Plant Physiology and Biochemistry 48 (2010) 893–902

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Symbiotic interactions between arbuscular mycorrhizal (AM) fungi and male papaya plants: Its status, role and implications

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ARTICLE INFO

Article history:
Received 7 January 2010
Accepted 18 August 2010
Available online 25 August 2010

Keywords:
AM fungi
Male *C. papaya*
Root colonization
Root phosphatases

ABSTRACT

Experiments were conducted to study the arbuscular mycorrhizal (AM) status and its role in P-uptake through assay of root phosphatases activities in four varieties of male *Carica papaya* L. viz. CO-1, CO-2, Honey Dew and Washington during flowering stages. In the present study, mean total root colonization of AM fungi recorded peak increase in flowering stage-II while mean root phosphatase (acid and alkaline) activities recorded peak increase in flowering stage-I. Unlike root colonization and root phosphatase activities, spore density did not exhibit any definite patterns and recorded a narrow range of fluctuation during different flowering stages of male *C. papaya*. The study brought out the fact that root colonization and spore density of AM fungi along with root phosphatase activities varied significantly within the four varieties of male *C. papaya* plants during each flowering stage. The study also recorded consistently higher acid root phosphatase activity than alkaline root phosphatase activity under P-deficient, acidic soil conditions during all flowering stages of male *C. papaya* plants. Studies revealed that the root colonization of AM fungi influenced root phosphatase activities (acid and alkaline) positively and significantly during all flowering stages of male *C. papaya* plants. A total of twelve species of AM fungi belonging to five genera viz. *Acaulospora*, *Dentiscutata*, *Gigaspora*, *Glomus*, and *Racocetra* were recovered from the rhizosphere of male *C. papaya* plants.

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1. Introduction

Carica papaya L. belongs to family *Caricaceae*. The genus *Carica* has about 48 species of which only *C. papaya* is grown for its edible fruits. *C. papaya* is a polygamous plant and has many sex forms. The three basic sex types are: staminate or male, hermaphrodite and pistillate or female. In India, large numbers of varieties are found for cultivation. This variability is seen due open pollination and indiscriminate multiplication using open pollinated seeds. In *C. papaya* there are two basic types of varieties. Those varieties, that produce only female and male plants are said to be *dioecious* and those that produce female and hermaphrodite plants are called *gynodioecious*. Some of the important gynodioeciuos varieties are Coorg Honey Dew, Sunrise Solo, CO-7 and Surya. The varieties like CO-1, Washington and Pusa Dwarf come under dioecious types [26].

Interestingly, India is the one of the largest producer of *C. papaya* in the world after Brazil with production (in thousand metric tones) up to 1500 [26]. It is cultivated on commercial scale in states of Karnataka, Bihar, Gujarat, Kerala, Tamil Nadu, Andhra Pradesh, Maharashtra and Madhya Pradesh. In fact in southern region, the area under cultivation has increased by 24.37% whereas the production has increased from 1.65% to 32.04%. However, in Goa, *C. papaya* cultivation is mostly confined to kitchen garden and along the bunds due to constraints in productivity resulting from fungal and viral diseases. Therefore at present, most of the demand for *C. papaya* is met by importing the fruit from neighbouring states [26].

The wide spread symbiotic arbuscular mycorrhizal (AM) fungal association with host plants is gaining importance due to the fact that they help in plant productivity. The AM fungi were found to be associated with different varieties of fruit bearing *C. papaya* in agro-based ecosystem of Goa, India. Here, the root colonization by AM fungi ranged from 26 to 77%, while the spore density ranged from 100 to 236 spores.100 g of rhizosphere soil^{-1} [30]. The AM fungi also reported seasonal variation in the rhizosphere of fruit bearing *C. papaya* from Goa, India [28]. Further, AM fungi were spatially [29] and spatio-temporally [33] distributed in rhizosphere of fruit bearing *C. papaya* plants in Goa, India. So, far AM fungi belonging to the genera *Acaulospora*, *Glomus*, *Gigaspora*, *Dentiscutata* [30], *Cetraspora* and *Racocetra* [33] were found to be associated with fruit bearing *C. papaya* in Goa, India. *C. papaya* is known to exhibit a strong growth response to colonization by AM fungi. Inoculation of vegetative *C. papaya* with AM fungi, under greenhouse conditions

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doi:10.1016/j.plaphy.2010.08.010

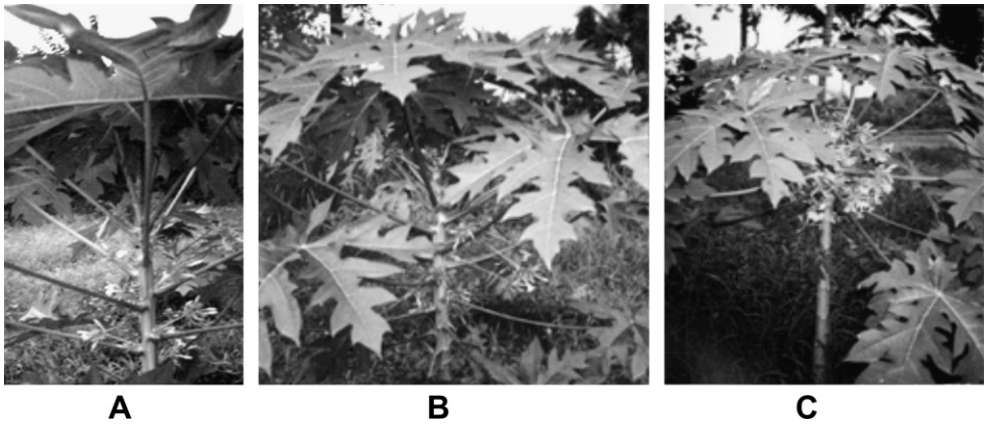

Plate 1. A) Flowering stage-I of male *C. papaya*. B) Flowering stage-II of male *C. papaya*. C) Flowering stage-III of male *C. papaya*.

significantly increased all the growth parameters viz. plant height, stem girth, leaf area and root length [31]. Similarly, vegetative mycorrhizal *C. papaya* plants exhibited significant increase in fresh and dry weights of shoot and root systems [31]. Further studies revealed that total potassium and total phosphorus content was higher in vegetative mycorrhizal *C. papaya* plants as compared to non-inoculated controls [32].

