20
	AM +	14.8	1.0+ 0.36	0.37	6.54	4.77	11.31	1.76
Jhingasail (V4)	AM -	**19.4**	0.29	0.25	8.10	10.07	**18.17**	0.69
	AM +	**4.5**	0.67	1.98	1.66		**1.66**	0.19
MTU 7029 (V7)	AM -	**69.8**	0.92	10.92	18.62	34.98	**53.60**	4.36
	AM +	**26.0**	0.59	10.57	3.08	3.78	**6.86**	7.98
IR 64 (V8)	AM -	**18.0**	0.51	1.63	2.88	9.59	**12.47**	**3.39**
	AM +	**14.6**	0.42	1.27	3.29	7.72	**11.01**	**1.90**

Values in bold print show significant changes in activity by densitometry

Results of the analysis showed significant differences in the titer values of whole root peroxidase activity among the varieties, both under inoculation and no-inoculation. Estimation of the peroxidase activity by densitometric analysis of the isozyme bands showed rise in whole root peroxidase titers at 10 -13 days in the roots of the 3 rice varieties following infection (Black Gora, ARC 12737 and Yamuk) all of which responded negatively to AM inoculation at the corresponding period (Table 42). Three varieties which responded positively at the same period (IR 64, MTU 7029 and Jhingasail) showed a lowering of the titer value. The variety Dular which showed no or very low response to AM had no perceptible change in the titer value. This confirmed the results obtained by colorimetric analysis of whole root peroxidase of the rice varieties presented earlier (Table 41, Figure 21).

There was no perceptible qualitative change in the composition of the peroxidase isozyme profile (banding pattern) of the differently responsive rice varieties. Characteristically, however, percent composition of the 5 identified bands of the varieties changed due to inoculation. The most significant change was in bands number 3 and 4 at Rf positions 0.60 and 0.65 which were found as predominant root peroxidase isozmes of rice at the particular growth stage. These two bands appeared in close proximity and their presence as percent of total of all bands increased in the negatively responding varieties, declined in the positively responding varieties and remained virtually unchanged in the less responding variety. Out of the 7 varieties, largest significant changes in the percent occurrence of these two bands were observed in MTU 7029 and Jhingasail, both of which were positively responding varieties.

These results, confirming the previous results of colorimetric estimates of whole root peroxidae acivity of rice roots due to AM inoculation, showed that apart form the quantitative change in whole root peroxidase activity due to AM infection at early seedling stage, extent of presence of the individual isozymes may also change significantly. The two isozymes which occur in largest amounts in rice roots were more prone to change in quantity due to infection. Moreover, their change was found to be differential – rise in the negatively responding varieties and decline in the positively responding varieties.

Based on the results of analysis of whole root peroxidase activity and of the peroxidase isozyme profile, it appeared that during the initial stages of AM colonization regulation of peroxidase enzyme (and isozymes) may change in roots suggesting for possible association/ involvement of the enzyme with the colonization process. Root peroxidase activity in the low AM responsive varieties which do not or negatively respond to AM infection at the early growth stage might rise significantly following initial attempts for colonization. With progress of time and success of

colonization the rising trend might be reversed to show a decline in the increased activity. In the positively responding varieties the activity might be suppressed right at the initial stage of colonization and such suppression might become more significant with time and progress of infection. Apparently, the regulation of the two most predominant peroxidase isozymes of rice roots was most involved in such differential response of peroxidase enzyme to AM colonization, which were either up or down in expression quantity depending upon the response character of the varieties.

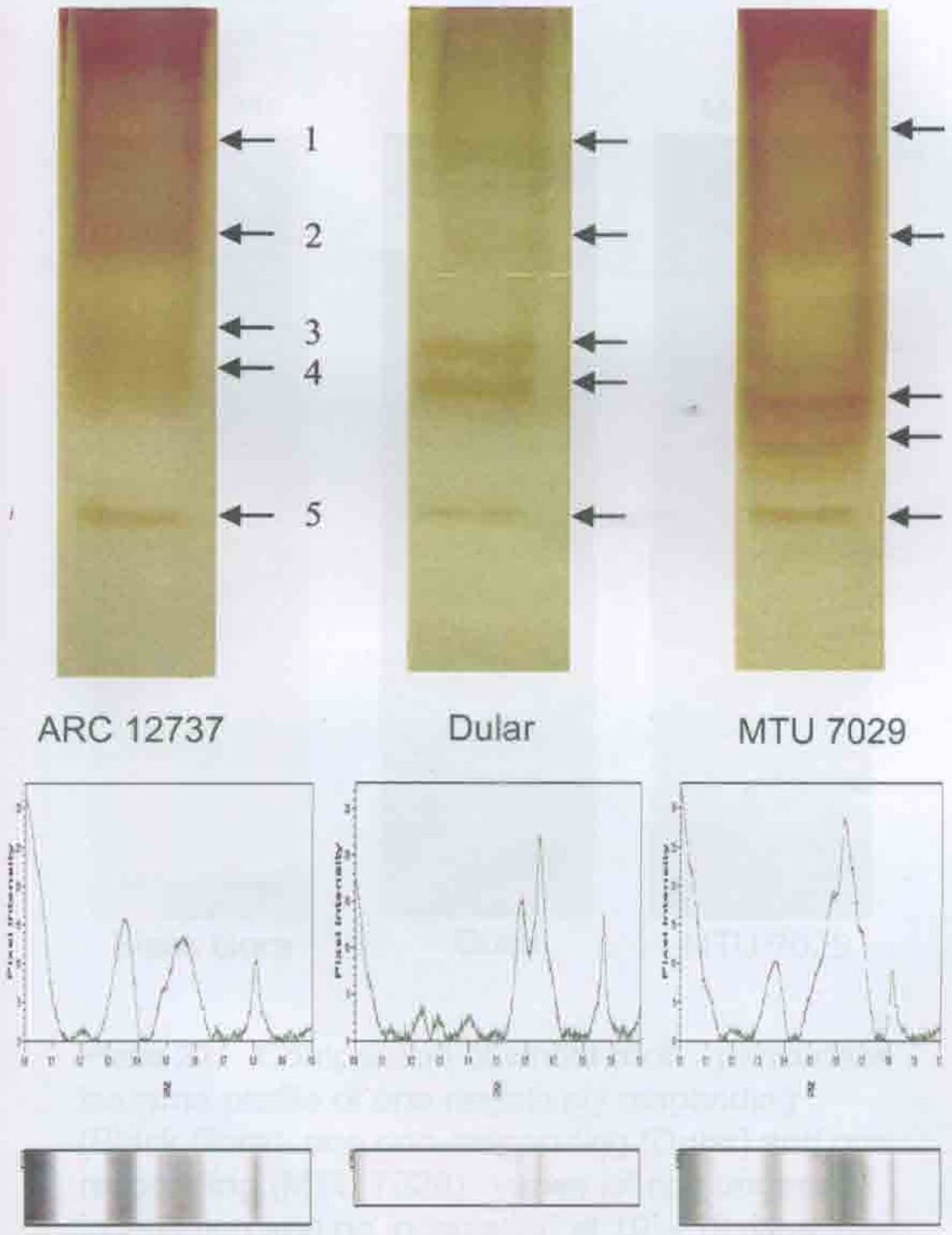


Plate 21: Peroxidase isozyme bands of rice root at 10 – 13 days.

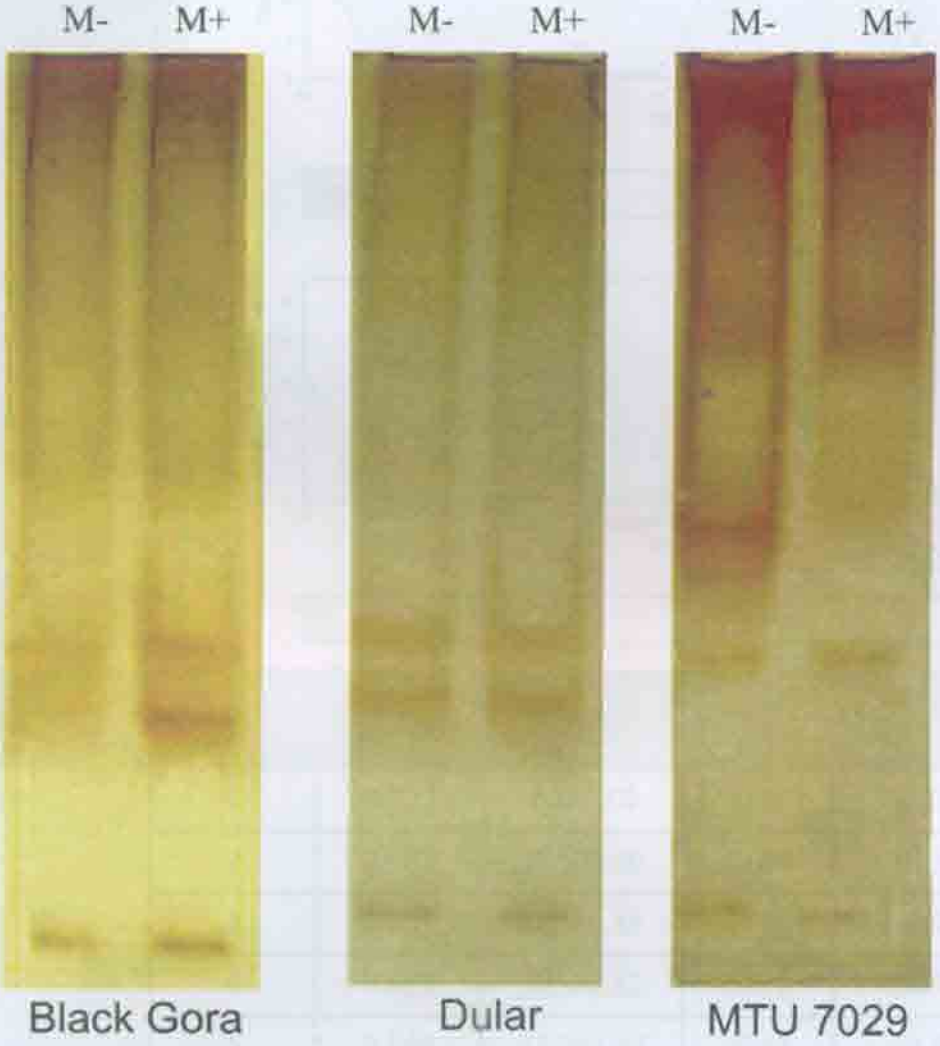
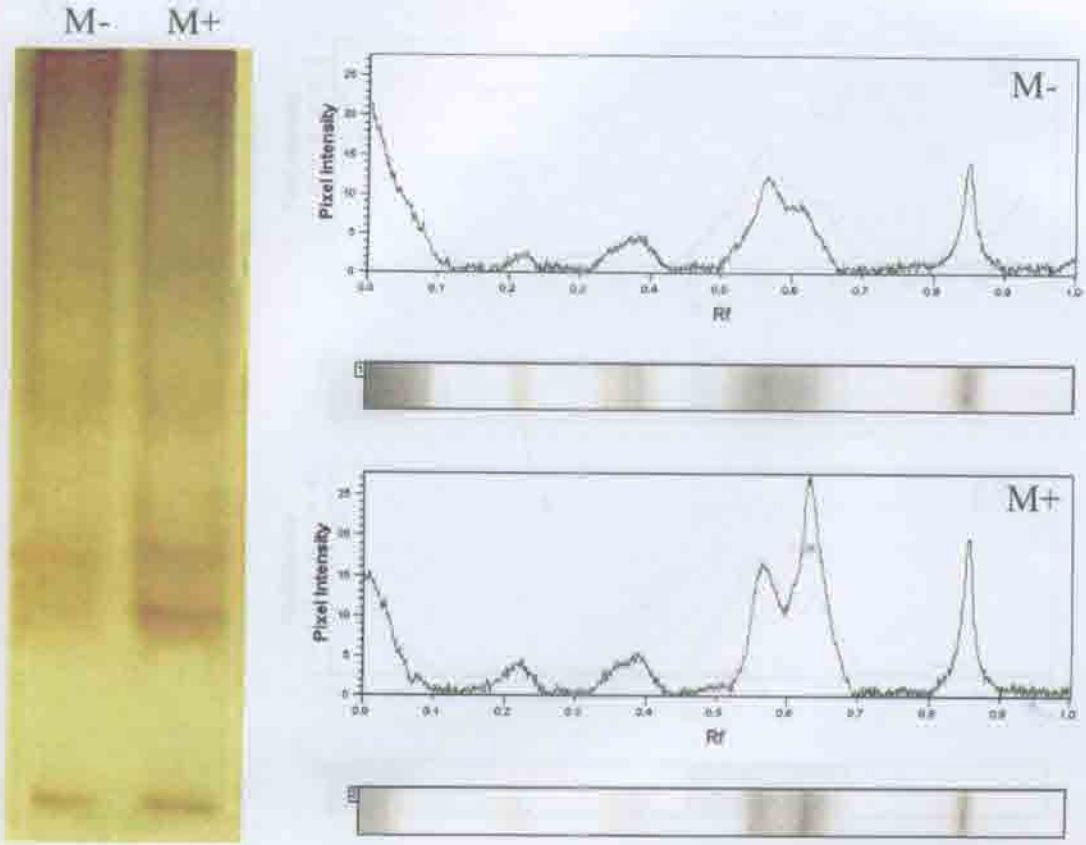
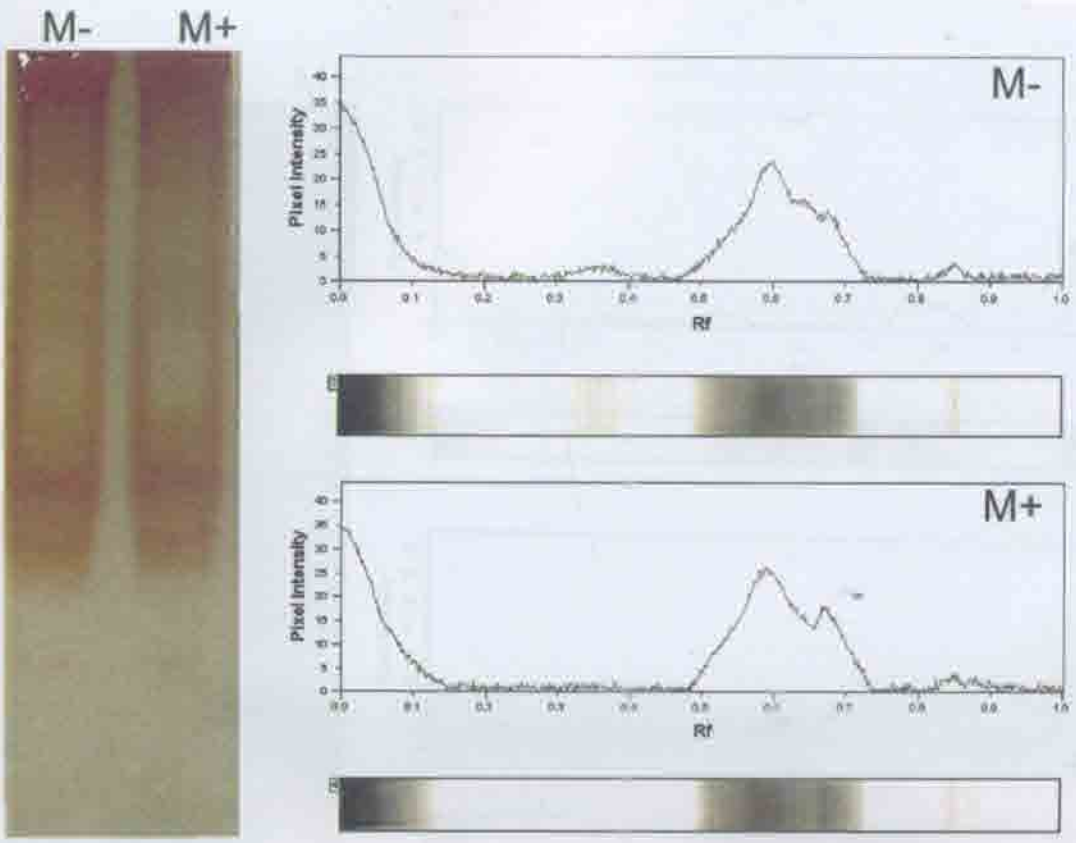


Plate 22: Comparison of whole root peroxidase isozyme profile of one negatively responding (Black Gora), one non-responding (Dular) and one responding (MTU 7029) variey of rice under AM inoculation and no inoculation at 10 – 13 days.



