Table 1: AMF colonization intensity – pre- and post infection responses of the rice varieties between 10 days after seed germination in inoculated soil

Features	*4 low/ 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; Table t at 3 df - 0.05 *p* 3.182, 0.01*p* 5.841

Table 2: Pre-penetration AM structures on root surface of differently dependent rice varieties between 10 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± .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± .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 2. 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 of 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 3) 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 3: AMF penetration response of differentially AM responsive varieties at 10 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 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 4) 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 4: Post-penetration AMF development response of differentially AM responsive varieties at 15 days of seed germination in inoculated soil

Features (by 0-4 point scale)	*Average of 2 low responsive varieties*	*Average of 2 high 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; Table t at 5 df - 0.05 *p* 2.571, 0.01 *p* 4.032

4. Discussion

Results of the previous studies on inter-variety differences in AMF response reviewed by many researchers [8] - [11] and read with the early data on mutational origin of resistance to AMF colonization [12] have shown the possible existence of two sets of independently acting genes behind the functional compatibility of the AMF with their hosts and identification of several novel nutrient transporters has revealed some of the cellular processes that underlie symbiosis [13]. There may be one set of genes that control whether or not colonization occurs [14] and another set that controls the degree of benefit derived from the symbiosis [10]. 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 [10] observed that wheat cultivars which do not benefit from AM symbiosis may suffer from growth depressions. Hetrick *et al*. (1995) [15] 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.

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 [16] and the recently described low AMF penetration, and low response mutant phenotype of pea which has low nodulation phenotype also [17]. 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 cannot be overruled among the plant species.

5. Future Scope of Study

Results of the investigation have helped in identification of physiological correlates of variable of AM responsiveness or dependency of rice, chosen as the model plant, in a low nutrient soil and have provided a base for further genetic studies relating to selection and breeding of high mycorhiza responsive varieties for low input cultivation with arbuscular mycorrhiza. Results of the studies on inter-variety differences in AMF response have provided basis for further study on the presence of two sets of independently acting genes behind the functional compatibility of the symbionts and the role of elicitor – receptor in the host – parasite interactions.

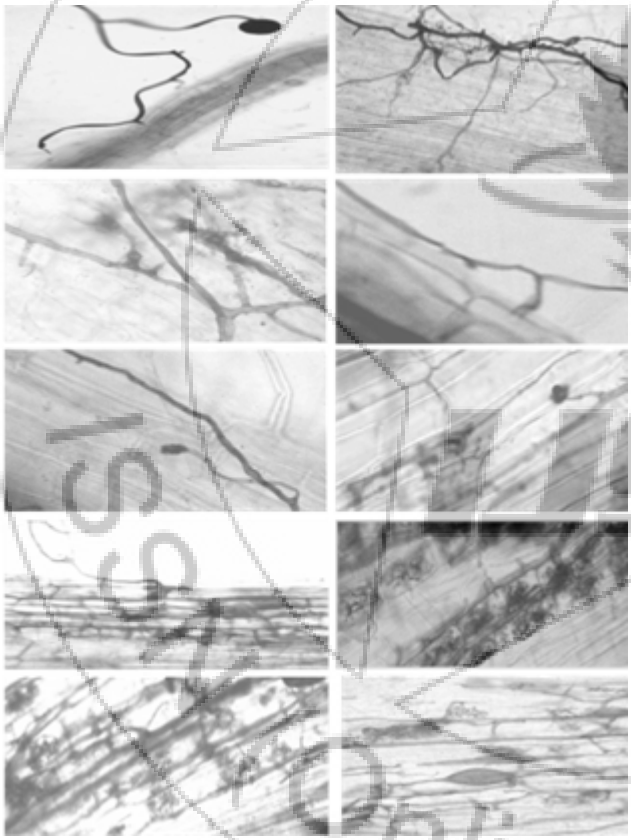

Plate 1: Microscopic observation of surface colonization, appresoria formation, penetration, post-penetration colonization, arbuscule and vescicle formation in rice root by AMF

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