So, the AM fungi can enhance plant uptake of inorganic phosphorus from soil through hyphal scavenging of soil volumes that are not accessed by roots [23]. The fungi exploits the same pools of inorganic phosphorus that are exploited by roots [5], but bypass the slow process of diffusion by translocating soluble inorganic phosphorus from non-rhizosphere soil to their host through extraradical hyphae [45]. However, when the plants grow under P-deficient conditions, plant roots secrete organic acids and acid phosphatases [52]. Mycorrhizal colonization could also alter inherent phosphorous supply by either increasing the activity of alkaline phosphatase in roots and in the rhizosphere [10] or by reducing acid root phosphatase activity [1]. Since prior studies on *C. papaya* plants with respect to AM fungi have only focused on vegetative [31,32,34] and fruit bearing plants [28–30,33], in present study AM status and root phosphatase activities of *C. papaya* was investigated under field conditions in flowering male plants. The study was carried out to test the hypothesis, that, whether to compare the activity of root phosphatase enzyme and root colonization of AM fungi in male *C. papaya* plants and relate their roles in supply of nutrients to host plants.

2. Results

2.1. Edaphic factors

Data on edaphic factors recorded during the different flowering stages of male *C. papaya* plants revealed that the soil pH was acidic (4.6–6.0), while electrical conductivity was satisfactory (0.07–0.28 mmhos cm^{-1}). Available phosphorus recorded low levels (4 kg ha^{-1}) in the rhizosphere of male *C. papaya* throughout the study except for few instances during flowering stage-III (12 kg ha^{-1}) and flowering stage-IV (25 kg ha^{-1}) whereas, total nitrogen content of the soil was low (0.28–1.0%). Potassium levels were mostly ranging from optimum (58 kg ha^{-1}) to high (200 kg ha^{-1}). Micronutrients in general were present in high concentrations.

2.2. Root colonization of AM fungi

Four stages of host development viz. flowering stage-I (Plate 1A), flowering stage-II (Plate 1B, Plate 2A), flowering stage-III (Plate 1C) and flowering stage-IV (Plate 2B) were considered for the study. All the root samples collected during the study period exhibited the presence of AM colonization which was characterized by the presence of hyphae, hyphal coils, arbuscules (Plate 3A) and vesicles (Plate 3B). Arbuscular mycorrhizal colonization was prevalent during the study period. Data on total root colonization and root length colonized by hyphae, arbuscules and vesicles of AM fungi in different varieties of male *C. papaya* flowering $stage^{-1}$ is represented in (Fig. 1). The data on average values for total colonization and root length colonized by hyphae, arbuscules and vesicles during different flowering stages but irrespective of varieties of male *C. papaya* is represented in (Table 1). It was observed that the root colonization of AM fungi varied within the varieties at any particular flowering stage of male *C. papaya* plants.

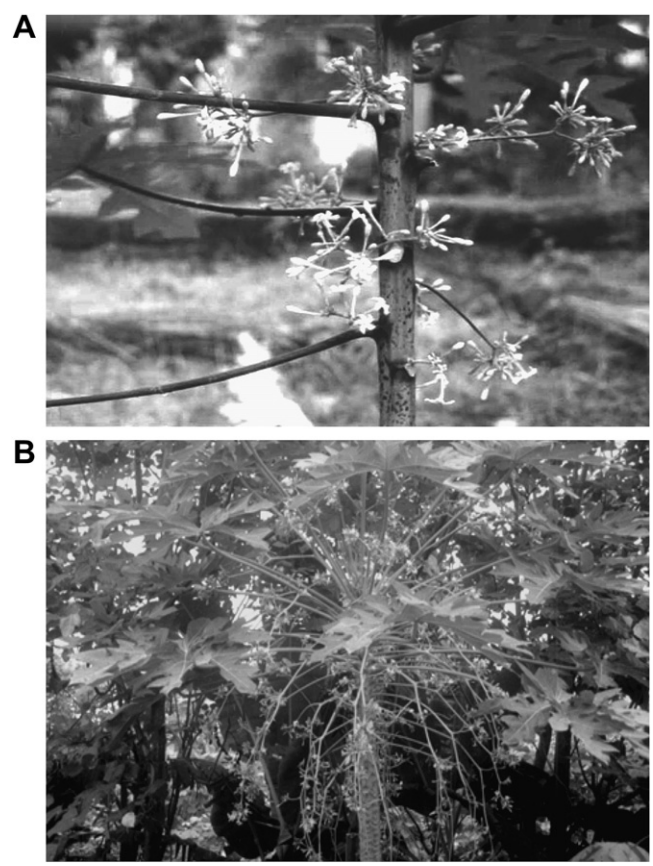

Plate 2. A) Magnified view of flowering stage-II of male *C. papaya*. B) Flowering stage-IV of male *C. papaya*.