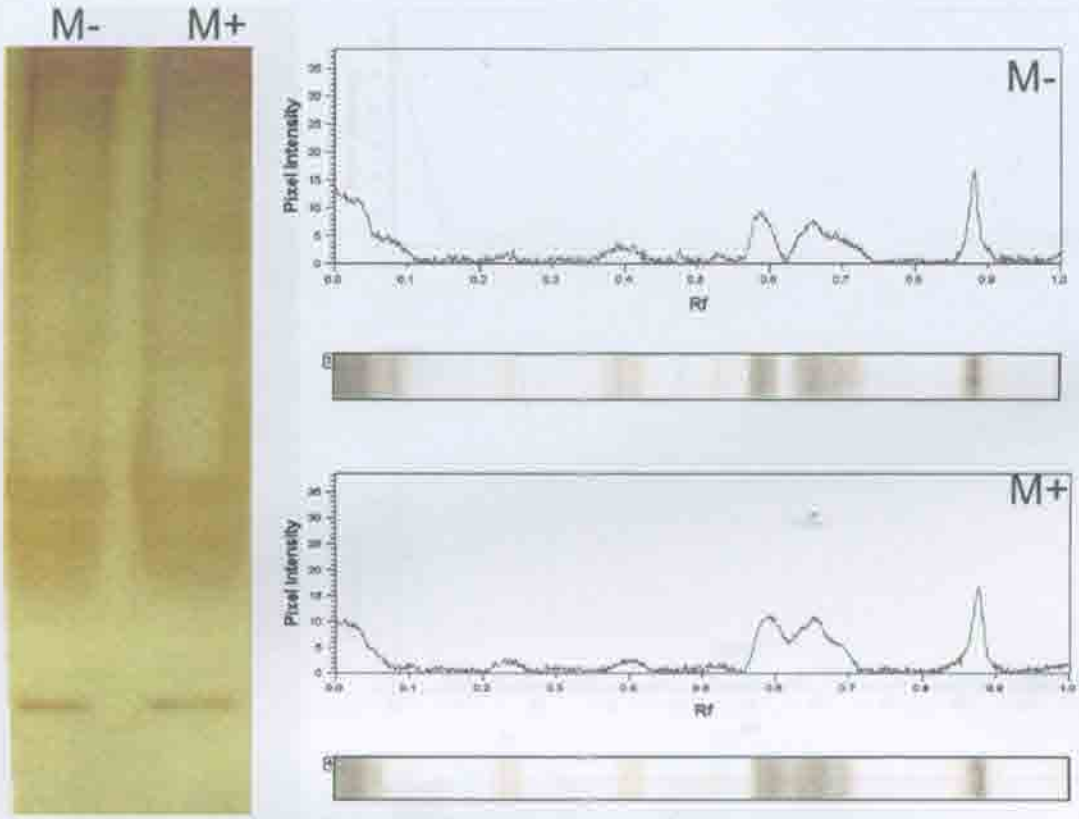
Treatment	Band number	Rf	Volume	Area	Band %
M-	1	0.223	6052.10	3740	4.28
	2	0.381	23106.00	8160	16.34
	3	0.561	46556.45	6800	32.93
	4	0.613	32380.82	5372	22.90
	5	0.848	33.281.48	7752	23.54
M+	1	0.229	14539.10	5236	6.26
	2	0.392	23725.05	7344	10.22
	3	0.567	55274.02	5440	23.81
	4	0.630	97794.19	7276	42.12
	5	0.852	40859.89	7140	17.60

Plate 23: Whole root peroxidase isozyme profile together with densiometric analysis of zymogram of variety Black Gora (V1) at 10 – 13 days.



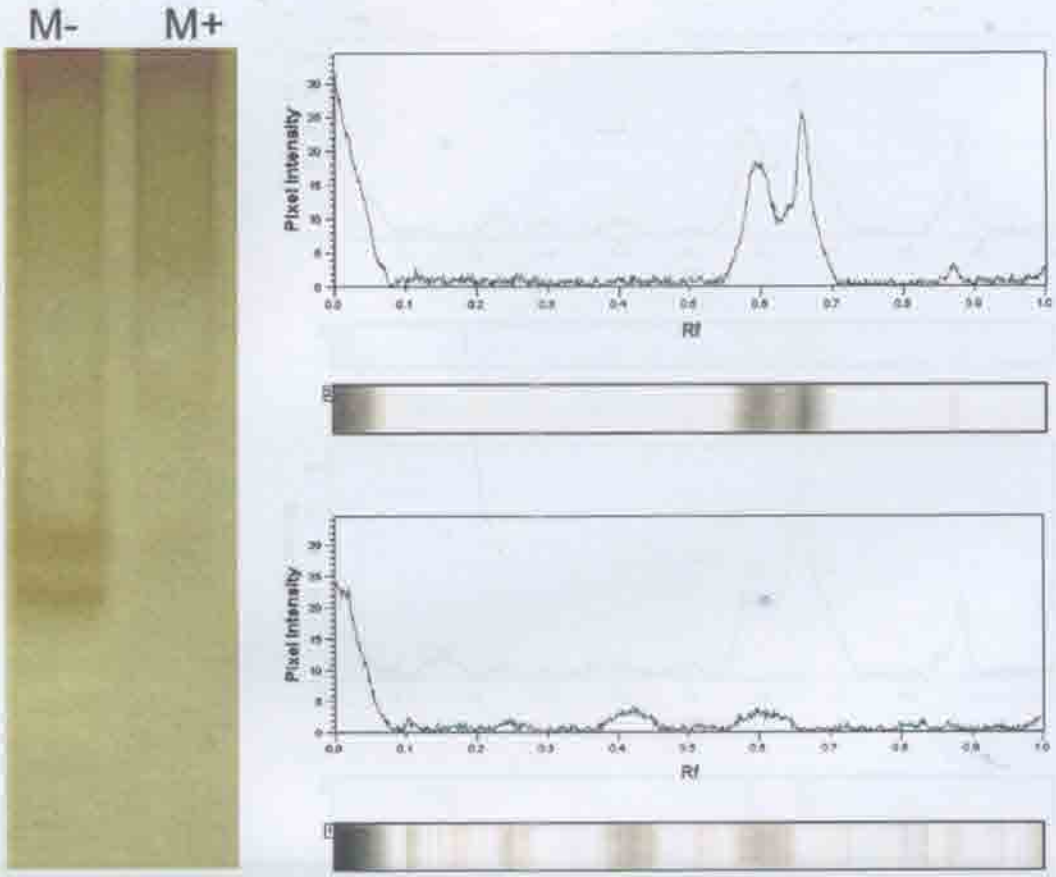
Treatment	Band no.r	Rf	Volume	Area	Band %
M-	1	0.200	2806.20	2736	1.31
	2	0.365	12712.20	5904	5.93
	3	0.599	154047.58	12384	71.87
	4	0.676	36894.97	4248	17.21
	5	0.852	7873.32	4320	3.67
M+	1	0.197	1687.50	1904	0.72
	2	0.353	4226.10	3536	1.81
	3	0.588	161009.09	10608	69.15
	4	0.670	55777.69	5032	23.95
	5	0.849	10147.27	4692	4.36

Plate 24: Whole root peroxidase isozyme profile together with densiometric analysis of zymogram of variety ARC 12737 (V2) at 10 – 13 days.



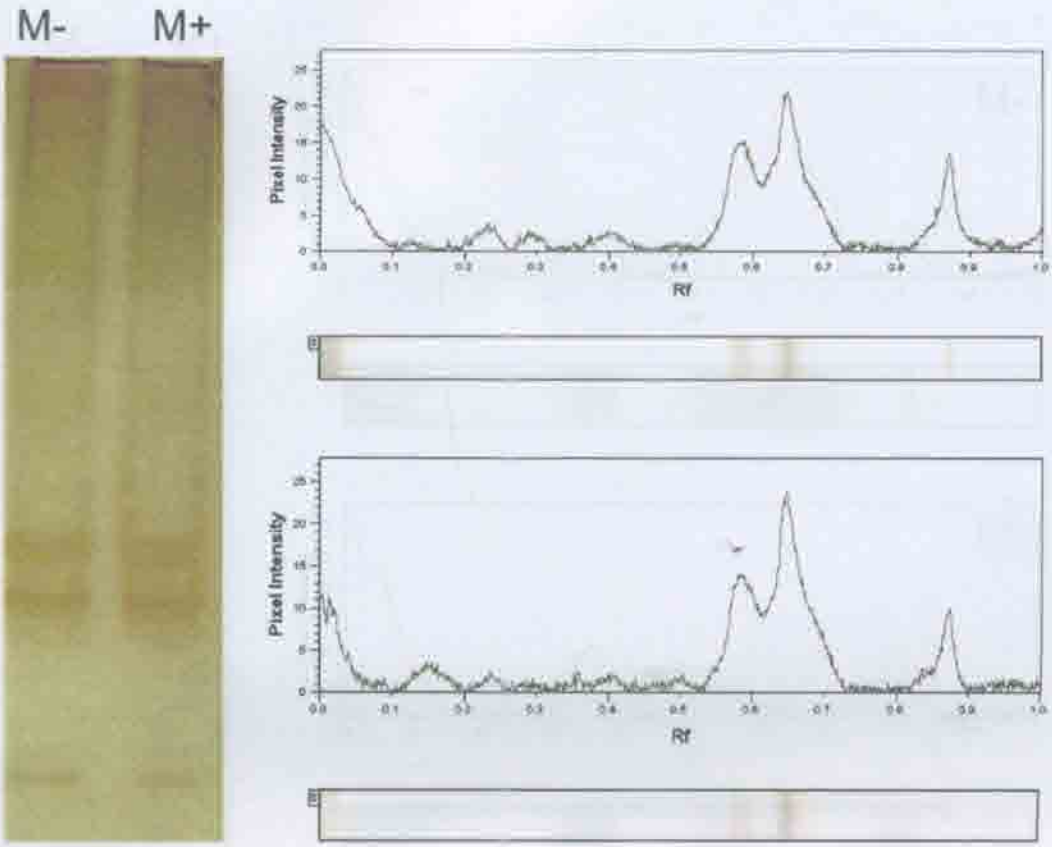
Treatment	Band no.	Rf	Volume	Area	Band %
M-	1	0.218	4293.04	3102	4.48
	2	0.378	9125.50	4686	9.53
	3	0.562	22340.15	4554	23.34
	4	0.633	36711.91	8514	38.35
	5	0.846	23251.33	4554	24.29
M+	1	0.220	7301.50	4284	5.49
	2	0.378	12009.00	5916	9.03
	3	0.567	33243.24	4080	25.00
	4	0.624	50218.82	7140	37.77
	5	0.843	30193.71	6324	22.71

Plate 25: Whole root peroxidase isozyme profile together with densiometric analysis of zymogram of variety Yamuk (V3) at 10 – 13 days.



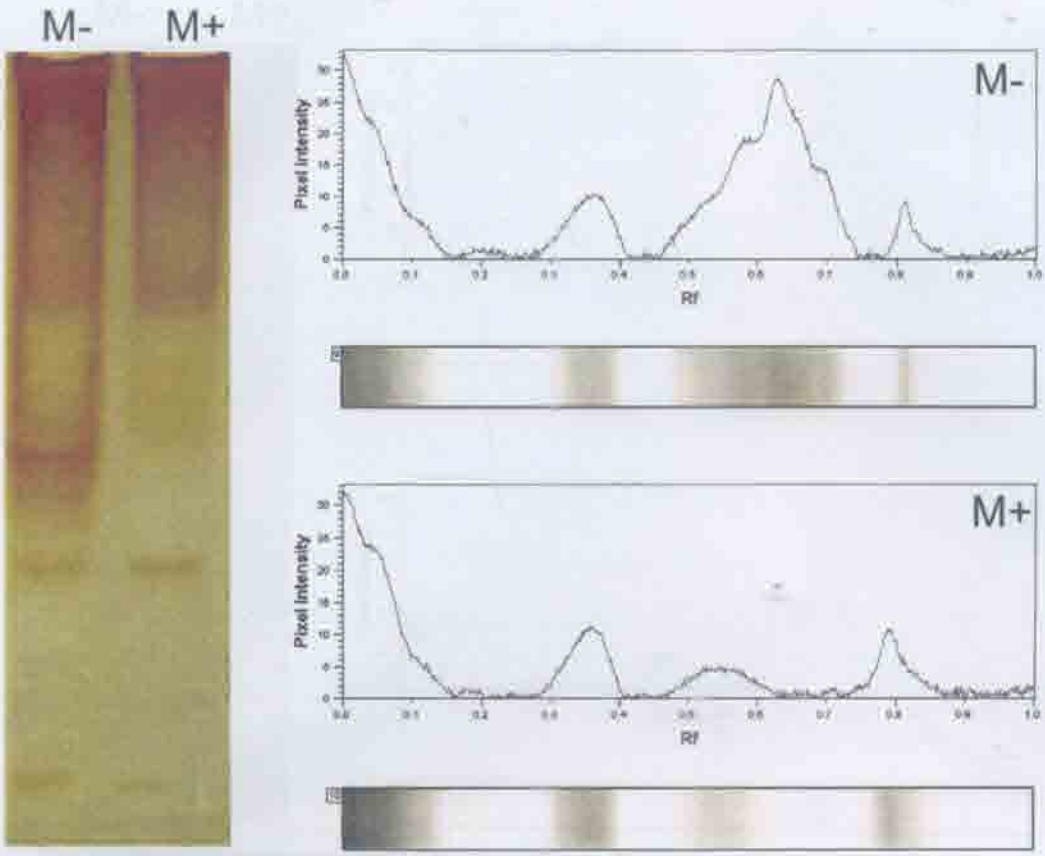
Treatment	Band no.	Rf	Volume	Area	Band %
M-	1	0.183	3334.00	3200	1.51
	2	0.338	2902.37	4320	1.32
	3	0.602	91976.08	8080	41.73
	4	0.664	114405.40	9600	51.91
	5	0.879	7791.67	5600	3.54
M+	1	0.245	7639.79	5688	14.86
	2	0.423	22590.50	8424	43.95
	3	0.604	18930.52	8064	36.83
	4				
	5	0.877	2236.26	1944	4.35

Plate 26: Whole root peroxidase isozyme profile together with densiometric analysis of zymogram of variety Jhingasal (V4) at 10 – 13 days.



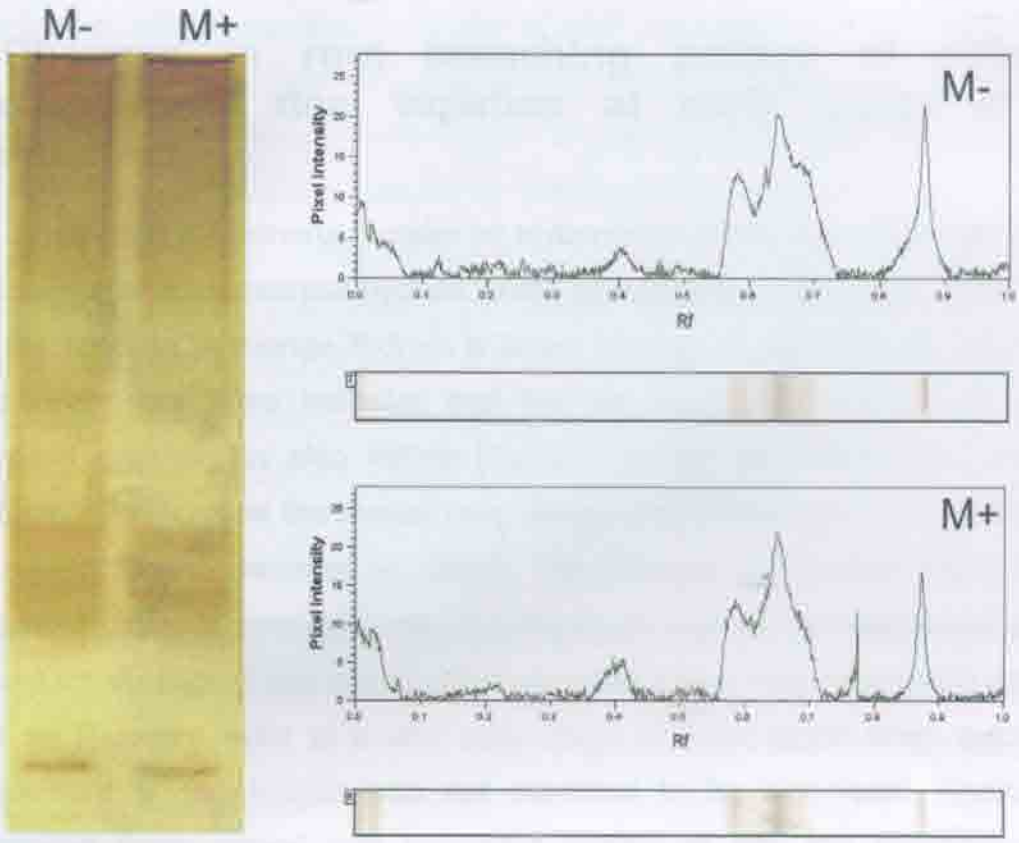
Treatment	Band no.	Rf	Volume	Area	Band %
M-	1	0.230	9333.83	4692	5.45
	1'	0.286	5259.79	3468	3.07
	2	0.389	9966.63	6120	5.82
	3	0.562	46616.03	5780	27.20
	4	0.625	75034.82	7616	43.78
	5	0.838	25164.33	6800	14.68
M+	1	0.152	11311.33	5848	6.73
	1'	0.234	4082.16	3128	2.43
	2	0.396	4195.50	2856	2.50
	3	0.566	74269.24	7888	44.21
	4	0.626	54155.09	5712	32.23
	5	0.840	19996.00	5372	11.90

Plate 27: Whole root peroxidase isozyme profile together with densiometric analysis of zymogram of variety Dular (V5) at 10 – 13 days.



Treatment	Band no.	Rf	Volume	Area	Band %
M-	1	0.201	5236.85	4346	1.32
	2	0.361	62165.58	10332	15.64
	3	0.578	106066.68	10660	26.68
	4	0.630	199199.83	11562	50.11
	5	0.812	24.856.32	7380	6.25
M+	1	0.186	3365.00	3116	2.27
	2	0.358	60361.90	9512	40.63
	3	0.520	17593.62	5658	11.84
	4	0.556	21620.59	6888	14.55
	5	0.791	45623.20	11070	30.71

Plate 28: Whole root peroxidase isozyme profile together with densiometric analysis of zymogram of variety MTU 7029 (V7) at 10 – 13 days.