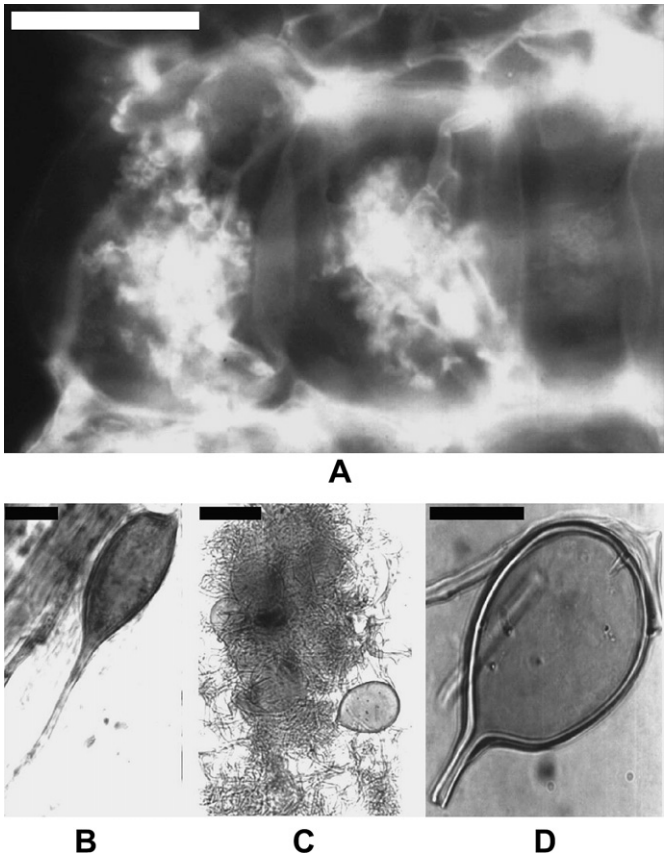

Plate 3. A) Arbuscular colonization in Honey dew variety (Bar = 50 μm). [Note hypha entering the host root cell and repeatedly branching to form haustoria like structure in host root cells]. B) Single ellipsoidal vesicle of AM fungi (Bar = 50 μm). C) Spores of *Glomus coremioides* enclosed within peridial hyphae (Bar = 50 μm). D) Single spore of *Glomus coremioides* (Bar = 50 μm). [Note the absence of septum at the spore base].

In flowering stage-I, values for hyphal, arbuscular, vesicular and total root colonization of AM fungi are depicted in (Fig. 1A) respectively. During this flowering stage-I, arbuscular (C. $D_1 = 3.735$; $P = 0.05$), vesicular (C. $D_1 = 4.752$; $P = 0.05$) and total root colonization (C. $D_1 = 5.08$; $P = 0.05$) of AM fungi varied significantly in four male *C. papaya* varieties. However, hyphal colonization did not vary significantly within the varieties.

In flowering stage-II, the values for hyphal, arbuscular, vesicular and total root colonization of AM fungi in this stage is depicted in (Fig. 1B). During this flowering stage-II, the hyphal (C. $D_2 = 3.758$; $P = 0.05$), arbuscular (C. $D_2 = 4.549$; $P = 0.05$), vesicular (C. $D_2 = 2.701$; $P = 0.05$) and total root colonization (C. $D_2 = 6.366$; $P = 0.05$) of AM fungi varied significantly in four male *C. papaya* varieties.

In flowering stage-III and flowering stage-IV, the values for hyphal, arbuscular, vesicular and total root colonization of AM fungi in this stage is depicted in (Fig. 1C and D) respectively. Further, the total root colonization of AM fungi (C. $D_3 = 4.747$; $P = 0.05$ and C. $D_4 = 4.040$; $P = 0.05$) and root length colonized by hyphae (C. $D_3 = 3.641$; $P = 0.05$ and C. $D_4 = 3.264$; $P = 0.05$), arbuscules (C. $D_3 = 3.179$; $P = 0.05$ and C. $D_4 = 3.657$; $P = 0.05$) and vesicles (C. $D_3 = 3.800$; $P = 0.05$ and C. $D_4 = 1.371$; $P = 0.05$) varied significantly among the four male *C. papaya* varieties during flowering stage-III and flowering stage-IV respectively.

Further, the highest average hyphal, arbuscular and total root colonization was recorded in flowering stage-II (Table 1). The highest average vesicular colonization was recorded in flowering stage-III and flowering stage-IV (Table 1). The lowest average hyphal colonization was recorded in flowering stage-III (Table 1). The lowest average arbuscular and total root colonization was recorded in flowering stage-IV (Table 1). The lowest average vesicular colonization is recorded in flowering stage-I (Table 1).

2.3. Acid and alkaline root phosphatase activities of male C. papaya varieties

Comparative account of alkaline and acid root phosphatase activities in four male *C. papaya* varieties flowering stage^{-1} is recorded in (Table 2). While, comparative account of average alkaline and acid root phosphatase activities in male *C. papaya* during different flowering stages but irrespective of varieties is recorded in (Table 3). Throughout the study period, Washington variety recorded the highest root phosphatase (alkaline and acid) activity followed by Honey dew, CO-2 and CO-1 variety (Table 2) respectively. Acid root phosphatase activity was consistently higher compared to alkaline root phosphatase activity in four male *C. papaya* varieties during all flowering stages (Tables 2 and 3). Both alkaline and acid root phosphatase activities varied significantly among the four male *C. papaya* varieties for any particular flowering stage (Table 3). Further, average alkaline and acid root phosphatase activities were highest during flowering stage-I, followed by flowering stage-II, flowering stage-III and flowering stage-IV respectively (Table 3).

2.4. Spore density of AM fungi

During flowering stage-I, maximum spore density was recorded in CO-2 variety and minimum was recorded in CO-1 and Washington varieties (Table 4). The maximum and minimum spore density of AM fungi was recorded in CO-1 and Honey dew variety respectively during flowering stage-II (Table 4). In flowering stage-III, maximum spore density was recorded in CO-2 variety whereas minimum spore density was recorded in Washington and CO-1 variety (Table 4). Further, in flowering stage-IV, maximum spore density was recorded in Honey dew variety, while minimum spore density was recorded in CO-1 variety (Table 4). The average spore density of AM fungi in male *C. papaya* plants was maximum during flowering stage-IV and minimum during flowering stage-II (Table 4). The spore density of AM fungi varied significantly among the four male *C. papaya* varieties during flowering stage-I (C. $D_1 = 19.373$; $P = 0.05$), flowering stage-II (C. $D_2 = 25.256$; $P = 0.05$), flowering stage-III (C. $D_3 = 16.601$; $P = 0.05$) and flowering stage-IV (C. $D_4 = 16.527$; $P = 0.05$) respectively.

2.5. Relationship between root colonization of AM fungi and root phosphatase activities of male C. papaya varieties

Multiple regression analysis was carried out to study the relationship between root colonization of AM fungi and root phosphatase activities at each flowering stage in male *C. papaya* plants. In the present study during flowering stage-I and flowering stage-III, all the four parameters of root colonization by AM fungi significantly influenced the alkaline and acid root phosphatase activity (Table 5). In flowering stage-II, it was observed that vesicular colonization did not influence root phosphatase activities, whereas the remaining three parameters viz. hyphal, arbuscular and total root colonization contributed significantly to root phosphatase (alkaline and acid) activities (Table 5). In flowering stage-IV, acid root phosphatase activity was significantly influenced by all the four parameters of root colonization while, alkaline root phosphatase activity was not significantly influenced by root colonization of AM fungi (Table 5). The values of regression coefficient revealed that the fit of the equations are most appropriate.

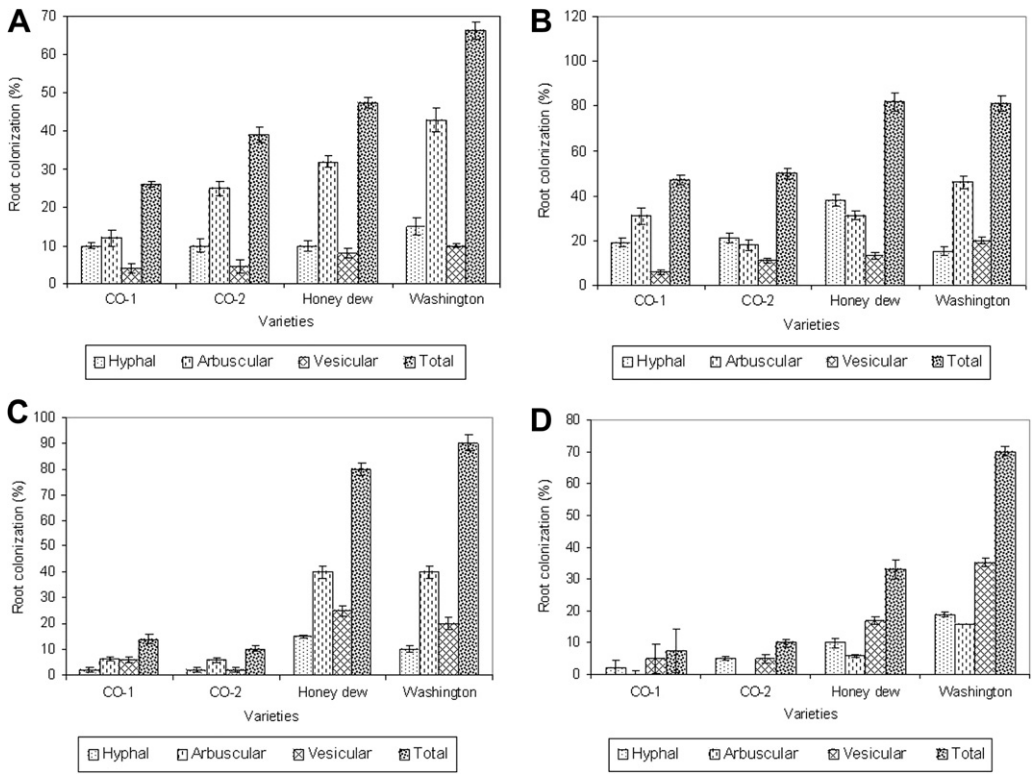

Fig. 1. A. Root colonization of AM fungi in different varieties of male *C. papaya* during flowering stage-I. Error bar indicate ± SE. B. Root colonization of AM fungi in different varieties of male *C. papaya* during flowering stage-II. Error bar indicate ± SE. C. Root colonization of AM fungi in different varieties of male *C. papaya* during flowering stage-III. Error bar indicate ± SE. D. Root colonization of AM fungi in different varieties of male *C. papaya* during flowering stage-IV. Error bar indicate ± SE.

2.6. Distribution of AM fungi

Arbuscular mycorrhizal fungi associated with the four male *C. papaya* varieties during different flowering stages is presented in (Table 6). A total of 12 species of AM fungi belonging to five genera viz. *Acaulospora* (4), *Dentiscutata* (1) *Gigaspora* (1), *Glomus* (4) and *Racocetra* (2) were recovered with number of species given in parenthesis. During the present study, all the species of AM fungi recorded from the original field samples, sporulated in pot cultures with an exception of two species viz. *Glomus coremioides* (Plate 3C, D) and *Glomus sinuosum*. Additionally, one species of AM fungi viz. *Glomus macrocarpum* was exclusively recovered from pot cultures and was not recorded in the original rhizosphere field samples. This species was recorded in the pot cultures established from rhizosphere soil collected during flowering stage-I (Table 6). Thus, in the present study, original field samples were used to extract and quantify the AM fungal spores and identify them to species level. Part of the original soil samples were used to establish pot cultures.

Table 1
Average values of root colonization by AM fungi irrespective of male *C. papaya* varieties but per flowering stage.

Flowering stages	*Average root colonization (%)			
	Hyphal colonization	Arbuscular colonization	Vesicular colonization	Total colonization
Flowering Stage-I	11.75 ± 1.810	28.00 ± 5.578	6.65 ± 1.628	44.70 ± 6.49
Flowering Stage-II	23.25 ± 4.502	31.50 ± 5.224	12.50 ± 2.571	65.00 ± 8.56
Flowering Stage-III	7.25 ± 2.705	23.10 ± 8.01	15.50 ± 4.654	48.55 ± 10.34
Flowering Stage-IV	9.00 ± 1.967	5.55 ± 1.531	15.50 ± 1.628	30.10 ± 8.34

*$n = 4 \pm$ SE.

After completion of one life cycle of the host plants used to culture the AM fungi, the spores were extracted from the pot cultures and these spores were used only to make diagnostic slides for species identification purpose. The identification of species recovered from original field samples were confirmed with help of species obtained from pot cultures.

Two species of AM fungi (*Acaulospora myriocarpa* and *Dentiscutata reticulata*) were encountered during all flowering stages of male *C. papaya* varieties and recorded the highest frequency of occurrence (100%) (Table 6). Species richness of AM fungi in male *C. papaya* plants varied from 6 to 9 species flowering stage^{-1} (Fig. 2). Maximum species richness was recorded during flowering stage-IV whereas minimum species richness was recorded during flowering stage-II.