Treaetment	Band no.	Rf	Volume	Area	Band %
M-	1	0.214	5866.00	3672	2.86
	2	0.393	18432.58	7480	9.00
	3	0.564	32808.21	3672	16.02
	4	0.623	109148.21	9452	53.28
	5	0.839	38600.47	6324	18.84
M+	1	0.211	5077.49	3264	2.89
	2	0.398	15178.00	5100	8.64
	3	0.566	39559.98	4148	22.53
	4	0.627	92855.43	7684	52.89
	5	0.840	22906.00	4420	13.05

Plate 29: Whole root peroxidase isozyme profile together with densiometric analysis of zymogram of variety IR 64 (V8) at 10 – 13 days.

7. Changes in root branching pattern of differently responding rice varieties at early stage of AMF infection

Increased phosphorus uptake by mycorrhizal plants is a consequence of the development of extrametrical hyphae which spread and ramify some distance away from the roots to scavenge P_i from a larger volume of soil (Schachtaman, 1998). Recent evidences have indicated that the AM, besides functioning as extended absorptive organs can also initiate changes in the root branching pattern and architecture to increase the overall root surface area (Tisserant *et al.*, 1996; Barker *et al.*, 1998; Bhattacharya *et al.*, 2002). The changed architecture might also help the plants to absorb more amounts of nutrients through the increased root surface – soil contact. Results of the investigation revealed a very high mycorrhiza inoculation effect on P-uptake even at a very early stage of colonization when extrametrical development of the fungus was not expected to be significant. Against these background facts, lateral root branching pattern of one low responsive variety (ARC12737) and one high responsive variety (MTU 7029) following root exposure to AMF by soil inoculation was studied at 15 days after seed germination to see whether any change in the branching pattern occurs following AM infection / early colonization.

The two varieties were grown in long cylindrical plastic tubes in solarized soil inoculated with root based AMF inoculum for 15 days and after whole plant harvest, the root mass was separated for counting and measuring the branch roots under low magnification stereoscopic microscope. For each variety 3 x 10 samples were used as replicates. Methods of plant growing and counting are presented in Chapter III (Materials and Methods). Results are presented in Tables 43 - 44; Figure 23 and Plates 30.

Results of the analysis showed that at 15 days after seed germination in AMF inoculated soil there were significant increases in the number and total length of branch roots per unit plant or per unit seminal root number per plant giving rise to a

significantly higher lateral root length density of AMF inoculated plants. While the changes in seminal root number and length were also not mostly significant, the changes in the number, hence total length of the branch roots were highly significant. Changes in average length of the branch roots were not mostly significant. Characteristically, levels of significance for the observed differences between the inoculated and non-inoculated plants were higher in the high AM responding variety MTU 7029 than ARC 12737. There were an average of 2 -2.5 times increase in length and number of lateral roots due to AMF infection in both the varieties giving almost similar increase lateral root length density in both the varieties

These results showed that the branching pattern and density of lateral roots were changed at the very early stage of AMF colonization in both AM responding and non-responding varieties providing for a changed root architecture whereby the mycorrhizal plants were expected to have a larger root surface contact with soil.

Tab e 43: Changes in root branching pattern of rice variety ARC 12737 at 15 days after seed germination in AMF inoculated soil

Primary metrical characters of lateral roots

Treatment	Seminal roots		1st order roots		2nd order roots		3rd order roots	
	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)
AM -	7.6± 0.5	84.7± 7.2	800± 86.3	2402± 80.9	340± 13.5	568± 77.4	0	0
AM +	8.0± 0.7	80.8± 6.3	1056± 54.0	3168± 161.9	1335± 171.2	2536± 324.5	397± 27.4	278± 18.6
Est t	2.547**	2.233**	13.773**	23.182**	31.735**	32.311**	-	-
Table t	0.05 *p* 1.960				0.01 *p* 2.576			

Unit measurements of lateral roots

Treatment	Seminal roots average Length (cm)	1st order roots			2nd order roots			3rd order roots		
		Per seminal root	Per unit length of seminal root	Average length (cm)	Per 1st order root	Per unit length of 1st order root	Av. length (cm)	Per 2nd order root	Per unit length of 2nd order root	Av. length (cm.)
Am -	11.1	105	9.4	3.0	0.42	0.14	1.7	-	-	-
AM +	10.1	132	13.1	3.0	1.26	0.42	1.9	0.3	0.16	0.70

Lateral root branching and lateral root length density

Treatment	Lateral branch root development				Lateral root length density cm /cc
	Number		Length (cm)		
	Per plant	Per seminal root	Per plant	Per seminal root	
AM –	1140± 392.2	150± 0.4	2970± 58.0	391± 5.7	6.4± 0.8
AM +	2788± 334.2	348± 41.6	5982± 496.3	748± 62.0	13.0± 2.2
Change	x 2.4	x 2.3	x 2.0	x 1.9	x 2.0
Est. t	17.518**	26.068**	33.016**	31.406**	15.442**
Table t	0.05 *p* 1.960		0.01 *p* 2.576		

Table 44: Changes in root branching pattern of rice variety MTU 7029 at 15 days after seed germination in AMF inoculated soil

Primary metrical characters of lateral roots

Treatment	Seminal roots		1st order roots		2nd order roots		3rd order roots	
	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)	Number	Length (cm)
AM -	8.3± 0.5	40.1± 3.1	310± 19.8	1085± 45.8	1075± 55.8	1397± 72.6	18.4± 4.4	13.0± 3.1
AM +	9.7± 0.5	49.0± 2.2	657± 34.5	1840± 102.1	2195± 115.9	2941± 155.3	1130± 75.1	768.0± 51.1
Est t	10.844**	12.824**	47.780**	36.954**	47.690**	49.331**	80.933**	80.778**
Table t	0.05 *p* 1.960				0.01 *p* 2.576			

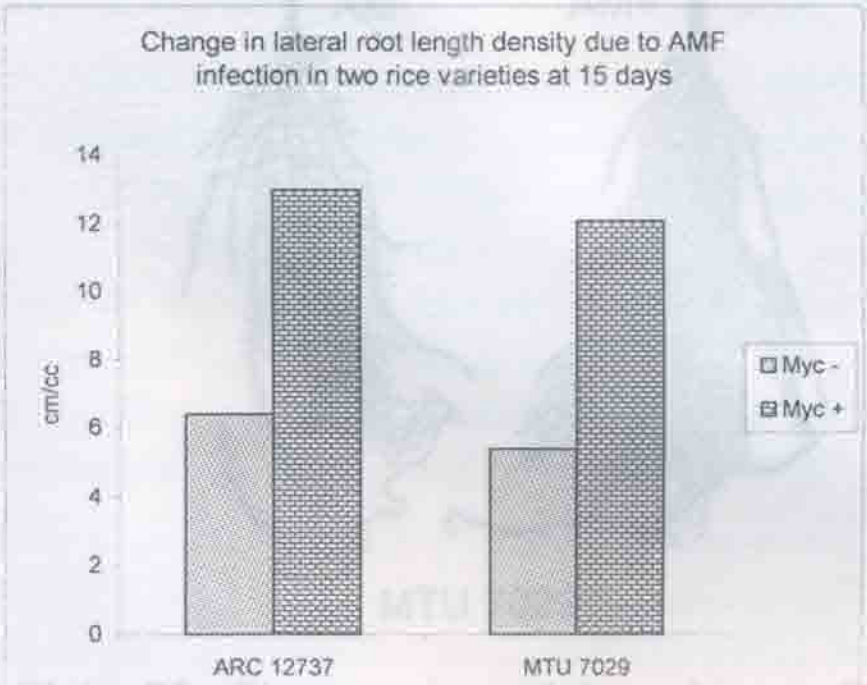
Unit measurements of lateral roots

Treatment	Seminal roots average Length (cm)	1st order roots			2nd order roots			3rd order roots		
		Per seminal root	Per unit length of seminal root	Average length (cm)	Per 1st order root	Per unit length of 1st order root	Av. length (cm)	Per 2nd order root	Per unit length of 2nd order root	Av. length
AM -	4.8	37.3	7.7	3.5	3.5	0.99	1.29	0.02	0.01	0.70
AM +	5.0	67.7	13.4	2.8	3.4	3.3	1.33	0.51	0.38	0.68

Lateral root branching and lateral root length density

Treatment	Lateral branch root development				Lateral root length density cm /cc
	Number		Length (cm)		
	Per plant	Per seminal root	Per plant	Per seminal root	
AM –	1403± 402.3	169± 20.6	2495± 210.9	301± 72.2	5.4± 0.7
AM +	3982± 432.6	410± 55.7	5549± 438.5	572± 61.5	12.1± 1.8
Change	x 2.8	x 2.4	x 2.2	x1.9	x 2.2
Est. t	23.912**	22.227**	34.378**	15.650**	19.001**
Table t	0.05 *p* 1.960			0.01 *p* 2.576	

Fig 23: Comparison of changes in lateral root length density of the differentially responsive rice varieties upon mycorrhiza inoculation at 15 days



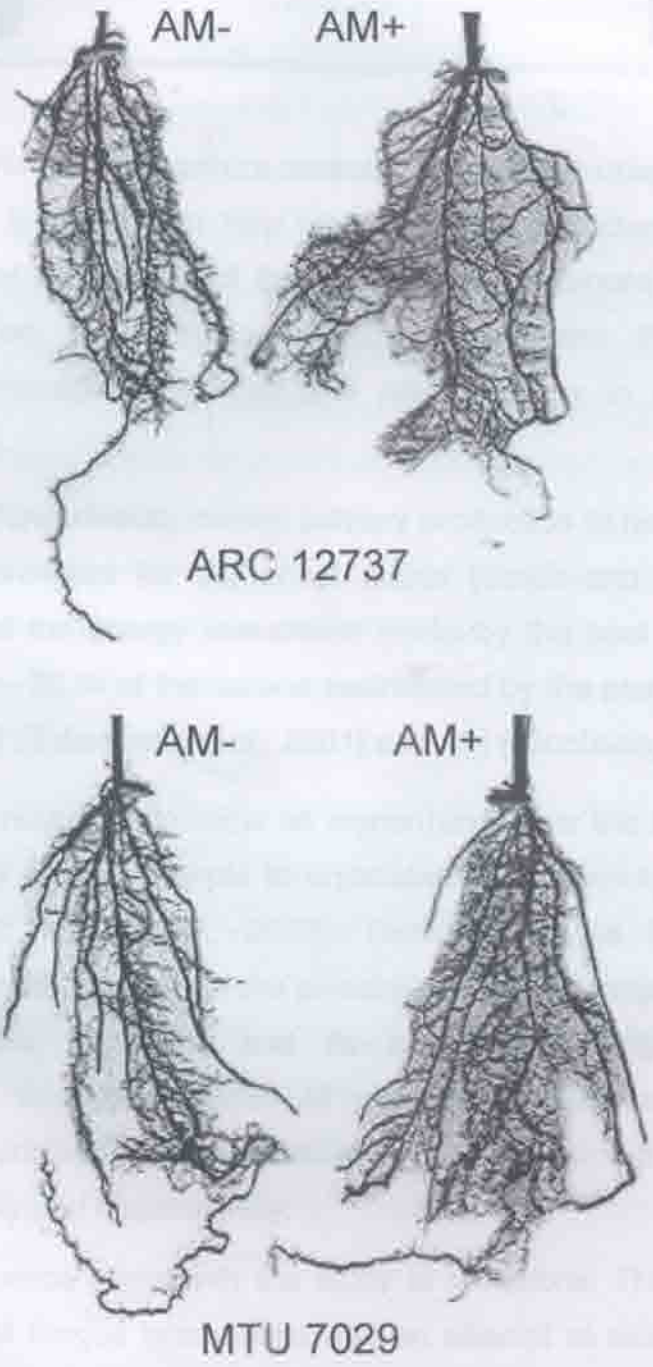


Plate 30: Changes in root branching pattern of one non-responding (ARC 12737) and one responding (MTU 7029) rice variety due to AM infection at 15 days

Chapter– V Discussion

One hundred years of mycorrhiza research has put this unique and elegant form of symbiosis in limelight in 'new biology' that is predicted to rule the present century. The critical importance of the mycorrhizal symbioses in nature *and their* strong influence on many ecological processes make them invaluable for maintaining and managing the crop and plant ecology in sustainable manner (Soderstrom, 2002).

Mycorrhizal fungi directly control primary production in nature as they are the major nutrient scavengers for their host plants (Smith and Read, 1997). That happens in spite of the energy investment made by the host for maintaining the fungus partner, 20 – 30 % of the carbon assimilated by the plant is shared with the fungus in both ECM (Bidartondo *et al.*, 2001) and AM (Jacobsen, 1995) symbioses.

The diverse research domains on mycorrhizas over the last 100-years have the underlying unity in the attempts to understand the diversity and integration in mycorrhizas (Smith and Smith, 2002). Genetics is the keystone for such understanding as knowledge about the primary genetic determinants controlling the development of the symbiosis and its metabolic activity is essential for understanding the ecological fitness of mycorrhizal symbiosis (Martin, 2001). Knowledge about ecological fitness determines how best the symbiosis can be exploited ecologically and economically.

Study of genetics starts with the study of variations. The present study on host-genotype – AM fungus interactions was an attempt to study the variations in functional compatibility between the genotypes of a plant taxon and the AM fungi in relation to the primary physiological attribute behind the functional role of mycorrhizas. Rice was chosen as the test plant in view of its emergence as a model species for dissection of the genetic pathways of AM symbiosis (Barker and Larkan, 2002) and the access to genetically diverse plant materials - from inbred land races to hybrid combinations of widely different genotypes. The tropical environment of the

experimental site and easy, all thorough out the year growing potential were the other considerations for the choice of rice as the test plant. Mixed species AMF inocula and mostly non-sterile, low infective soils were used to create a situation as near as natural environment.

The rice varieties used in the experiment representing land races, traditional and improved varieties and modern high yielding hybrid inbreeds were selected from a large collection of such genotypes. Results of the experiments on AMF response relations of the 8 selected varieties at two ages of early seedling phenological stage very clearly showed variations in growth and P-uptake responses (MIE%) among the varieties. Such variations being to some extent predictable, it was worthwhile to note that there was a clear separation of the varieties according to their origin and their AMF response. The land race selections and traditional accessions were comparatively less or low mycorrhiza responsive than their modern day counterparts.

Cultivar differences in response to mycorrhizas have been observed in crops like, wheat (Young *et al*, 1985; Vierheiling and Ocampo, 1991; Hetrick et al, 1993; Singh and Adholeya, 2004), maize (Toth *et al.*, 1990), *Vigna* (Mercy *et al.*, 1990), Lucerne (Lackie et al., 1988), Ground nut (Kesava Rao *et al.*, 1990), *Agropyron* (June and Allen, 1991) and some other crops (Smith and Read, 1997). There are two opinions about the possible evolutionary relationship of mycorrhizal response in varieties or genotypes within a species. Hetrick *et al.* (1993) observing that wheat cultivars released before 1950 were consistently more dependent on AM than those released later suggested that germplasm selection under high phosphorus fertilizer conditions could have reduced the frequency of genes for mycorrhizal dependence of wheat. Singh and Adholeya (2004) have recently observed that the old hexaploid wheat land races and tetraploid cultivars display more consistent and higher growth responses to AM symbiosis than the modern cultivars. Reviewing some of these evidences, Barker *et al.* (2002) commented that the beneficial traits like, enhanced response to mycorrhization, likely to have been present in the older accessions and cultivars that pre-date the heavy fertilization era, may well have been lost from

modern cultivars. However, Koide *et al.* (1988) compared wild and domestic oat and reported little response by the wild oats to AM inoculation and commented (Koide, 1991) that to the extent the wild plants are inherently more able to match phosphorus supply with demand the wild species or germplasms of plants may be less dependent on the symbiosis for phosphorus uptake than cultivated species.

The present observation that the ancient land races and traditional varieties of rice adapted to relatively low nutrient situations were less responsive to AMF inoculation than the modern improved and high yielding varieties would support that evolutionary adjustment and ecological adaptation to low nutrient stressful situations as is obtained in the lateritic Chhotonagpur Plateau (var. Black Gora) or degraded hills of Arunachal Pradesh and Assam (vars. Yamuk and ARC 12737) might have made the land races inherently less or non-dependent on AMF as their potential P-demand were likely to be met by matching inherent supply efficiency of phosphorus. It becomes important to recall here that the North-Eastern Hill Region of India which includes Arunachal Pradesh and upper Assam bordering Indo-China land mass is one of the recognized centres of domesticated origin of *indica* rice (*Oryza sativa*) from where the last named varieties were collected. The modern improved and HYVs were possibly more responsive to AMF in view of their selection and breeding for high nutrient response (P-demand), imposing the inability to adjust to low P-situations.

The hypothesis gets credence from the systematic observations about the morphological and physiological traits of the varieties relating to their nutrient (phosphorus) demand and supply relations. These experiments were carried out to determine the 'phosphorus deficit' of the varieties relative to their potential maximum growth in a low phosphorus soil. It was observed that the traditional land race varieties which were low mycorrhiza responsive had lower shoot growth rate (in terms of dry matter accumulation) as compared to the high AM responding modern varieties. The rate of shoot growth is one important physiological factor which determines the rate of nutrient demand (Fitter and Hay, 1987). For tissue phosphorus concentration to remain constant to support active growth the rate of

phosphorus uptake must be proportional to the rate of shoot growth (Koide, 1991). It was observed that the low AM responsive traditional land race varieties all had a lower shoot growth rate than high AM responding, modern varieties. It was characteristic that the potential P-demand rates of the low growth rate, low AM responsive varieties were found to be lower than that of the high growth rate, high AM responsive varieties. That substantiated the physiological truism about the relation between growth rate and nutrient demand and pointed out 'shoot growth rate' as one possible primary determinant of mycorrhizal response. It may be important to recall that modern cultivated varieties often have higher inherent growth rate than the older cultivars, land races or undeveloped ecotypes and the demand for nutrients and the need for fertilization to attain maximum growth in the modern cultivars is higher (Mengel, 1983; Koide, 1991).