3. Discussion

The bioavailability of soil phosphorous to plants depends on various mechanisms of P acquisition from the rhizosphere and on the strategies developed by the plants for taking P. A major process is the decrease in the concentration of phosphate ions in the soil solution which occurs within the rhizosphere as a direct consequence of removal of P by root uptake. This depletion gives rise to replenishment of P from solid phase and this process varies with the composition and physiochemical conditions such as soil pH [41]. To cope up with such adverse conditions plants have evolved various mechanisms that influence the chemical condition at the root–soil interface and enhance the solublization of soil P in the rhizosphere [3].

The ability of a plant to access soil P varies among species and genotypes within species and is affected by interactions of plants

Table 2
Comparative account of root phosphatase activities in varieties of male *C. papaya* per flowering stage.

Flowering stages	*Mean root phosphatase activities (n moles of p-NPP released min^{-1} μg of protein^{-1})							
	CO-1		CO-2		Honey dew		Washington	
	Alkaline	Acid	Alkaline	Acid	Alkaline	Acid	Alkaline	Acid
Flowering Stage-I	15.33 ± 2.934	34.98 ± 5.564	23.52 ± 4.662	42.60 ± 10.708	102.55 ± 12.699	137.04 ± 17.064	320.11 ± 39.327	767 ± 65.996
Flowering Stage-II	8.71 ± 0.317	23.33 ± 1.506	10.03 ± 1.686	69.34 ± 6.818	25.58 ± 3.562	86.35 ± 12.520	126.10 ± 28.330	166.69 ± 32.019
Flowering Stage-III	3.961 ± 0.626	9.09 ± 0.891	4.09 ± 0.671	11.37 ± 0.856	4.99 ± 0.525	17.45 ± 1.568	5.55 ± 0.350	35.743 ± 3.453
Flowering Stage-IV	0.71 ± 0.160	1.07 ± 0.276	0.91 ± 0.139	4.68 ± 0.208	1.04 ± 0.249	4.61 ± 0.247	2.46 ± 0.158	6.39 ± 0.230

*$n = 10 \pm$ SE.

and microbes. In addition to the inherent genetic potential of a plant to extract P from the soil, the ability to interact with microbes can also be a major determinant of the plant's ability to grow in soils with low levels of bioavailable P [24]. Arbuscular mycorrhizal fungi are plant symbionts that are known to enhance P-uptake in numerous plant species. Therefore, our study confirms that papaya genotypes can form beneficial symbioses with mycorrhizal fungi even though they were selected under conditions of P fertilization [30,33]. However, substantial variation exists among papaya varieties for responsiveness to AM fungi [31,32].

Usually the experiments that examine AM association at particular instant may produce conflicting or unexpected results [12] although consistencies in the results were reported by Khade et al. [34] when many replicates are considered. To overcome discrepancies in results, it is necessary to investigate the functioning of the association over an extended period of time [28,33]. The experiment reported here was therefore intended to monitor AM fungal contribution to male papaya plants under field conditions over the main growing season.

The present study was carried out in conventionally managed monoculture plantations of *C. papaya*. The limiting levels of phosphorus and nitrogen levels despite of fertilizer applications could be attributed to process such as phosphorus fixation in acidic soils and run off or leaching of soil nitrogen due to rainfall [53]. Throughout the study, feeder roots were sampled since they generally reported greater incidence and intensity of colonization than the main root axis [48].

In the present study all stages of root colonization by AM fungi were evident and their proportion varied with each flowering stage of male *C. papaya* plants. In the roots, hyphal colonization is responsible for uptake of nutrients especially P, arbuscular colonization or arbuscule is a key structure of AM symbiosis and it is responsible for exchange of nutrients from fungi to the plant in return of host carbohydrate, while vesicular colonization is resting and late growth phase of AM fungi inside the host root [27]. The present study, also recorded varietal differences in root colonization by AM fungi. In general CO-1 variety recorded the lowest levels while Washington variety recorded the highest levels of root colonization during flowering stage-I and flowering stage-IV. No trend in root colonization was evident between the varieties during flowering stage-II and flowering stage-III. In general, average hyphal, arbuscular and total root colonization values were highest in flowering stage-II, while average vesicular colonization values were highest during flowering stage-III and flowering stage-IV. Thus, the proportion of arbuscular mycorrhizal fungal structures inside the host plant reflected to certain extent, the fungal growth stage [6].

The present study was conducted during wet season (June–September) and collections of samples were carried out at the short interval (25–30 days). Thus, the present study upholds the view of Clarholm and Rosengren-Brinck [8] that root phosphatase studies should be performed during favourable moisture conditions and also it is important to minimize the time between sampling and analysis [49] since it is most likely to give the best information.

The present study recorded varietal differences in root phosphatase activities. Here, Washington variety recorded consistently highest root phosphatase activities (alkaline and acid) followed by Honey dew, CO-2 and CO-1 variety. Similarly, Helal [20] reported considerable variability in root phosphatase activity and P-uptake by young roots of *Phaseolus vulgaris* L. Yadav and Tarafdar [55] reported variability in time of secretion of acid root phosphatase activity in legumes, cereals and oil seeds and related it to the P-requirements of the plants.

The present study recorded consistently higher acid root phosphatase activity as compared to alkaline root phosphatase activity. Possible reasons for such results recorded in present study could be due to the fact that acid root phosphatase activity lacks substrate specificity [11] while alkaline root phosphates activity may be highly substrate specific [23]. P-deficiency is well known to enhance plant root exudation [18] or more correctly root leakiness with the possibility that enzyme enter the soil due to a loss of membrane integrity [23]. Thus the low levels of available phosphorus influenced both root colonization and root phosphatase activity in the present study. Similarly, Joner et al. [23] reported that the factors which adversely affect the mycorrhizal functioning and vitality, correlate negatively with alkaline root phosphatase activity. Similarly, Krishna et al. [36] reported consistently higher acid root phosphatase activity as compared to alkaline root phosphatase activity at the end of 15, 30, 45 and 60 days of growth in *Arachis hypogea* L inoculated with *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske. Further, in studies conducted on *Terminalia arjuna* L., grown in polythene bags in sterilized soil treated with *G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. fasciculatum,* and rock phosphate separately, or in various combinations results showed that acid phosphatase activity increased to a maximum in *G. fasciculatum* roots followed by *G. mosseae* + phosphorus and *G. mosseae* + *G. fasciculatum* treated roots. The acid phosphatase activity in shoots was maximum in *G. mosseae* + phosphorus treated plants. All other

Table 3
Comparative account of average values of root phosphatase activities in male *C. papaya* irrespective of varieties but per flowering stage.

Flowering Stages	*Average root phosphatase activities (n moles of p-NPP released min^{-1} μg of protein^{-1})			
	Alkaline root phosphatase activity	**C. D ($P = 0.05$)	Acid root phosphatase activity	**C. D ($P = 0.05$)
Flowering Stage-I	211.33 ± 59.34	36.29	452.39 ± 142.06	60.24
Flowering Stage-II	42. 60 ± 26.442	24.904	126.52 ± 28.91	30.546
Flowering Stage-III	7.14 ± 2.365	0.971	26.59 ± 11.92	3.541
Flowering Stage-IV	1.75 ± 5.501	0.317	5.50 ± 2.46	0.421

*$n = 4 \pm$ SE; **F test significant at 0.05 level of probability.

Table 4
Comparative account of spore densities of AM fungi during flowering stages of male *C. papaya* varieties.

Flowering stages	*Mean spore density. 50 g soil^{-1} variety^{-1} flowering stage^{-1}				**Average spore density. 50 g soil^{-1} flowering stage^{-1}
	CO-1	CO-2	Honey Dew	Washington	
Flowering Stage-I	10.00 ± 0.838	34.00 ± 1.645	23.00 ± 3.278	10.00 ± 1.139	19.50 ± 5.192
Flowering Stage-II	59.00 ± 1.370	25.00 ± 1.520	24.00 ± 3.826	48.00 ± 2.764	17.33 ± 7.771
Flowering Stage-III	17.00 ± 1.793	39.00 ± 2.005	19.00 ± 1.582	17.00 ± 1.268	23.00 ± 4.801
Flowering Stage-IV	14 0.00 ± 1.227	37.00 ± 2.414	38.00 ± 0.448	37.00 ± 1.765	31.50 ± 5.233

*$n = 5 \pm$ SE; **n=4 ± SE.

combinations had reduced acid phosphatase activity. Alkaline phosphatase activity was considerably lower than the acid phosphatase activity in the roots and shoots of all the AM fungi treated *Terminalia* plants. Alkaline phosphatase activity was maximum in roots of *G. fasciculatum* + phosphorus and in shoots of *G. mosseae* + phosphorus, followed by phosphorus treated plants [4].

In the present study, maximum acid and alkaline root phosphatase activity was recorded during flowering stage-I followed by flowering stage-II, flowering stage-III and flowering stage-IV respectively. Similarly Krishna et al. [36] studied the acid and alkaline root phosphatase activities in *A. hypogea* inoculated with *G. fasciculatum* at four harvests (15, 30, 45 and 60 days) under acidic soil conditions. They reported that only alkaline root phosphatase activity was significantly higher from the second harvest and that the acid root phosphatase activity did not vary significantly within the four harvests. Further, in greenhouse pot experiments conducted at the Universidad de la Frontera, Tamuco, Chile, seedlings of wheat cultivars, Dalcahue, Malihue, Carahue and Naofen were grown in the typical low phosphorus volcanic soil of South Chile with and without 300 kg of phosphorus pentaoxide per hectare and harvested at 21, 42, 63, 84, and 96 days after planting. The AM colonization was similar among all genotypes. Root surface acid phosphatase activity did not differ significantly among cultivars and the enzyme was significantly influenced by phosphorus. When plants were grown without phosphorus fertilizer, maximum enzyme activity was reached at 63 days for Carahue and Dakahue and 43 days for Naofen and Malihue. Phosphatase activity was highest at 21 days but quickly declined in the later samplings [44].

In the present study, total root colonization of AM fungi recorded peak increase in flowering stage-II while root phosphatase activities recorded peak increase in flowering stage-I. The present study provides new information that, at each flowering stage, total root colonization and root length colonized by hyphae, arbuscules and vesicles positively and significantly contributed to acid and alkaline root phosphatase activities even though peak increase in root colonization and root phosphatase activity was seen in two different stages. Further, the significant positive regression coefficient recorded between root colonization of AM fungi and root phosphatase (acid and alkaline) activities in the present study, corroborates with findings of Krishna et al. [36] who reported that increased root phosphatase activity is caused by mycorrhizal root colonization. More recently Khade et al. [34] also reported that acid root phosphatase activity increases with root colonization of AM fungi in vegetative *C. papaya* varieties under field conditions.

Microbes, such as AM fungi, are themselves affected by environmental factors [13,22,51] and vary in density and population composition from environment to environment. In the present study, unlike root colonization and root phosphatase activities, spore density did not exhibit any definite patterns within the four male *C. papaya* varieties during different flowering stages. Also spore density of AM fungi recorded a narrow range of fluctuation during the study period as compared to root colonization and root phosphatase activity. Further, finding few spores yet appreciable root colonization could mean the presence of non-sporulating types of AM fungi [9]. However, the species reported in the present study (if not all) were largely sporulating types since propagules in the soil or root fragments from the soils with few apparent viable spores eventually produced spore after pot culture in glass house with susceptible host. The species, *G. macrocarpum* recovered in open pot cultures was probably not sporulating at the sampling time but were existed in root fragments or in soil as mycelium [40].

Thus, in the present study low levels of root colonization viz. total root colonization, root colonized by hyphae, arbuscules and vesicles were recorded in CO-1 and CO-2 while high levels were recorded in Honey dew and Washington variety. The present study also recorded varietal differences in root phosphatase activities with Washington variety recording highest root phosphatase activities and CO-1 recording the lowest root phosphatase activities. Further, the acid root phosphatase activity was consistently higher than alkaline root phosphatase activity throughout the study. The present study also revealed that acid and alkaline root phosphatase activities were influenced by root colonization significantly and positively during all the flowering stages of male papaya varieties under field conditions.

Table 5
Comparative account of relationship between root phosphatase activities (Y) and root colonization of AM fungi (X) during flowering stages of male *C. papaya*.