The inter-variety differences in root growth rate were observed to be reverse to that of the shoot growth rate. Root growth rate can be considered as the primary index of nutrient absorption (supply) efficiency and apparently the low AM responsive, low P-demanding traditional land race varieties in having higher root growth rate were expected to have higher supply (absorption) efficiency of immobile nutrients, like phosphorus than the modern, high AM responsive, high P-demanding varieties. Within a limit, the rate of phosphorus uptake of a plant is correlated with the rate of its root growth in a fixed volume of soil (Newman and Andrews, 1973).

Root : shoot ratio or the specific shoot growth rate is a morpho-physiological index for the nutrient supply –demand potentials of plants. In a given soil, a plant with higher root : shoot ratio than another will be more efficient to supply nutrients to support the nutrient demand rate for production of equal amount of shoot mass by the two varieties. In other words, nutrient demand held constant, a plant with a higher root : shoot ratio is expected to suffer less 'nutrient deficit' than another plant having lower root : shoot ratio than the former. Similarly, a plant producing a higher shoot mass per unit root mass than another shall suffer from higher nutrient deficit in a given soil than the latter. That the traditional land race rice varieties with higher root : shoot ratios and low specific shoot growth rates were found to be less responsive to AM than their modern counterparts were indications for their lower P-

deficit in the experimental low P soil. This was later substantiated by actual measurements of P-demand and supply potentials of the varieties. Rate of phosphorus inflow (phosphorus absorption per unit root length per unit time) and P-demand remaining constant a plant with a higher root : shoot ratio responds less to mycorrhiza than another having lower root : shoot ratio (Koide, 1991). Onion and wheat both having low P-inflow rates respond differently AM – high for onion and low for wheat - due to the difference in their root : shoot ratios – low for onion and high for wheat (Fohse, et al., 1988).

Root branching or architectural pattern was also different among the varieties giving rise to the differences in lateral root length density (LRLD) where LRLD was significantly higher in the low AM responding traditional land race varieties than the high AM responding modern varieties. Phosphorus uptake rate (plant P-content at a given time) is a function of root length density as this variable determines how far the phosphorus ion must diffuse in soil for absorption to occur (Baldwin, 1975). This substantiated the hypothesis that the traditional varieties have an inherently higher nutrient uptake (or acquisition) efficiency from a low nutrient soil than the modern varieties and given their apparent low P-demand as shown by low shoot growth rates they suffer less from P-deficit in a low nutrient soil and hence, respond less to AM.

The above interpretations were substantiated by comparison of P-uptake features of the two variety groups in the same low P-soil from where the above growth related observations were taken. During the early growth stage total plant P-uptake (absolute P-content) was at least 20% higher in the low AM responsive traditional varieties than the high AM responsive modern varieties, but the relation was reverse when specific plant or shoot P-contents were considered. This showed that absorption and transport rates of phosphorus to shoots per unit root mass of the varieties, even in the low P soil were higher in the high AM responsive modern varieties than the low AM responsive traditional varieties. Although not specifically determined, it seems that inherent P-inflow rates (phosphorus absorption per unit length of roots per unit time) were higher in modern than traditional varieties. It was also characteristic that higher proportion of the absorbed plant P concentrated in

shoots in the modern varieties as compared to the higher proportion concentrating to roots in the traditional varieties. This happened possibly to support their high P - demand to support their higher shoot growth rate. In spite of having a higher P-inflow rate, the modern varieties responded more to AM probably due to their lower root : shoot ratios and higher P-demands as observed in later parts of the investigation.

These interpretations were finally confirmed by actual estimates of P-deficit relative to maximum potential P-responsive growth rates of the varieties. The average maximum attainable per day growth rate of the 4 high AM responding modern varieties was 46 % higher than that of the 3 low AM responding traditional land race varieties (Table 19). Corresponding to that the optimum P-demand of the former group of varieties was 43% higher. The near one to one correspondence of the two estimates justified the physiological truism that P-demand rates of plants are directly correlated with growth rates of plants. With a lower efficiency of root P-uptake rates the modern varieties suffered a higher P - deficit at any level of soil phosphorus below the optimum than the group of traditional varieties where the deficit was less due to a lower P-demand and higher root P-supply efficiency. With a higher P-deficit in the low P-soil the modern varieties responded more to AM as compared to the traditional varieties where at the same soil P- level the P-deficit was less. It thus appeared that the AMF response potential of a variety or a plant genotype in a soil will depend primarily on its phosphorus deficit depending upon its (i) root P- uptake efficiency and (ii) potential P-demand relative to available P-status of the soil.

One improved traditional variety, Dular behaved as an intermediate in AM response between the two variety groups. Its relatively high P-demand than that of its closer allies was possibly matched by higher root P-supply (uptake) efficiency as justified by its very high root : shoot ratio, highest LRLD (Table 8) and high absolute plant P- uptake rates (Tables 10 & 12). In other words, it suffered from a lower P-deficit in the same low P soil than its counterparts in the traditional variety group not for a lower demand but for the higher P-supply (uptake) efficiency relative to the demand.

At this stage it becomes pertinent to consider the negative response of some of the traditional varieties to AM at very early stage of growth. The observed negative response can be interpreted as growth depression due to AM colonization. There have been many interpretations of the frequently observed negative responses of plants to AM colonization, reviewed by Marschner (1995), Smith and Read (1997), Burleigh and Bechmann (2002). The present results were suggestive of the fact that the growth depression resulted from host factors rather than the inability of the mycobiont(s) to provide phosphorus in amounts that are needed to stimulate growth, as increases in P-uptake of the plants effected by the AM fungi, even under negative growth response of some of the varieties was sizable, though not equal to that under positive response of some other varieties. Moreover, there were indications that not the whole of phosphorus taken up by the plants under AMF colonization, irrespective of their growth response, was either needed or utilized at that growth stage. That is there was excess or luxury consumption of phosphorus (Marschner, 1995) in all the varieties, irrespective of their growth response.

The varieties which responded negatively to AMF colonization at early seedling stage were all observed to have a low P-demand and high root nutrient supply (uptake) efficiency. These varieties showed a low P-deficit so that their incremental P-responsive growth rate was minimal. It would thus appear that their investment in carbon supply to support the developing fungus symbiont was not balanced or matched by their demand or need for phosphorus under the prevailing conditions of the experiment and phenological stage of the plant. It seems that the carbon drain to support the developing fungus in the root not evenly balanced by the low P-responsive shoot growth rate of the traditional varieties was at the root of the negative or growth depression response of these varieties at the early seedling stage. Marschner (1995) reviewed a few such cases of growth depression response. Whether these results can be interpreted as cases of functional incompatibility (Ravenskov and Jacobsen, 1995; Burleigh and Bechmann, 2002) between the AMF and host genotypes remains as a point to consider.

Results of the previous studies on inter-variety differences in AMF response (reviewed by Smith and Smith, 1997), especially those of Kesava Rao *et al.* (1990),

Hetrick *et al.* (1993), read with the early data on mutational origin of resistance to AMF colonization (e.g., Duc *et al.,* 1989) have shown the possible existence of two sets of independently acting genes behind the functional compatibility of the AMF with their hosts. There may be one set of genes that control whether or not colonization occurs (Barker et al., 2002) and another set that controls the degree of benefit derived from the symbiosis (Hetrick *et al.*, 1993). In the present case, the negatively or low AM responding varieties were all colonized by the AM, though at a slower rate than the high responding varieties. It seems that AMF colonization in these varieties was not blocked either at pre – or post-penetration stages, so that AM colonization responsive genes were likely to be present in these varieties also. It is possible that the genes that control AM responsiveness were not equally present or expressed in these varieties as in the high responding varieties. Hetrick *et al.* (1993) observed that wheat cultivars which do not benefit from AM symbiosis may suffer from growth depressions. Hetrick *et al.* (1995) from their data on formal genetic analyses of differently AM responding wheat cultivars suggested for the existence of 'mycorrhiza responsive' genes in the cultivars which respond to AM colonization. Whether the present results point out such a phenomenon being universal becomes a pertinent point to consider and may be important for considerations of exploitation of AMF in low input systems of cultivation through selection and breeding 'high AM symbiosis responding' varieties.

One important issue that arises here is the observation that the varieties, which responded negatively at the early seedling stage, showed some degree of positive response at late seedling stage. That seems to be a clear case of the impact of phenology on AMF response pattern. Phenology is known to influence the temporal pattern of nutrient demand (Koide, 1991) and hence, AM response pattern also. It would seem that irrespective of the varieties, and expectedly, P-demand of rice plants increased at the late seedling stage so that the degree of AMF inoculation response or MIE% of the varieties also increased at that stage. Whether this means a age dependent change in the expression pattern of the supposedly 'mycorrhiza responsive' genes becomes a point to ponder here.

These interpretations take us to consider the reasons for the delay or slower AMF colonization rates of the negative or low responding varieties. Results of the histological study on colonization features of the differently AM responding varieties showed that the structural pattern of colonization either at the pre- or post-penetration stage were not different between the two variety groups. The difference that was there was in the relative rapidity with which colonization was established in the two variety groups. The results were inadequate to understand the reasons for the slower rate of colonization in the negative or low AM responsive varieties. The only possible speculative explanation that can be offered is the expression of some degree of incompatibility between the host varieties and the AMF, especially at the early seedling stage. We are not aware of any previous example of such delayed or slower rate of histological colonization by the AMF of varieties of a plant species, except the cases of low or reduced AMF colonization of the *rmc* mutant of tomato (Barker *et al.*, 1998) and the recently described low AMF penetration, and low response mutant phenotype of pea which has low nodulation phenotype also (Resendes *et al.*, 2002). The latter has two blocks to AMF colonization, one at penetration of epidermis and the other at cortex invasion stage. Both the *rmc* and the low *Pen*, low *coi* phenotypes are however, arbuscule positive. Although these phenotypes are not exactly comparable to the presently observed slow colonization phenotype of rice varieties, existence of varietal or genotypic differences in compatibility to AMF colonization rates can not be overruled among the plant species.

Results of the studies on the changes in whole root peroxidase activity of the differently responding rice varieties become interesting in the context of both low or negative growth response and slower colonization rates of the traditional varieties. There are evidences of transitory increases of enzymes of post –recognition defense pathways of hosts (Gianinnazi-Pearson *et al,* 1999), including the whole root peroxidase activity, at initial stages of colonization of host roots by the AMF (Spanu and Bonfante-Fasolo, 1988; McArthur and Knowels, 1992). Our observations in the context showed a clearly different response of the two variety groups, responding negatively and positively to AMF colonization at the initial stage. While the early

negatively responding varieties which were colonized at slower rate showed a significant rise in whole root peroxidase activity, the varieties which were colonized at a faster rate and had a positive growth response showed a decline in peroxidase activity. The two peroxidase isozymes which were present in rice roots at higher amounts at that age were observed to be up or down regulated in the negatively and positively responding varieties respectively. Accepting that activation of peroxidase enzyme in roots under initial AMF colonization is an early expression of defense reaction through possible reinforcements of cell wall (Dixon and Harrison, 1997), the increased peroxidase activity in the negatively responding, and slowly colonized varieties can be interpreted as a possible activation of defense response to AMF colonization at the initial stage in these varieties. That would mean that root peroxidase activity in the high responding, rapidly colonized varieties were suppressed at the initial stage. Clearly, these were indications for some degree of incompatibility of the host-AMF combination in case of traditional varieties which responded negatively to AMF inoculation and such response may mean that the host perceives the invading AMF as a potential pathogen.

One of the earliest gross morphological changes in the host to AMF colonization is the changes in root branching pattern under possible activation of auxins like IAA and/or IBA which are involved in lateral root branching (Tisserant *et al.*, 1996; Barker *et al.*, 1998; Kaldorf et al., 2001). Results of the study on post-AM infectional changes in root branching pattern of the two rice varieties confirmed that root branching, hence architectural pattern of roots may change at a very early stage of AMF colonization, as observed by this laboratory in some other hosts also (Bhattacharya *et al.*, 2002). This observation becomes especially significant in the context of increased P-uptake of both negatively and positively responding varieties even at the very early stage of colonization, when the extramatrical hyphae mediated P-uptake due to AM is not expected to be highly significant, at least for the negatively responding and slowly colonized varieties. This would mean that the increased lateral root length density induced in host by the AM, since the very stage of colonization can be at the root of increased P-uptake, hence, AMF response of plants. That is in addition to the usual hyphal pathway of nutrient absorption and

may add to the competence of mycorhizal plants to perform better under nutrient-poor situations.

Results of the investigation aiming to elaborate inter-genotype variations in AMF interaction responses of rice have allowed the identification of physiological correlates of AMF responsiveness or dependency and refurbished the physiological truism that plants suffering from a higher P-deficit in a low P soil will respond positively to AM symbiosis. The magnitude of response will vary according to the P-demand related phenological state of a plant. In times of and in genotypes with low P-demand but high root P-supply there may be negative response of AM colonization. In such cases plants may perceive the invading AM fungus as a potential pathogen to respond with an activated defence response at cellular level.

Chapter– VI Summary and Conclusions

Arbuscular mycorrhizal symbiosis of plants has emerged as a frontier discipline of 'new biology' for management of crop agriculture in ecologically sustainable manner. The role arbuscular mycorrhiza plays in nutrient mobilization of plants, plant tolerance to abiotic and biotic stresses, soil resilience to physical perturbation, and the structure and dynamics of plant communities has provided the impetus for studying the AM symbiosis in its basic details. Knowledge about the ecological fitness of the symbiosis is being synthesized from the information gained from such studies. Genetics of AM symbiosis has emerged as the principal area of such knowledge gain to answer the unresolved questions of this ecologically unique ancient biological system of nature.

The thesis presents the results of an investigation into inter-variety (genotype) variations of rice in interaction response with selected high performing AM fungal isolates in a low nutrient laterite soil. The study aimed at defining the morphological and physiological correlates of variations in AM response of a model plant. The knowledge gained was expected to help starting planned breeding and selection of high arbuscular mycorrhiza responsive crop genotypes that might perform better with AM inoculation replacing phosphorus fertilization, especially in stressed soils. Results of the studies are summarized here.

1. Eight rice genotypes – modern dwarf, high fertilizer responsive cultivars, improved traditional varieties cultivated with low fertilizer rates and ancient land race germplasms were selected for the study from a large collection of such temporally and spatially originated and acclimatized plant materials. Experiments were carried out in a nursery house under ambient environment. Mixed species high performing root based AMF inoculum was used in a low nutrient, low infective laterite soil.
2. The 8 rice varieties showed variations in their AM inoculation response, both for growth and P-uptake at two early seedling ages. At the very early seedling stage of 15 days, 3 land race selections (Black Gora, ARC 12737, and Yamuk) showed negative response or growth depression due

to AMF infection. Four varieties representing modern high yielding hybrid inbreds and improved traditional varieties (Jhingasail, Taichung Native-1, MTU-7029 and IR-64) showed moderate positive response. One traditional variety (Dular) showed no significant response. Even at such an early stage of colonization the varieties, irrespective of their growth responses showed highly significant improvements in P-uptake. Improvements in P-uptake as AM colonization response also varied among the varieties, but apparently there was no relation between P-gain magnitude and growth response, as the traditional variety Dular in spite of a relatively high P-gain failed to show a parallel gain in shoot growth.

3. At the later stage of seedling growth at 30 days, the magnitude of AMF growth response increased in all the varieties and the early negatively responding land race selections showed a moderate positive response. The modern varieties and the traditional variety Dular showed increases in their response, but the difference in the magnitude of response between them still remained. There were corresponding increases in P-uptake, but the magnitude of growth response was not correlated with the magnitude of increase in P-uptake in any of the varieties.

4. Root growth response of the varieties to AMF colonization showed increase with a difference among the varieties. The magnitude of increased root growth response of the land race selections and the traditional variety Dular was only marginal and non-significant at the early 15- day growth stage, but that of the modern HYV and the improved traditional variety, Jhingasail was significant. At 30 days, corresponding to the increases in shoot growth response there were significant increases in root growth response also, with a higher magnitude than that at 15 days.

These results were interpreted as genotypic differences in AM response or dependency of rice where evolutionarily older genotypes or germplasms, adjusted and adapted to low nutrient soil situations through the ages were inherently more capable to match their nutrient demand with supply potential of soils and also plant root characters, so that they did not need mycorrhiza formation for uptake of nutrients. The modern varieties bred and selected for high

nutrient response suffered from greater nutrient deficit than the traditional varieties in the low nutrient soil and hence, responded to mycorrhiza formation as an added organ for nutrient absorption.

5. Analysis of inherent shoot growth characters of the varieties in two different soils at 2 different ages showed that the 3 ancient land race selections and germplasms and the traditional variety Dular which responded either negatively or insignificantly were all slow growing and have a low shoot growth rate as compared to the 4 positively responding modern varieties all of which showed higher and faster absolute and specific shoot growth rates. This reflected a possible difference in their nutrient demand to support their variable shoot growth rates.

5. Analysis of root growth characters of the varieties in the same soil at the corresponding ages revealed a contrasting pattern from that of the shoot growth characters. Root growth rate of the non- or low AM responding land race selections and the traditional variety Dular were all higher than that of the high AM responding modern and improved varieties.

These results showed that at the given period of seedling growth stage (25-50 days) the evolutionarily older land race selections and traditional varieties adjusted to low nutrient situations had a higher root development efficiency giving them the capacity to absorb more nutrients than the modern varieties, bred and selected for high nutrient situations. Conversely, the latter group of varieties in view of their higher shoot growth rate shall have higher nutrient demand as opposed to the possible lower nutrient demand of the land race selections generated from a lower shoot growth rate.

7. The low or non-AM responding land race selections and traditional varieties showed a higher root : shoot ratio than the high AM responding modern varieties to reflect further the above contrasting feature of root and shoot growth dependent nutrient supply and demand potentials of the two variety groups. Characteristically, root: shoot ratios of both the variety groups showed increases under AMF colonization, but with a different magnitude in the two variety groups. At later age of 30 days when full establishment of mycorrhiza was expected, the magnitude of positive

change in root: shoot ratio was lower in the high responding modern varieties than the low or non-responding land race and traditional varieties. This indicated higher functional competence of AM in case of the modern than the traditional varieties.

8. Between 25-50 days, total P-uptake efficiency or absolute plant P-content was at least 20 % higher in the low AM responding land race and traditional varieties than that of the high responding modern varieties. Characteristically, the whole or a major proportion of this 20 % higher phosphorus in the traditional varieties went to roots. Results showed that specific plant or shoot P-contents or the amount of P absorbed per unit amount of root mass of the modern varieties was higher than that of the traditional varieties and land race selections. This suggested that these varieties were inherently efficient to transfer more amount of absorbed phosphorus to shoots, possibly to satisfy their high shoot growth rate dependent high P-demand. In spite of the possible higher P-inflow rates (P-uptake per unit root length per unit time) these varieties might suffer from higher P-deficit due to a lower root: shoot ratio as evident from the analyses.

9. Lateral root length density or the correlate of inherent nutrient absorption capacity of plants, as measured during early growth stage of the low or non-AM responding varieties was significantly higher than that of the high AM responding modern varieties. This justified the interpretation that the evolutionarily older land race selections and traditional varieties were inherently more efficient to absorb nutrients from a low nutrient soil than their modern counterparts.

10. Results of the above studies were followed by measurements of potential P-demand optimum to attain the maximum potential growth rates of the varieties by regression analysis of growth and P-supply rates under increased soil P-levels. The estimates showed that the group of 4 high AM responding modern varieties had 46 % higher potential maximum shoot growth rate than the 3 low AM responding, land race selections and traditional varieties. The corresponding optimum P- demand rate was 43 % higher in the former than the later group. The near one to one

relationship between the P-demand and shoot growth rates, justifying the variations in principally demand driven P-deficits of the varieties showed that magnitude of P-deficit in a low P soil, will be higher in the modern varieties than the traditional land race selections / varieties. The higher P-deficit in the soil was at the root of their higher P-deficit. The traditional variety, Dular, in spite of having a relatively high P-demand, higher than that of its closer allies did not suffer from an equivalent P-deficit as of the modern varieties due to its higher root supply efficiency as reflected from its very high lateral root length density, highest root: shoot ratio among the varieties and very high absolute plant P-content.

These results were used to confirm the hypothesis that AM response of genotypes shall depend on the potential P-deficit of the genotypes in a particular soil P-level as determined by their growth rate driven P-demand and root length density dependent P-supply efficiency. Based on these, the inherent plant factors including the phonological stage which determine both demand and supply of phosphorus might appear as possible physiological correlates of AMF response in the field, especially in low nutrient soils.

11. The negative response of the land race selections and non-response of a traditional variety to AMF colonization were followed by histological study of pre- and post- penetration colonization features of the varieties at the corresponding growth stage of such plant response. Results of the detailed study showed that there were no differences among the two differently responding variety groups in the pattern of both pre- and post-penetration colonization, neither there was any easily perceptible difference among the varieties in histological response to colonization. But, there was a significant difference between the two variety groups in the relative rapidity of establishment of colonization at both pre- and post-penetration stages. Per cent root colonization with both pre- and post penetration structures, intensity of pre-penetration surface colonization structures (infection hyphae, appresoria etc.) per unit root surface area and intensity of intra- and intercellular hypha and arbuscule development in the cortex were all significantly lower in the negatively responding varieties at the given time of early seedling stage exposure to AMF in soil.

This was interpreted as possible incompatibility of the host- AMF combination, linked with the host rather than the fungal symbiont. Such delayed or slow colonization response of these varieties has not been observed or described earlier but has some parallel in some spontaneous or induced low AM or AM negative mutants of plants.

12. Examination of whole root peroxidase activity as an expression of possible post-recognition, non-specific defense response of plants to pathogens showed considerable increase of activity in the roots of the negatively responding varieties. Compared to that, the peroxidase activity appeared to be significantly suppressed in the roots of high responding varieties. With time and progress of colonization the magnitude of increase in peroxide activity in the former and decrease in the latter variety groups declined. Analysis of peroxidase isozymes at the corresponding age period by gel electrophoresis showed there were no qualitative changes in the isozyme profile of the variety groups due to colonization. But, the expressions or intensities of the two most predominant isozymes of rice roots of that age showed corresponding increases or decreases depending upon the negative or positive response characters of the varieties. These results were interpreted as possible activation and suppression of defense response at the initial colonization stage in the negatively and positively responding rice varieties. The former varieties possibly showed incompatible host – AMF interaction through activation of defense response that generally operates by cell wall reinforcement utilizing peroxidase enzyme. The same may be a normal phenomenon of AMF colonization response, suppressed in compatible and activated in incompatible combination.

13. Possible changes in root branching pattern under AM colonization were studied by counting and measuring branch roots of two differently responding varieties at 15 days of exposure to AMF inoculum in soil. Results of the study showed that in both the varieties lateral branch root number, hence total branch root length significantly increased even at the very early stage of colonization. The change resulted in a higher lateral root length density of the mycorrhizal plants than that of the non-

mycorrhizal plants. Although the magnitude of change was *almost the* same in both the varieties the levels of significance of the changed response was higher in the high AM responding variety than the low responding variety. Such changed root architecture may provide for higher nutrient uptake capacity to mycorrhizal plants over and above to that provided by extramatrical hyphae as nutrient absorbing organ. At the very early stage of colonization when extarmatrical hyphae development was expected to be minimal, such increased lateral root length density might have provided both the negatively and positively responding varieties with increased ability of nutrient (phosphorus) absorption, as observed from plant P-content measurements.

Results of the investigation have helped identification of physiological correlates of variable AM responsiveness or dependency of rice, chosen as the model plant, in a low nutrient soil and have provided a base for further genetical studies relating to selection and breeding of high mycorhiza responsive varieties for low input cultivation with arbuscular mycorrhiza as a substituent of chemical phosphorus fertilizer.

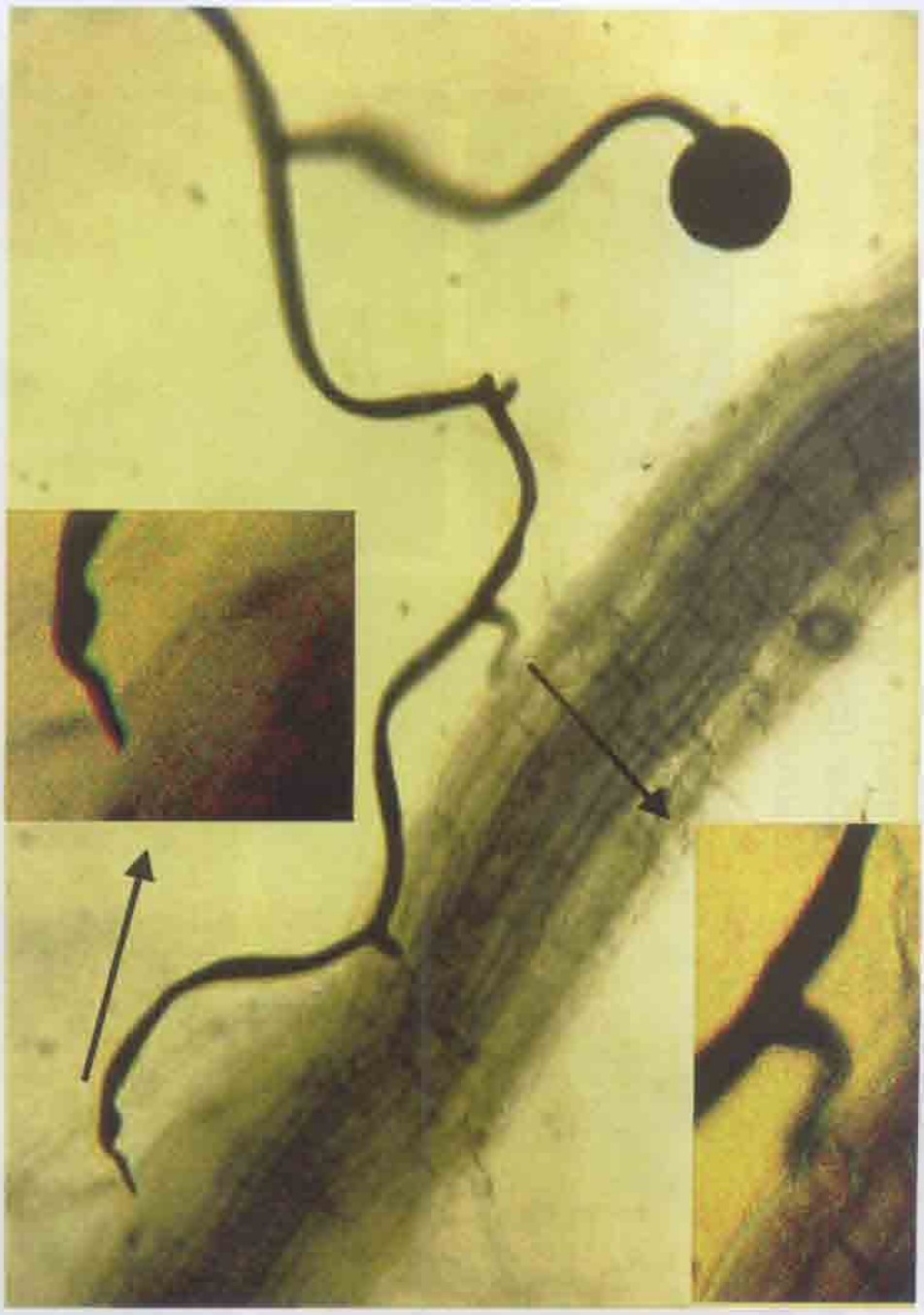


Plate 3: A germinated chlamydospore of *Glomus* sp. on root surface of rice variety ARC 12737 showing penetration hyphae attached to epidermis (Insets- enlarged attachment).

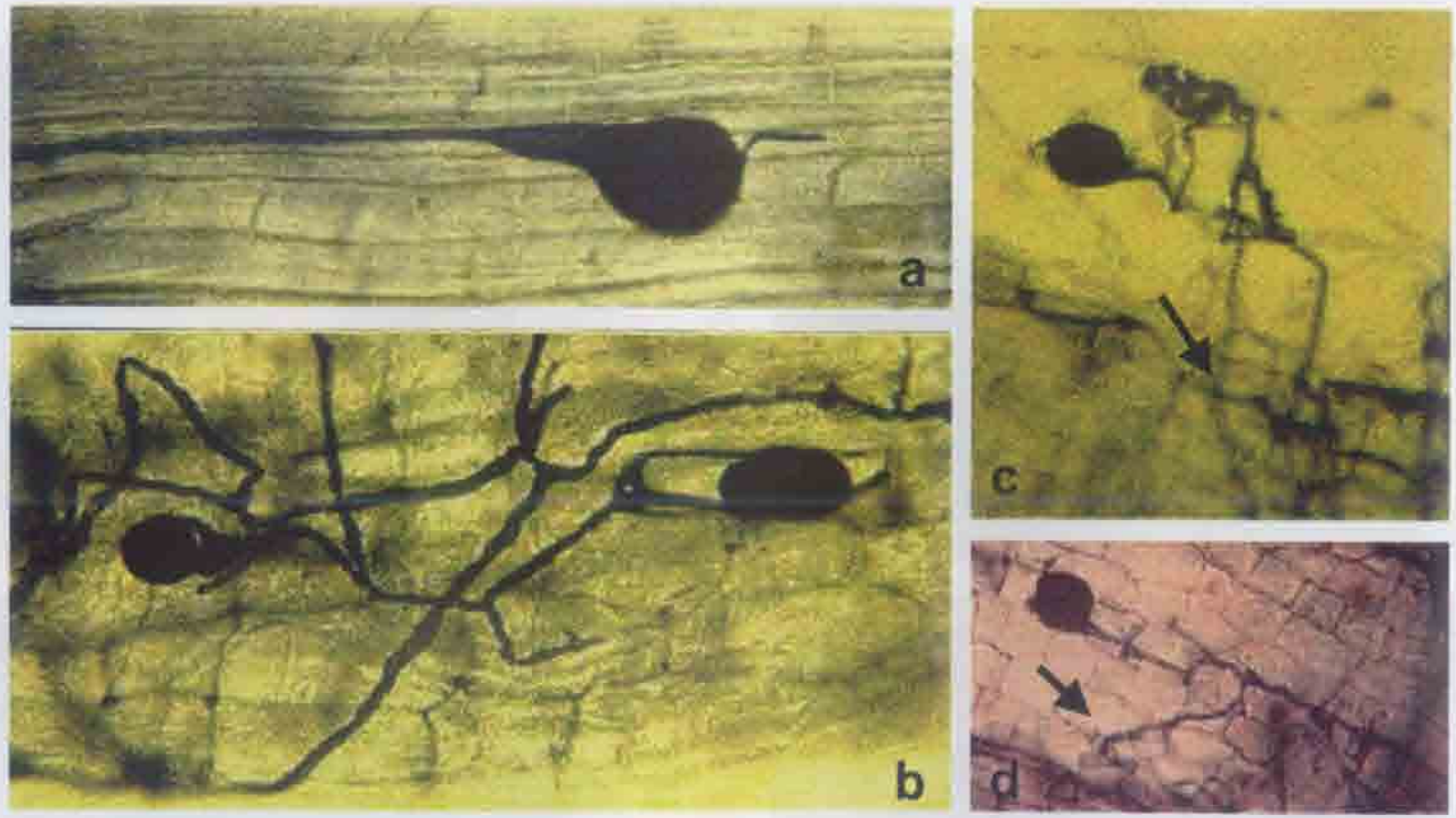


Plate 4: AMF spores and vesicles germinated on root surface of rice vari eties; (a) germinated azygospore of *Gigaspora*, (b) two vesicles forming colonization hypha on root surface, (c & d) germinated *Glomus* spores on root surface forming infection hyphae (→)

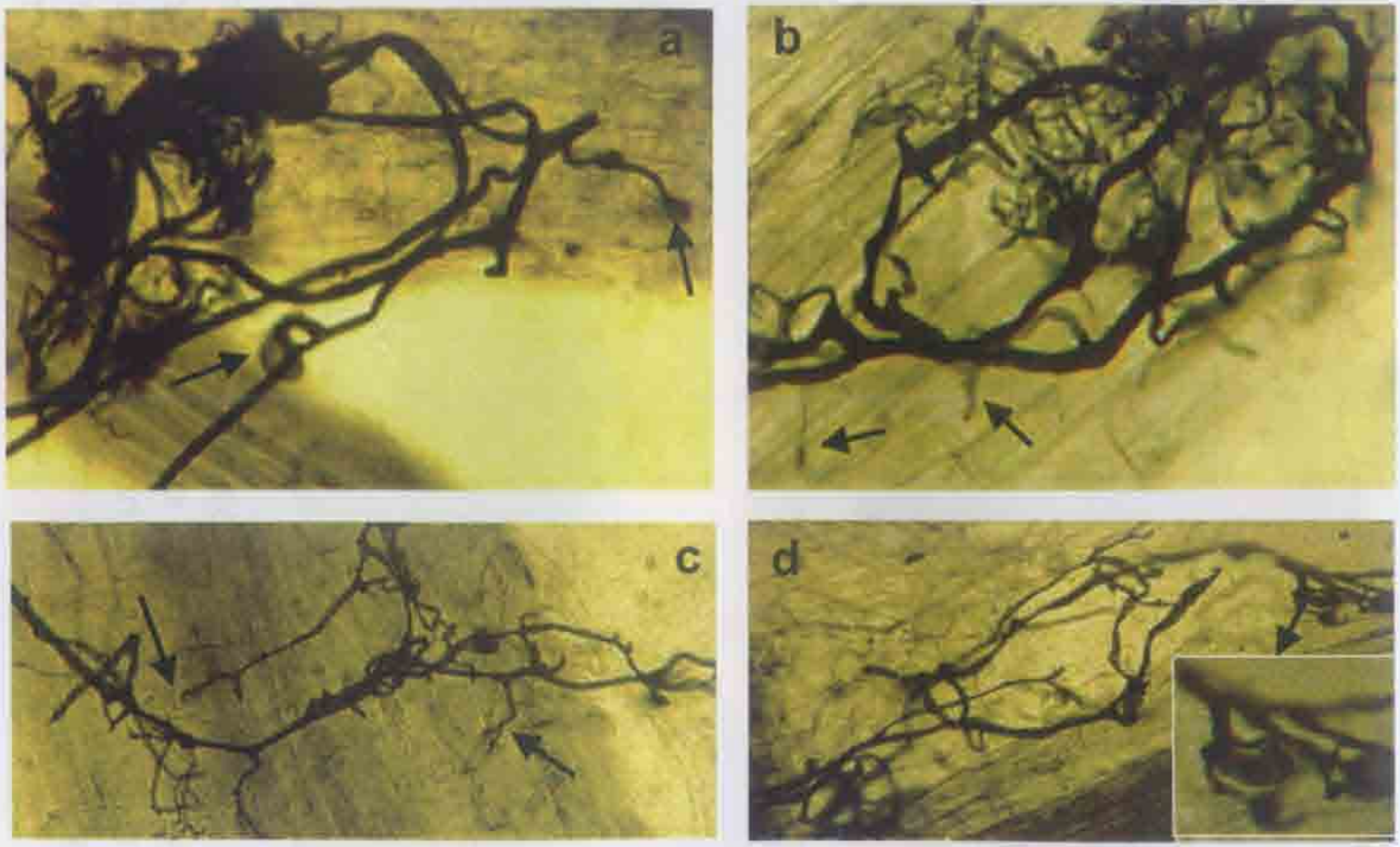


Plate 5: Colonization hyphae on root surface making penetration attempts.