Flowering Stages	Root phosphatase activities	Equations[b]	Regression coefficient (R^2)
Flowering Stage-I	Alkaline[a]	$Y_M = -177.22 + 4.18\ H + 1.50A + 1.69\ V + 3.83\ T$	0.706*
	Acid[a]	$Y_M = -445.80 + 11.29\ H + 0.051A + 7.\ 60V + 0.77\ T$	0.668*
Flowering Stage-II	Alkaline[a]	$Y_M = -28.06 - 1.98\ H + 0.83\ A + 1.33\ T$	0.903*
	Acid[a]	$Y_M = -38.69 - 0.83\ H - 0.21\ A + 1.88\ T$	0.757*
Flowering Stage-III	Alkaline[a]	$Y_M = 2.36 - 0.05\ H + 0.05\ A + 0.006V + 0.0003\ T$	0.872*
	Acid[a]	$Y_M = 8.43 - 0.\ 656H + 0.53\ A - 0.03\ V + 0.000\ 1T$	0.651*
Flowering Stage-IV	Alkaline[a]	$Y_M = 2.97 - 0.16H - 0.062\ A - 0.20\ V + 0.10\ T$	0.273
	Acid[a]	$Y_M = 10.74 - 0.12\ H - 0.19\ A - 0.\ 72V + 0.19\ T$	0.704*

*Regression significant at 0.05 level of probability.
[a] Y_M = Equation for male *C. papaya* plants.
[b] T = Total root colonization. Root length colonized by hyphae (H), arbuscules (A) and vesicles (V).

Table 6
Distribution of AM fungi in male plants of *C. papaya* during flowering stages.

Arbuscular mycorrhizal fungi	Flowering stages[d]				Frequency of occurrence (%)
	FL-I	FL-II	FL-III	FL-IV	
Acaulospora mellea Spain & Schenck[a]	−	+	+	+	75.00
Acaulospora myriocarpa Spain, Sieverding & Schenck[a]	+	+	+	+	100. 00
Acaulospora scrobiculata Trappe[a]	−	−	−	+	25.00
Acaulospora spinosa Walker & Trappe[a]	+	+	−	−	50.00
Dentiscutata reticulata (Koske, Miller & Walker) Sieverd., De Souza & Oehl[a]	+	+	+	+	100.00
Gigaspora margarita Becker & Hall[a]	+	−	+	+	75.00
Glomus claroideum (Smith & Schenck) Vestberg & Walker[a]	−	+	+	+	75.00
Glomus coremioides (Berk. & Broome) Redecker & Morton[c]	+	−	−	+	50.00
Glomus macrocarpum Tulasne & Tulasne[b]	+	−	−	−	25.00
Glomus sinuosum (Gerd. & Bakshi) Almeida & Schenck[c]	+	−	+	+	75.00
Racocetra gregaria (Schenck & Nicol.) Oehl, De Souza & Sieverding[a]	+	+	−	−	50.00
Racocetra verrucosa (Koske & Walker) Oehl, De Souza & Sieverding[a]	−	−	+	+	50.00

+ Presence of arbuscular mycorrhizal fungi; − Absence of arbuscular mycorrhizal fungi.
[a] AM fungal species recovered from the original field samples and pot cultures.
[b] Recovered from pot cultures only.
[c] Recovered from original field samples only.
[d] FL-I = Flowering Stage-I; FL-II = Flowering Stage-II; FL-III = Flowering Stage-III; FL-IV = Flowering Stage-IV.

In conclusion, phosphatases are known to catalyze the hydrolysis of ester bonds between phosphate and C compounds in organic substrates and increased production of phosphatase enzyme by plant roots is induced when phosphorus is limiting. Consequently increased root phosphatase activity may reflect high demand for phosphorus [47]. Therefore it can be inferred that the high P-demand of Honey Dew and Washington variety was compensated by increased root phosphatase activities and increased root colonization by AM fungi and under acidic P-deficient conditions, acid root phosphatase activity is more pronounced than alkaline root phosphatase activity. These phosphatase activities of the roots are responsible for mineralization of organic P in order to make the inorganic P available for plants. This uptake of inorganic P is enhanced or increased due to presence of root colonization of AM fungi since it is a well known fact that these fungi explore the portion of soil inaccessible to host roots and translocation of P takes place through passive diffusion while active transfer of P from fungus to the plants takes place in the host root colonized by arbuscules which is the key structure of AM symbiosis.

Thus the study supports the contention that root length colonized by hyphae, arbuscules, vesicles and total root colonization by AM fungi contribute positively and significantly to both acid and alkaline root phosphates activities in male *C. papaya* plants under field conditions. Therefore it is confirmed that there is positive relationship between root colonization and acid and alkaline root phosphatase activities of host plant throughout different flowering stages of male *C. papaya* under field conditions. Further, the study recorded maximum spore density and species richness of AM fungi at the end of the study period. The study supports the earlier findings that AM fungal association varies with different developmental stages of the host plants under tiels conditions. Therefore, the study proves the hypothesis that these AM fungi aid in plant uptake of mineralized P by influencing activity of root phosphatase which is shown by elucidating positive relationship between root colonization of AM fungi and root phosphatase activities and that these parameters responsible for P-uptake vary with different varieties of male *C. papaya* plants at each flowering stage under field conditions.

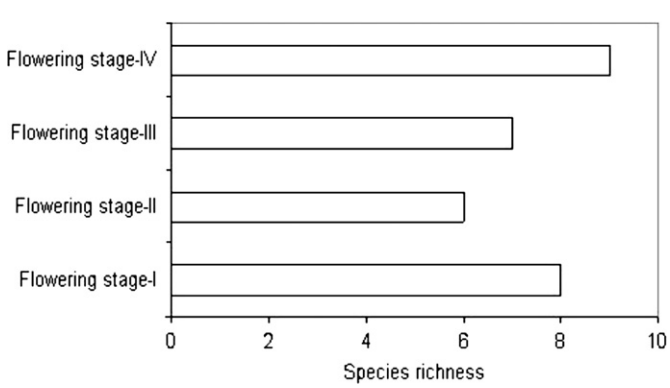

Fig. 2. Species richness of AM fungi in male *C. papaya* during different flowering stages.