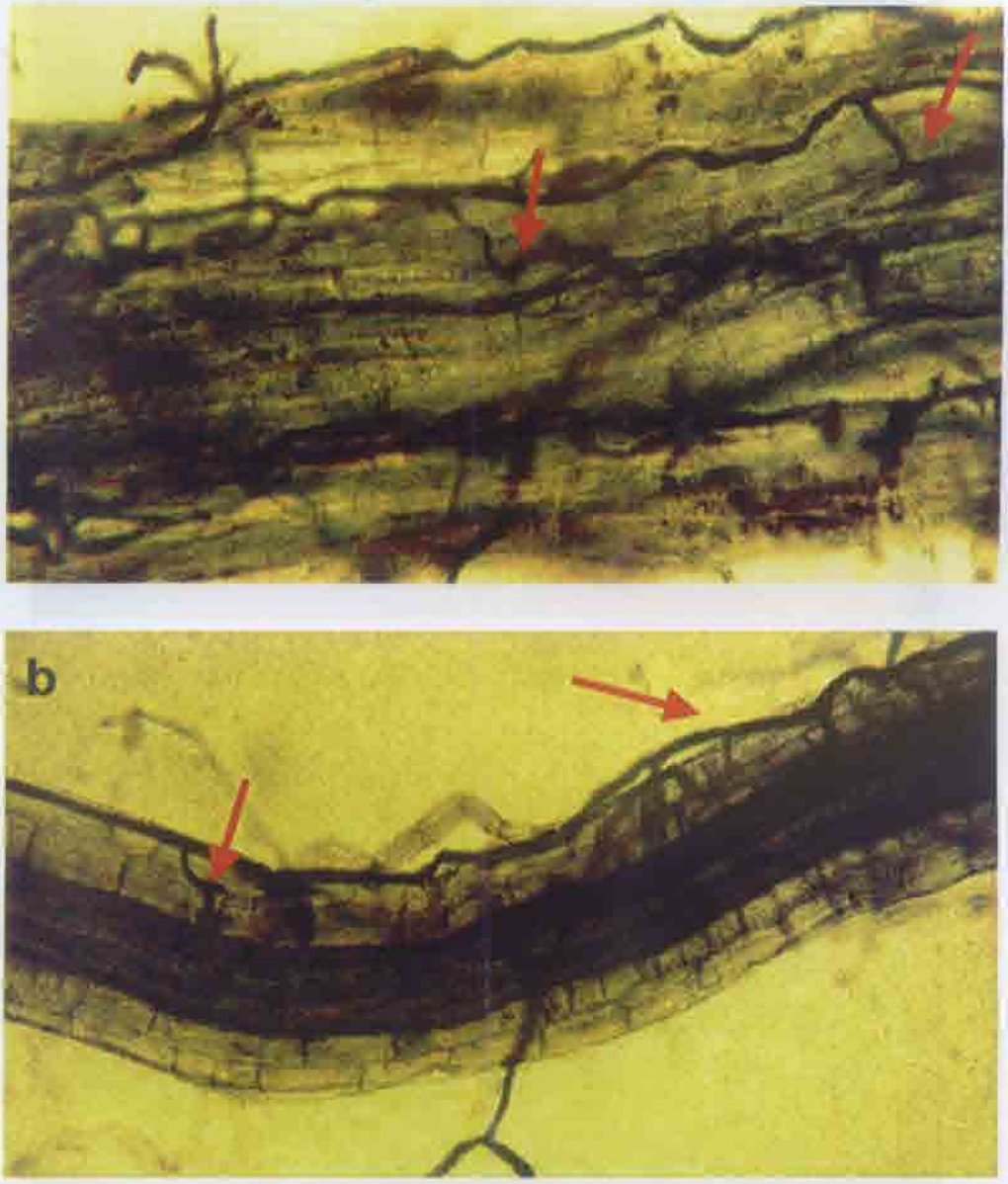


Plate 6: Surface colonization of root prior to penetration; (a) AMF hypahe running length wise on root surface forming infection hyphae (→), (b) hypha running over epidermis forming infection hyphae at many points (→)

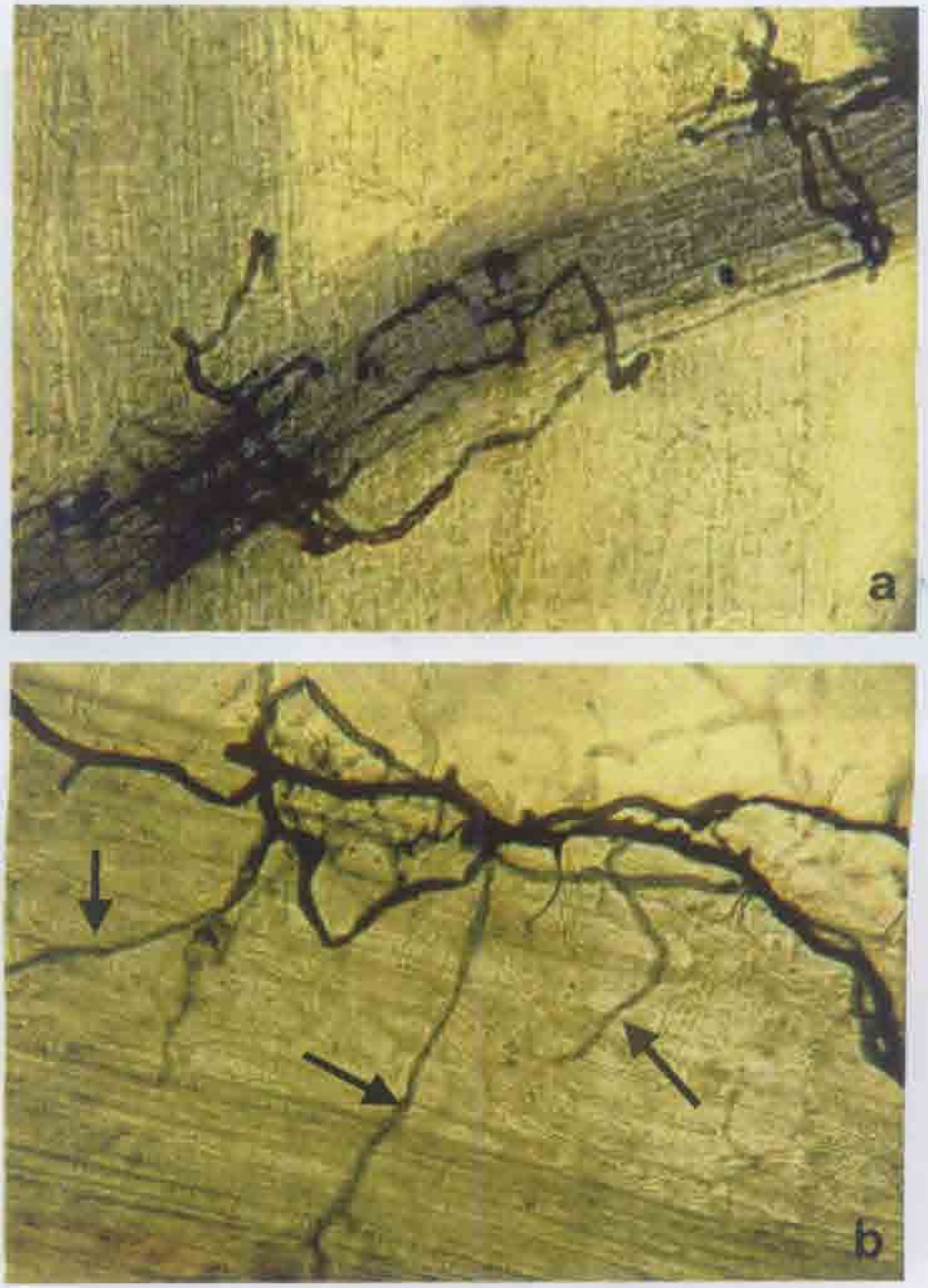


Plate 7 : AMF hyphae colonizing root surface at many points and forming infection hyphae (→), a- surface view, b- enlarged view.

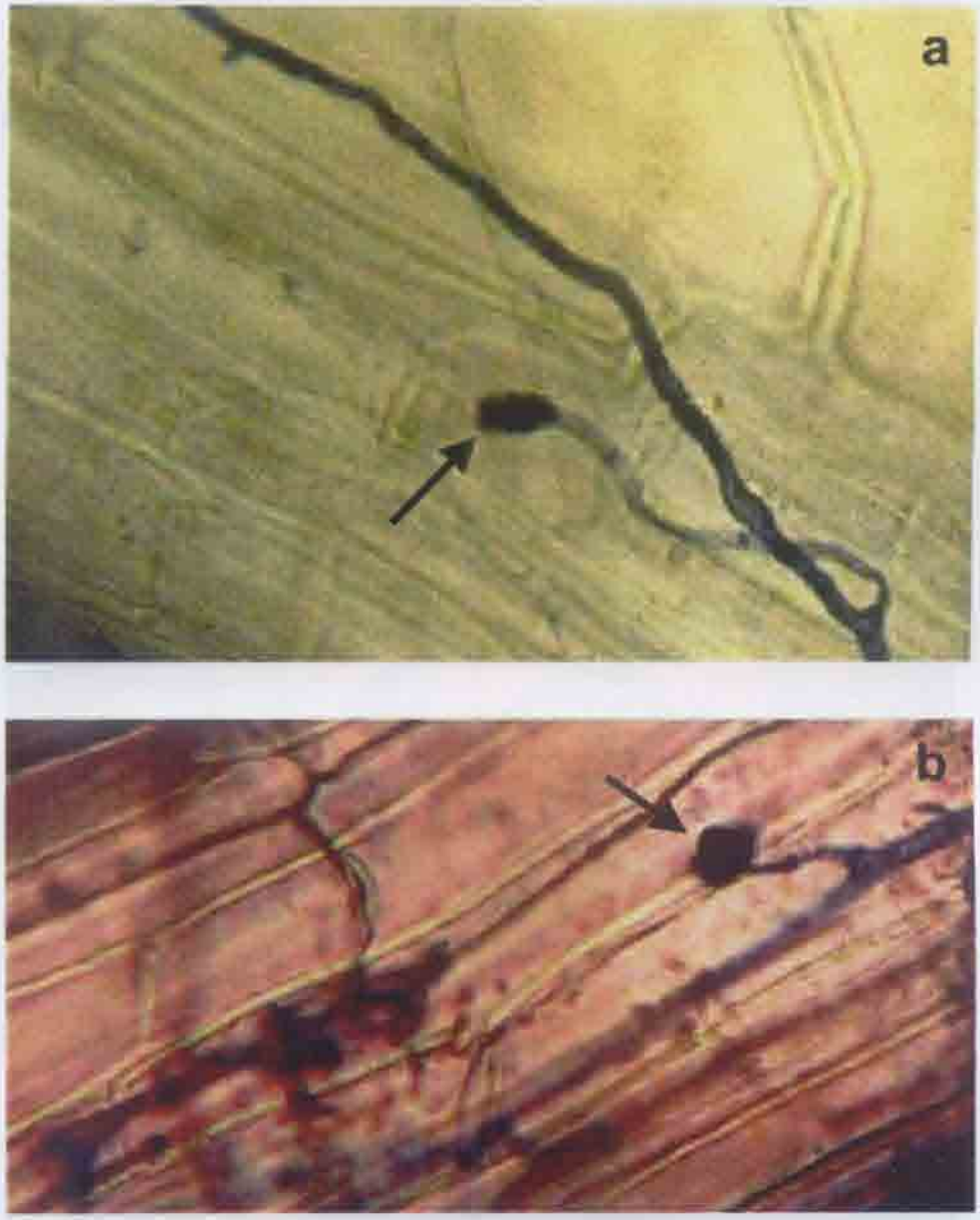


Plate 8: Infection hyphae forming appresoria on root surface (→)

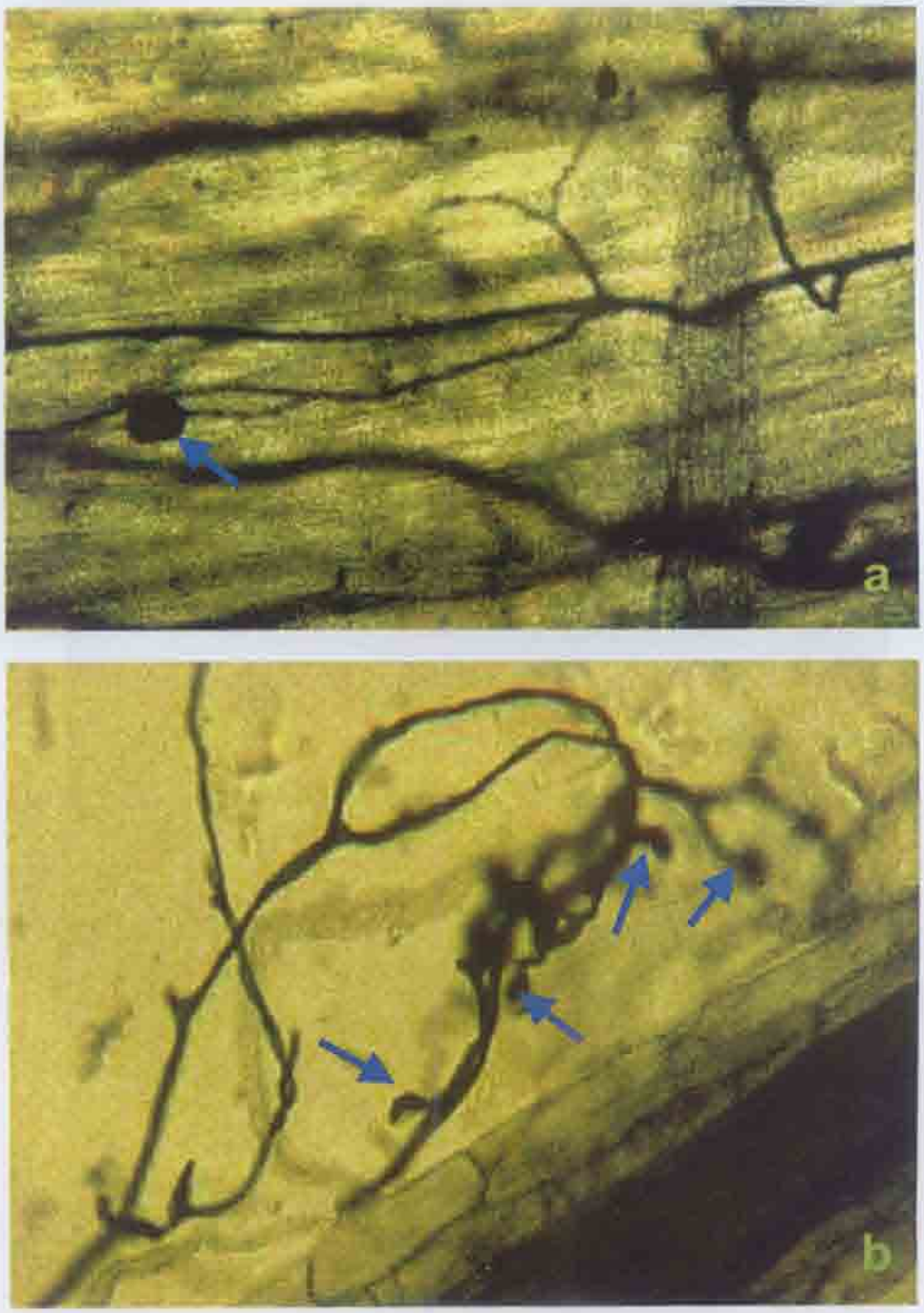


Plate 9 : (a) Appresorium formation on root surface (→), (b) appresoria initiation on tips of infection hyphae

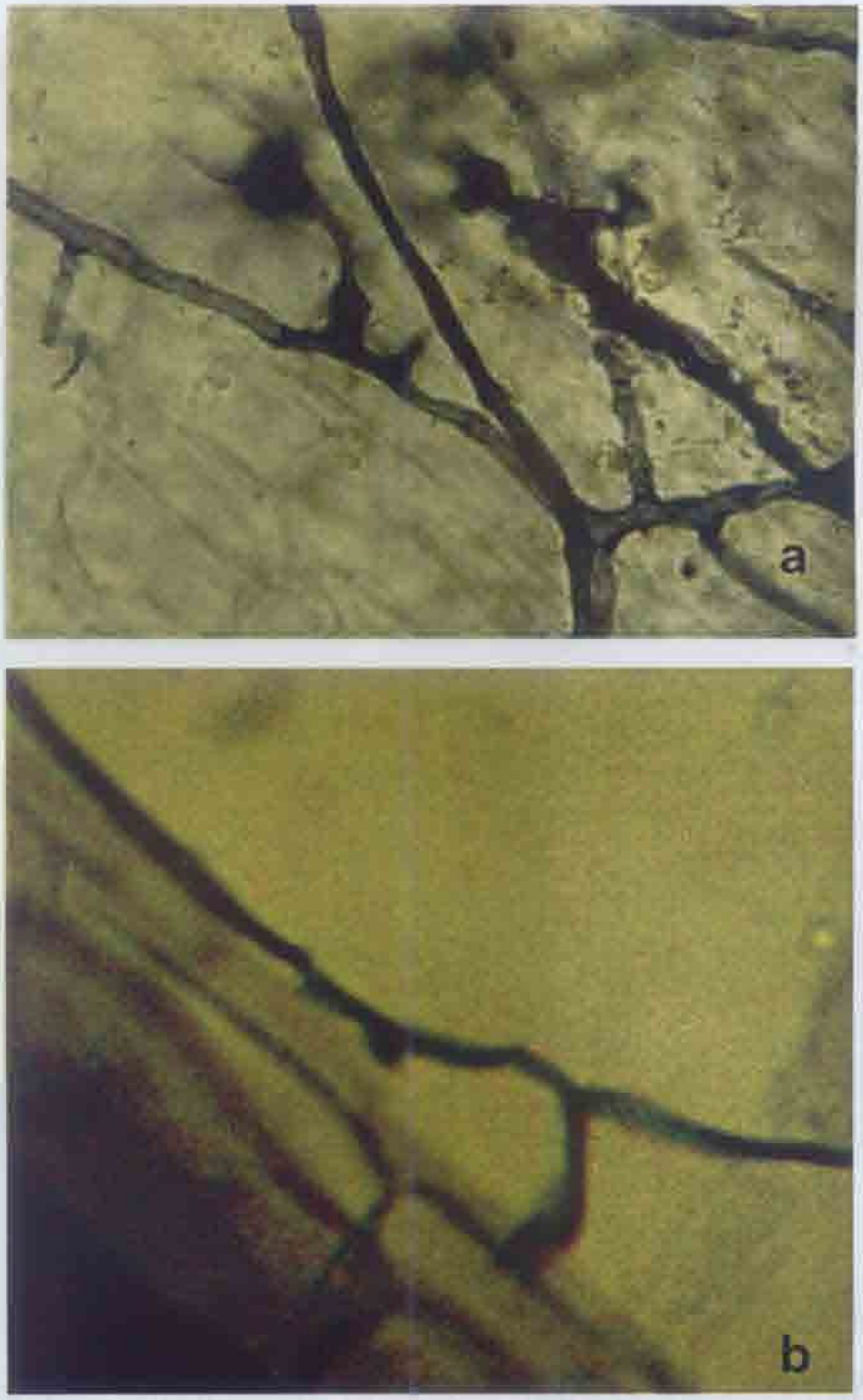


Plate 10: Appresoria formation on root surface; (a) many appresorial attachments from infection hyphae, (b) an appresorium initial in contact with epidermis

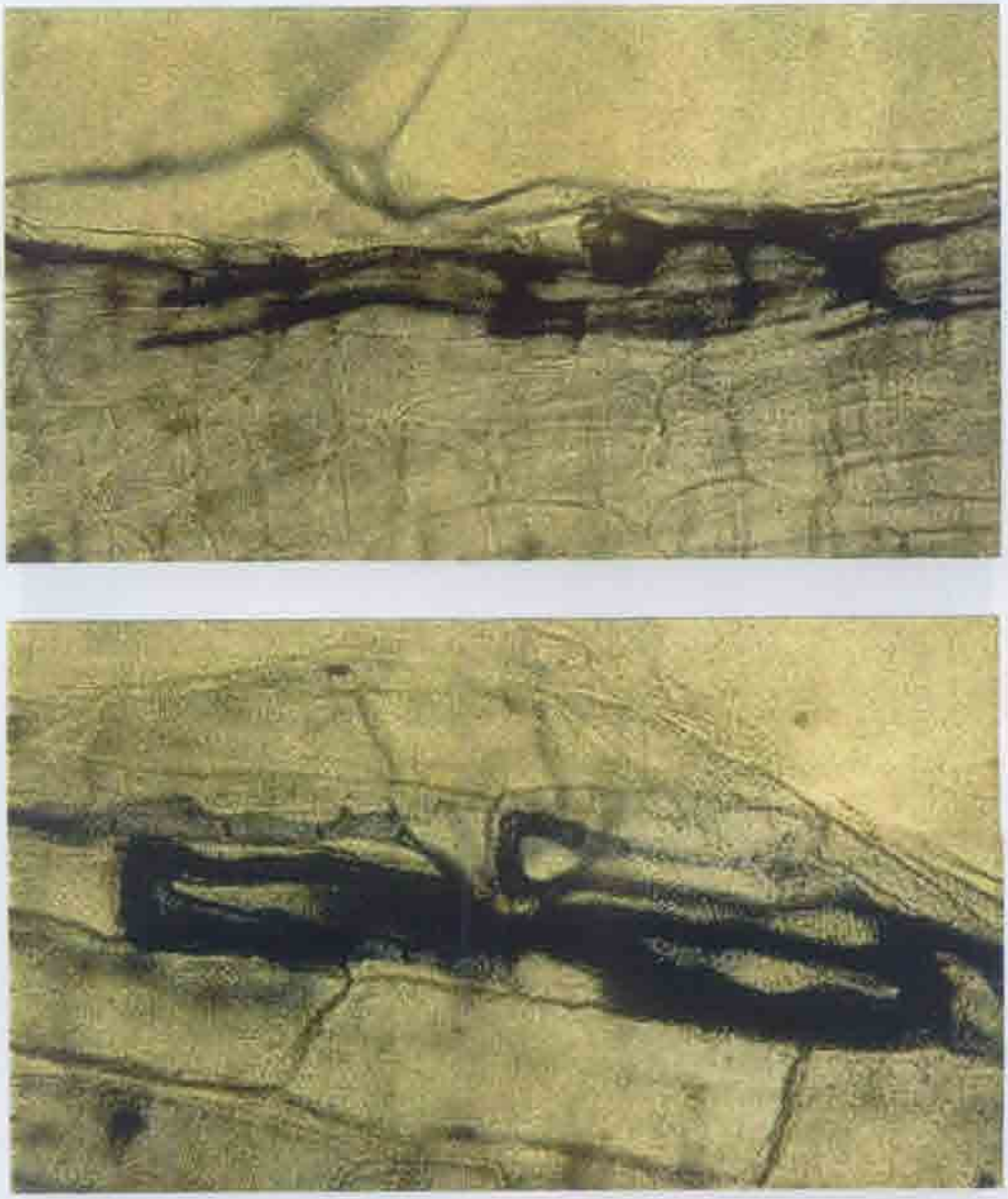


Plate 11: Initial stage of epidermal colonization after penetration showing intracellular hyphae in the hypodermis

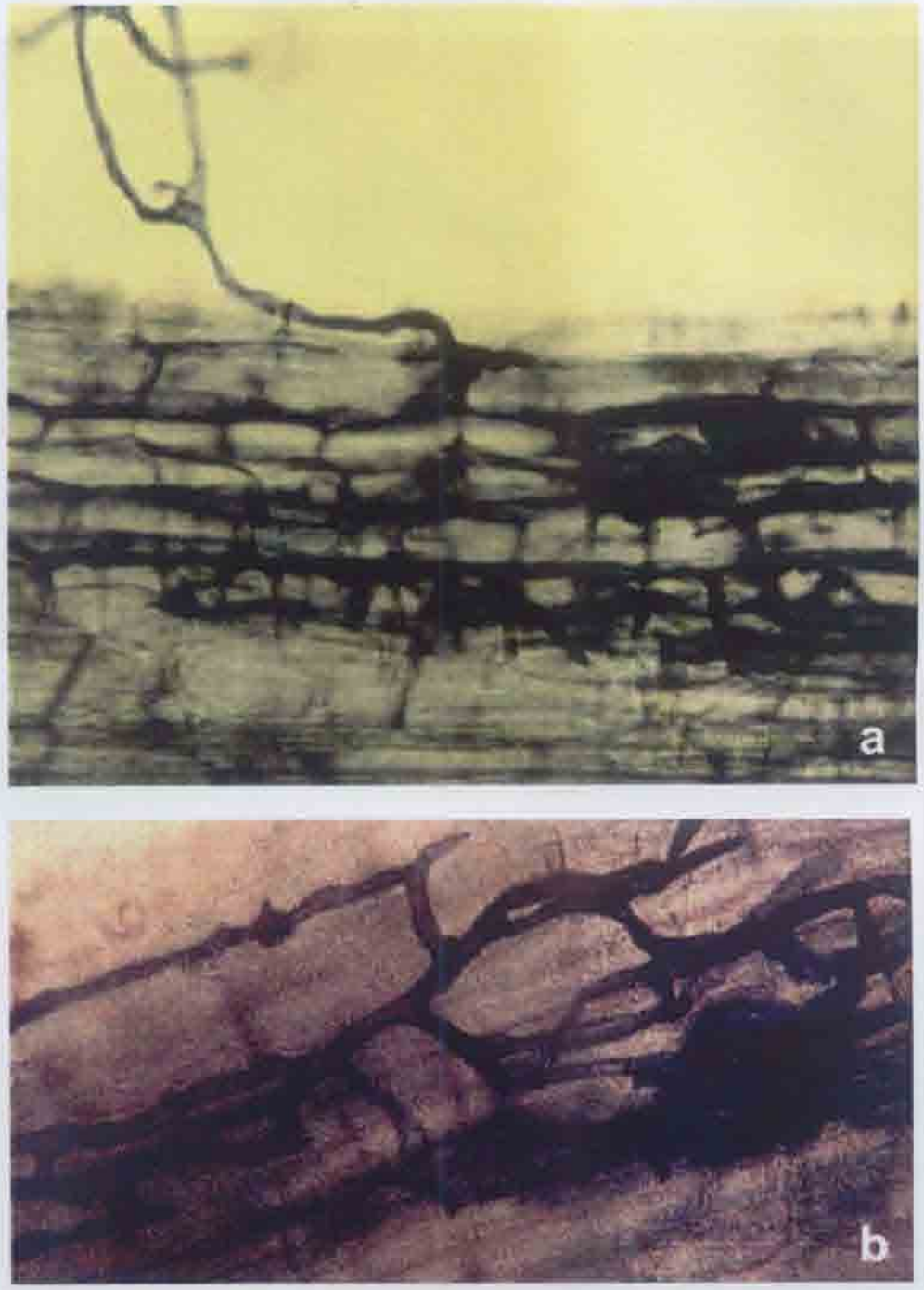


Plate 12: Post-penetration colonization showing hyphae moving through intercellular spaces; (a) surface view, (b) enlarged view.

Plate 13: Post-penetration colonization, intercellular hyphae in the outer cortex

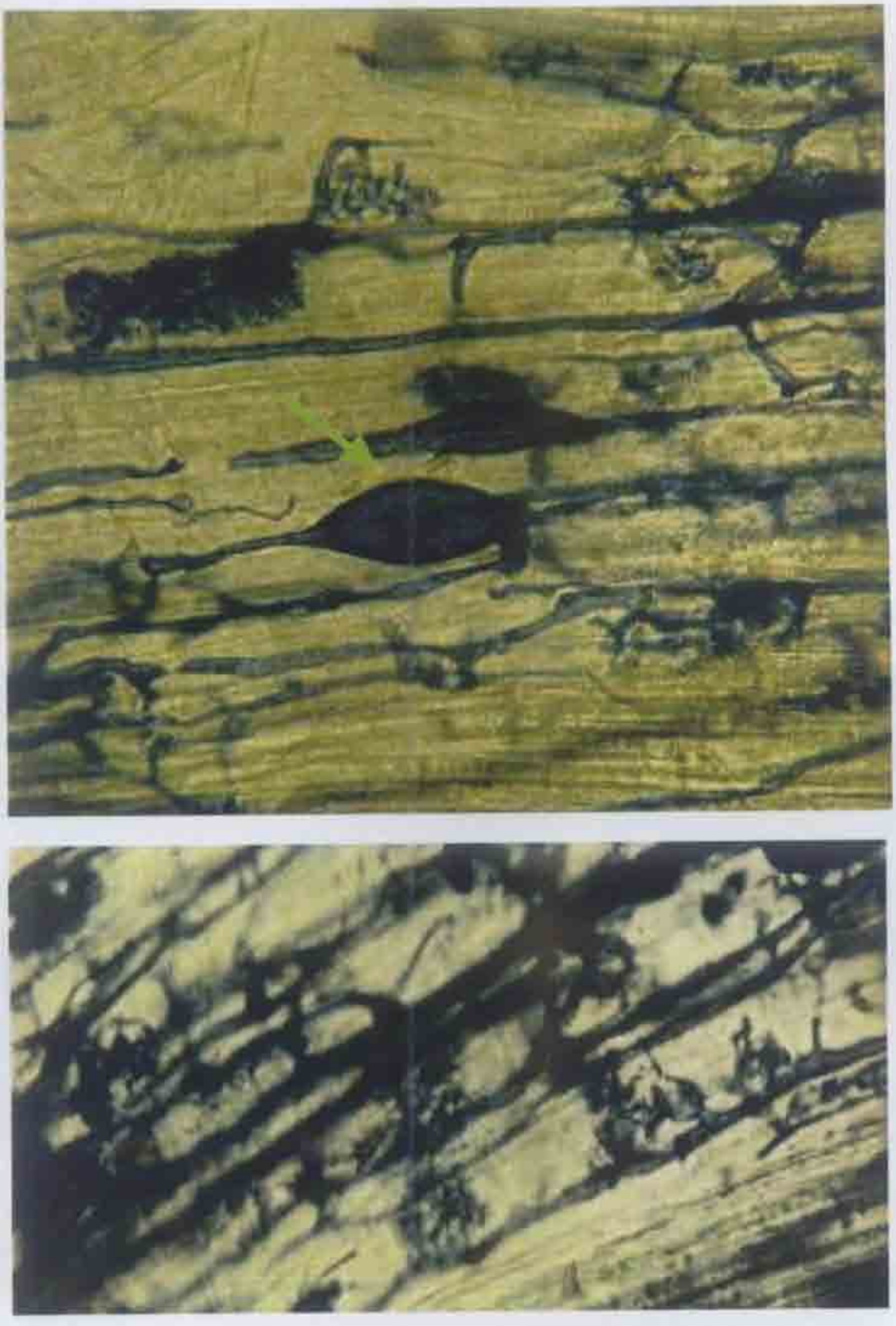


Plate 14: 'Arum' type colonization of cortex showing intercellular hyphae, arbuscule initials and a vesicle (→)

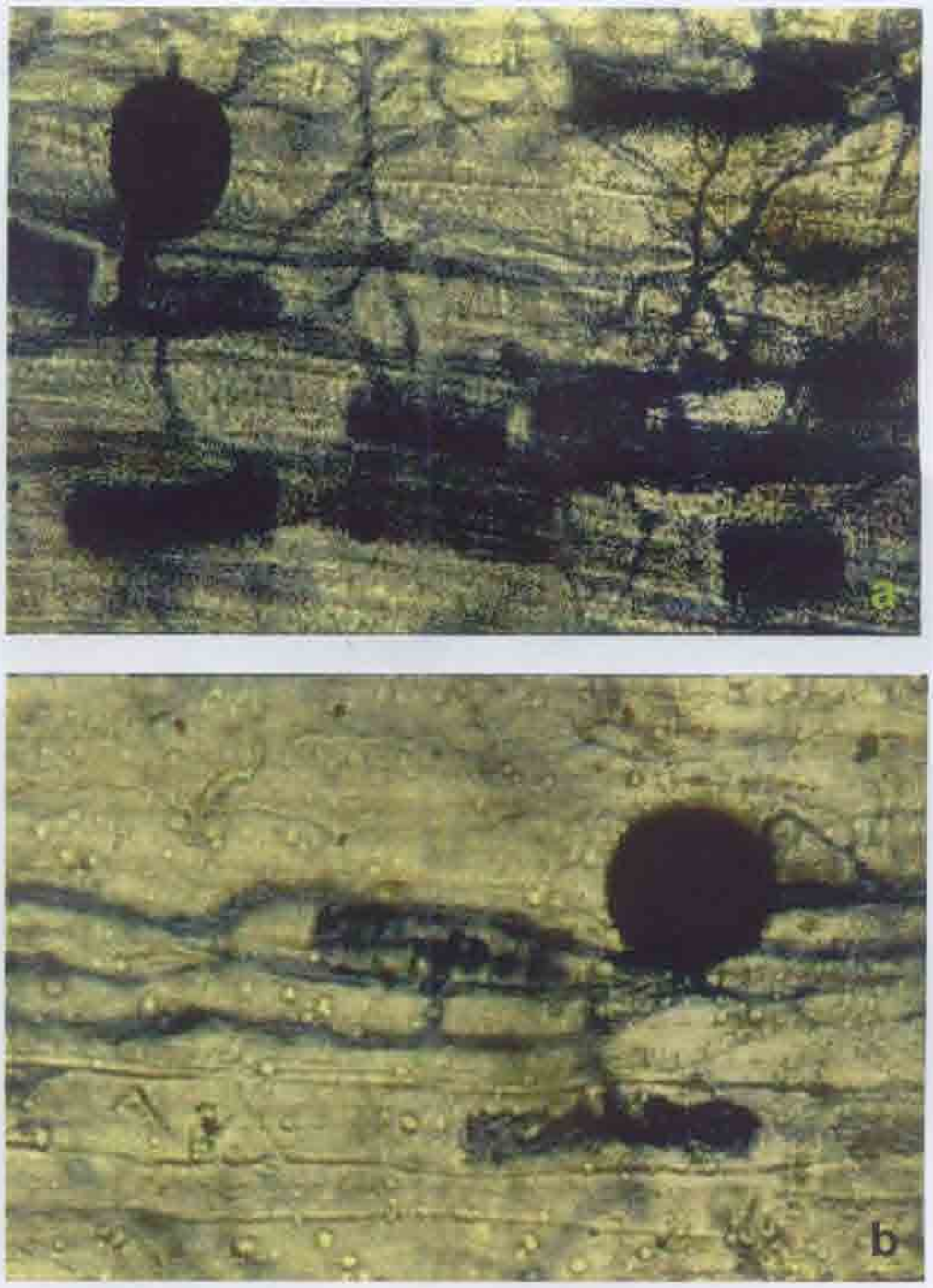


Plate 15: (a) Paris type colonization showing intracellular coils and extramatrical chlamydospores of *Glomus* sp, (b) enlarged view of intracellualr hyphal coil

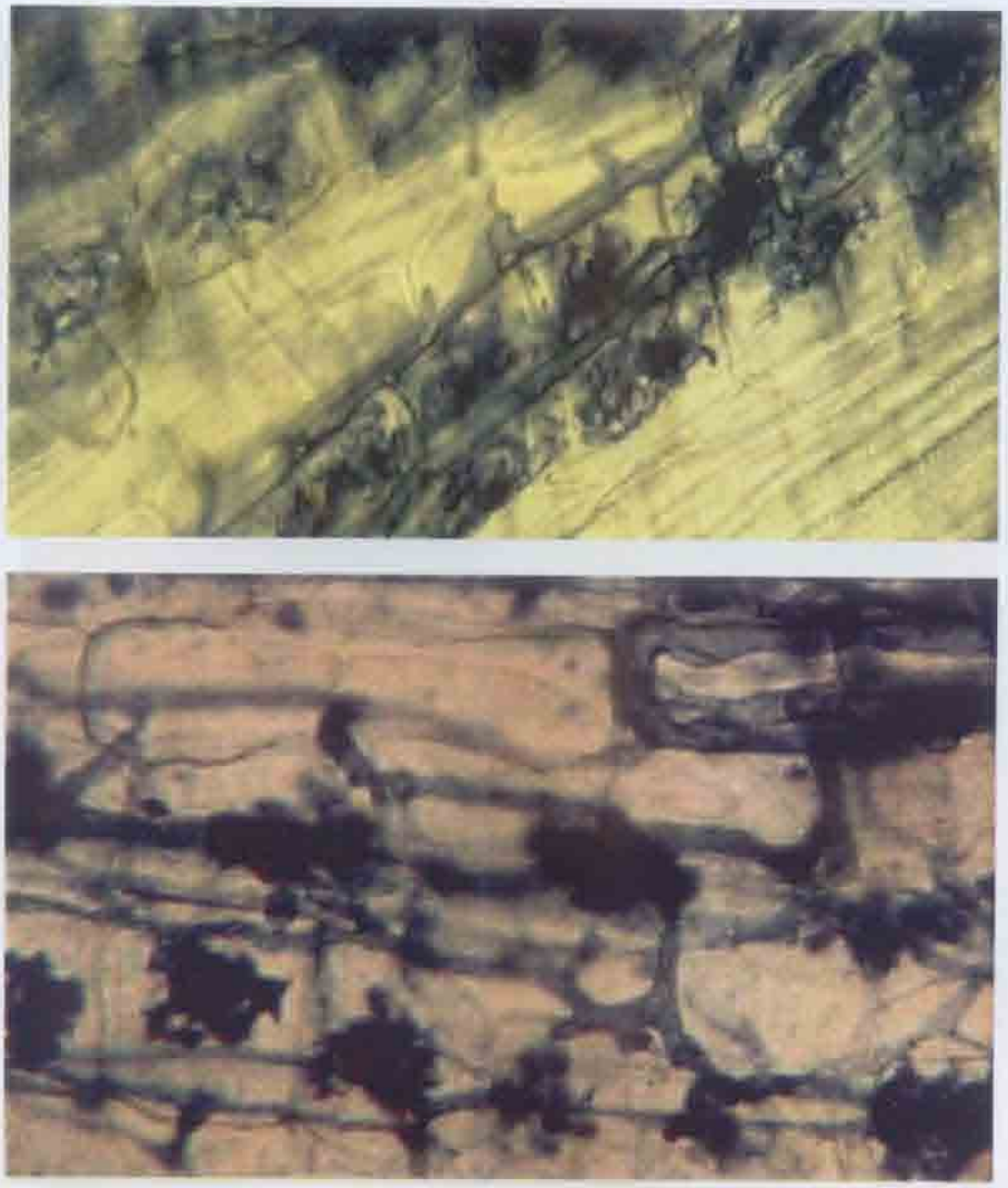


Plate 16: 'Arum' type arbuscules in inner cortex

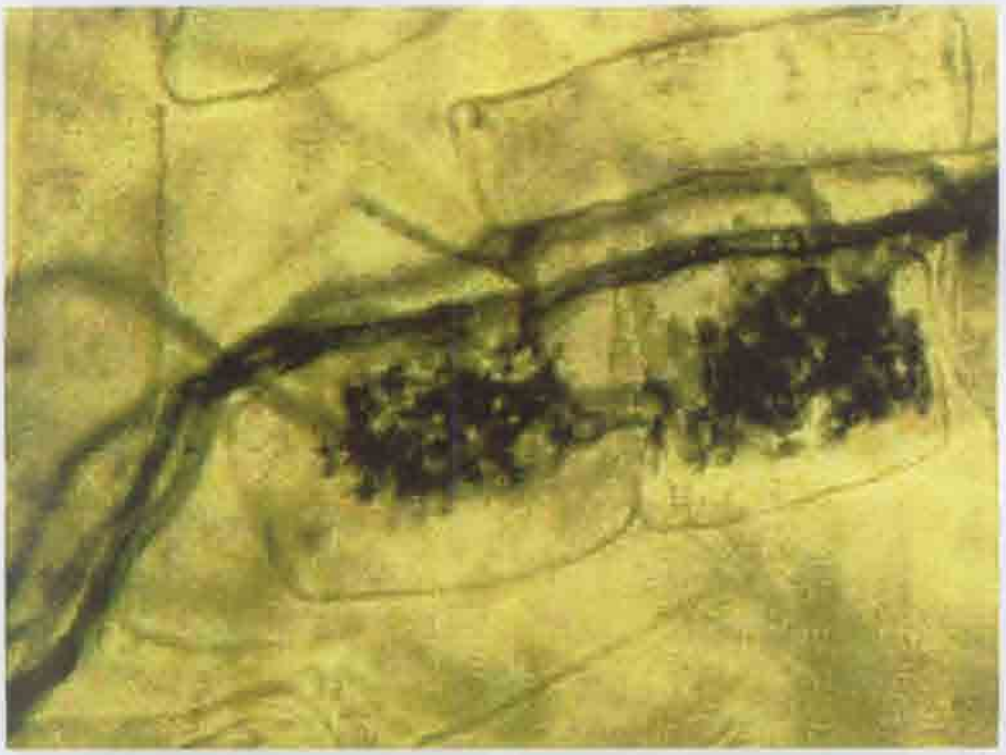


Plate 17: 'Arum type' arbuscule developing from intercellular hypha on terminal Intracellular projections.

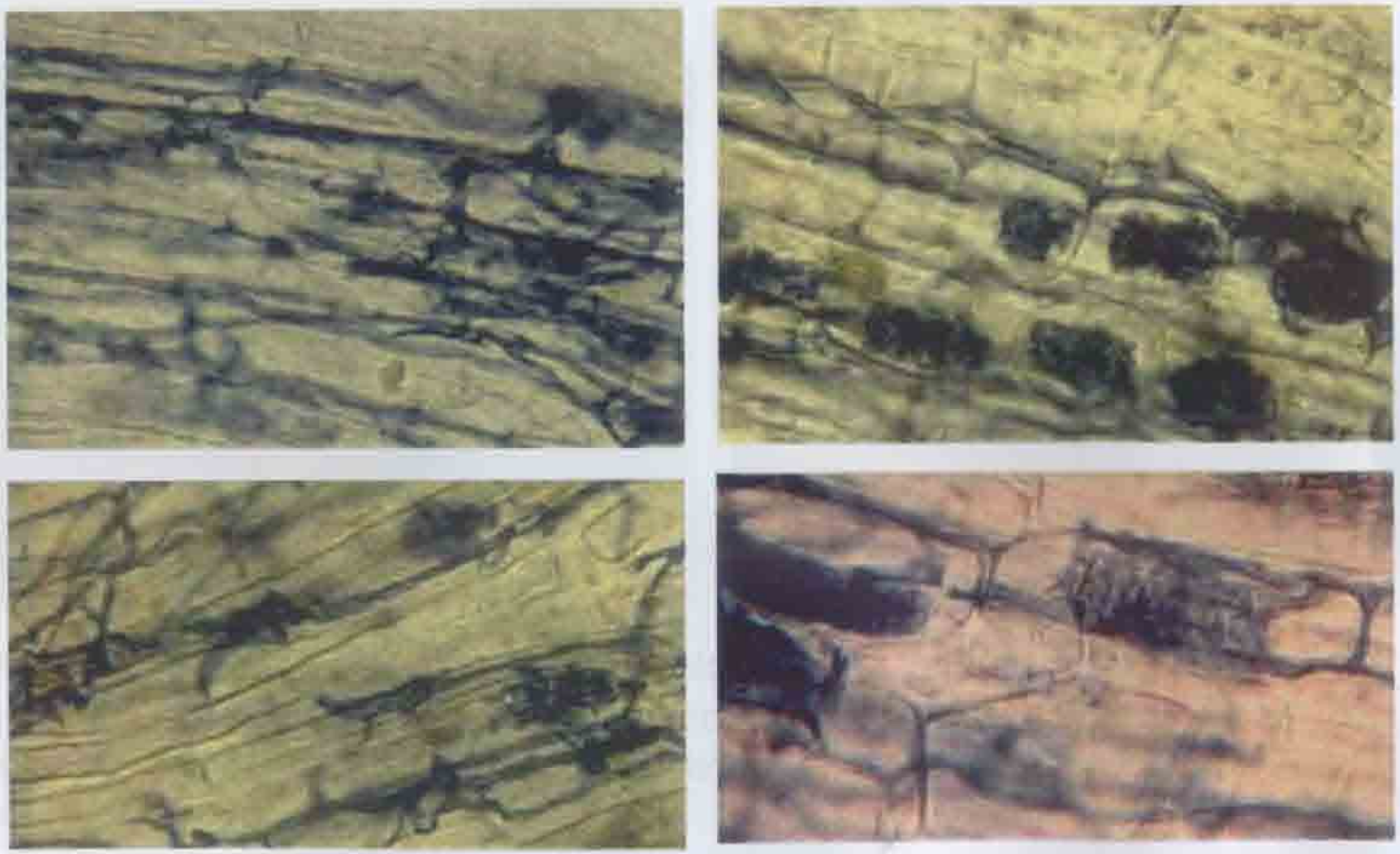


Plate 18: 'Arum type' arbuscule development in the cortex, various stages

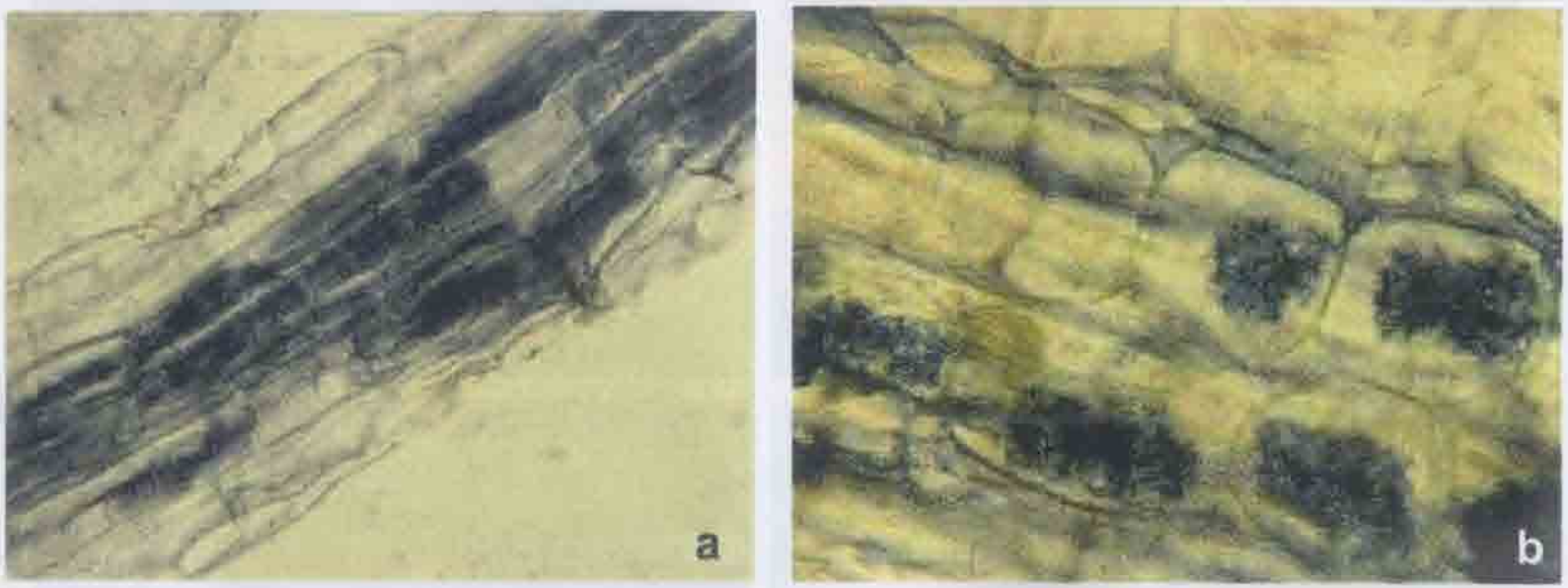


Plate 19: 'Paris' type and 'arum' type colonizations (arbuscules) compared; (a) 'Paris' type colonization forming predominantly intracellular coils by cell to cell penetration, (b) 'Arum'type colonization forming terminal arbuscules from intercellular hypahe

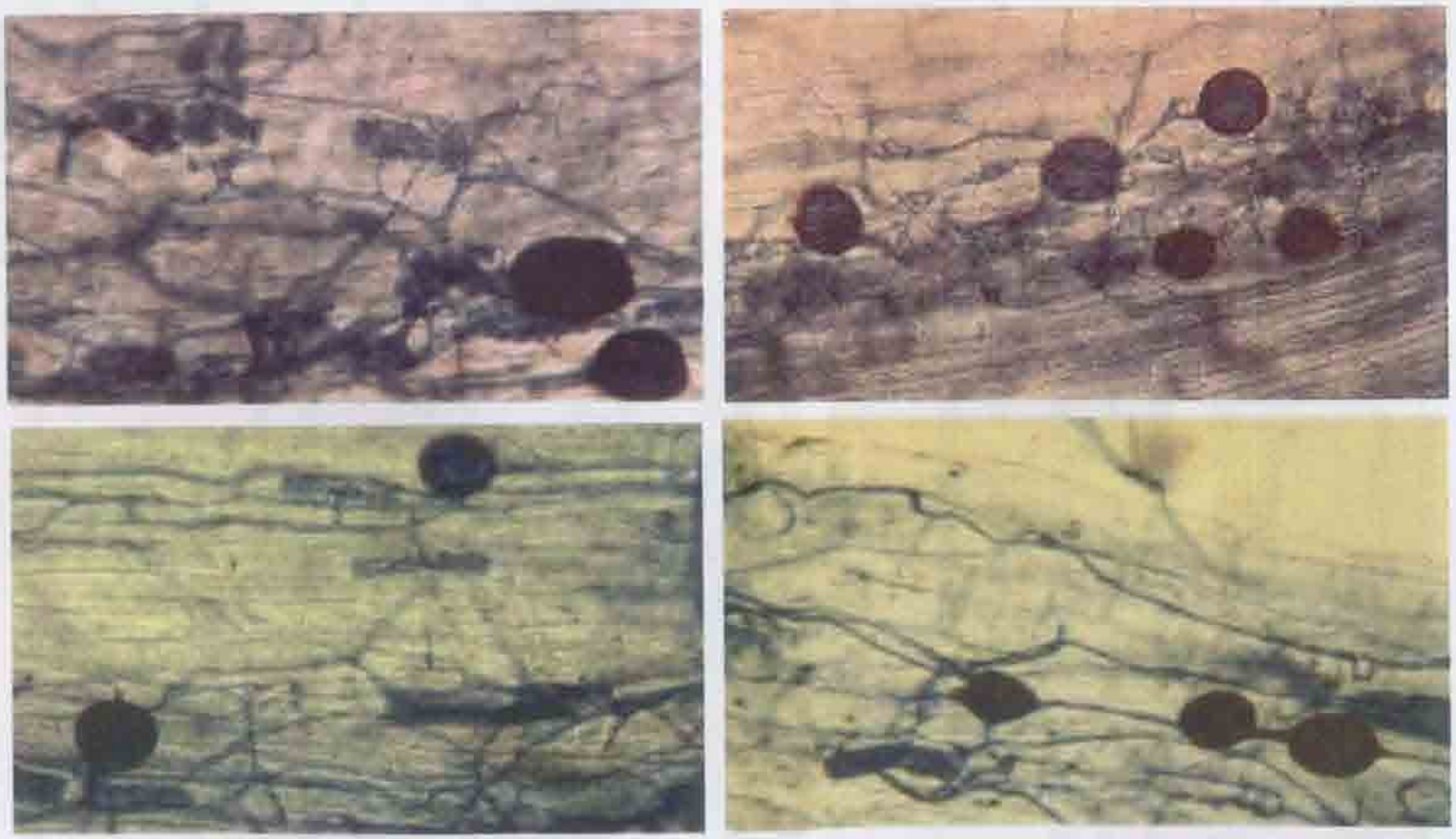


Plate 20: Surface view of vesicles developing in the cortex

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