4. Materials and methods [21]

4.1. Study site and management regimes

C. papaya plantations in agricultural farm located at Old Goa (North Goa), India was selected for the study. The soil at this site was well drained, dark grey brown to dark brown gravelly sandy loam to sandy clay loam with medium water holding capacity. The study was carried from June 2001 to September 2001. The maximum and minimum temperatures recorded during this period were 31 °C and 25.5 °C respectively with relative humidity ranging from 82 to 87%. Rainfall recorded during study period was 120 mm (September) to 832.4 mm (July).

Four dioecious *C. papaya* varieties viz. CO-1, CO-2, Honey Dew and Washington were planted in monoculture. *C. papaya* plantations were managed conventionally. Under this type of management, inorganic fertilizer (19:19:19) was applied in six split doses per year at an interval of two months. *C. papaya* plants received 250 g N, 250g P_2O_5 and 50g K_2O per plant per year. So during the study period, inorganic fertilizer was applied once *i. e.* in September. The entire study was carried out in the wet season and therefore no irrigation was carried out during this period. Also it was observed that *C. papaya* produces numerous feeder roots on the surface in response to soil moisture. So the collections were carried out at the depth of 0–25 cm throughout the study period.

4.2. Collection of samples

In the present study, *C. papaya* seedlings were raised from seeds of four dioecious viz. CO-1, CO-2, Honey dew and Washington in the nursery for two months and than they were transplanted in the field. The early vegetative stage (two-four months old plants) mainly comprises of tap root system with feeble primary branches making it difficult to collect root (feeder roots) samples to carry out mycorrhizal studies. The differences in male plants and female plants can be observed only during flowering stages when the plants were seven months old plants and exhibited extensive root system with ample of feeder roots available for collection. Generally, the male plants are maintained in the field for pollination purpose and at the end of growing season they are destroyed. So the present study was carried out during flowering stages when the male *C. papaya* plants were seven (June) to ten (September) months old.

In the present study, sampling was carried out four times at short intervals when the *C. papaya* male plants were in the flowering stages viz., last week of June (Fl-I), last week of July (Fl-II), mid August (Fl-III) and mid September (Fl-IV). Sampling procedures were carried out according to Tews and Koske [54] except that the core size was bigger (15 cm in diameter).

Two healthy plants variety^{-1} flowering stage^{-1} were randomly selected for the collection of rhizosphere soil and root samples. For each plant, three random soil cores were collected from within 60 cm of each plant, each at the depth of 0–25 cm. Immediately, samples collected from both plants were then combined to give approximately 400g moist soil after thorough mixing in laboratory. From this, 250 g air-dried soil was employed for extraction of AM fungal spores, 100 g was utilized for nutrient analysis and 50 g soil was utilized for setting open pot cultures. From each composite sample, five sub-samples were made for quantification of spore density, two sub-samples for nutrient analysis and two sub-samples for establishment of pot cultures.

Roots were packed separately for assay of phosphatases and for estimation of root colonization plant^{-1} variety^{-1} flowering stage^{-1} in the field. Root samples collected for assay of phosphatases, were transported in ice to the laboratory. These root samples (feeder roots) were washed in ice cold water and rinsed with double distilled water, packed in polyethylene bags, labeled and stored at −80 C. For estimation of root colonization and establishment of pot cultures, the root samples of both the plants were combined. From this composite sample, five sub-samples were utilized for estimation of degree of root colonization and two sub-samples were utilized for pot cultures.

4.3. Establishment of pot cultures

Baiting of native AM fungi were carried out by using open pot cultures [16]. Two pot cultures were maintained variety^{-1} flowering stage^{-1}. For establishing single pot culture, 25 g of rhizosphere soil along with root bits of *C. papaya* was mixed with equal quantity of sterilized sand and placed in 12.5 cm diameter pots. Seeds of *Eleusine coracana* (L.) Gartner were sterilized with 0.1% $HgCl_2$ washed thoroughly with distilled water and placed over the soil sand mixture and covered with 2 cm of soil. Cuttings of *Coleus* sp. were also used as host plants for baiting the native AM fungi. The plants were maintained under glass house conditions and watered adequately. The roots of host species were checked for AM colonization after 45 days since, this stage represents the vesicular colonization by AM fungi which is easily detectable after root staining procedure. Pots showing successful mycorrhization were maintained for period of six months and application of water was reduced at final three weeks to maximize spore production. While other pots which lacked AM colonization were rejected. At the end of six months the plants were cut near the base, the growing substrate (cultures) were air-dried and checked for the presence of spores. Spores isolated from pot cultures were used to make diagnostic slides only. Further, these slides were used for verifying the identification of AM fungi recovered from original field samples.

4.4. Soil sample analysis

Soil samples were analyzed for 11 edaphic factors. Soil pH was measured in 1:2 soil water suspension using pH meter (LI 120 Elico, India). Electrical conductivity was measured at room temperature in 1:5 soil suspension using Conductivity meter (CM-180 Elico, India). Standard soil analysis techniques viz. micro-Kjeldahl method [21] and Bray and Kurtz [7] method were employed for determination of total nitrogen and available phosphorus respectively. Available potassium was estimated by ammonium acetate method [19] using Flame Photometer (Systronic 3292). Available zinc, copper, manganese and iron were quantified by DTPA–$CaCl_2$–TEA method [37] using Atomic Absorption Spectrophotometer (AAS 4139, Electronic Corporation of India Ltd, India).

4.5. Estimation of root colonization by AM fungi

Roots were cleared in 10% KOH, acidified in 1N HCL and stained in 0.05% trypan blue in lactoglycerol [43]. Total root colonization and length of the root colonized by hyphae, arbuscules and vesicles by AM fungi were estimated by of modification of grid intersection method [39] and slide method [17]. Tertiary roots of 1 cm length were randomly selected for estimation of root colonization. Stained root bits were placed on the microscopic slides and the field of the view was moved across the slide. The hairline graticule inserted in the eyepiece acted as a line of intersection. At each intersection, presence of hyphae, arbuscule and/or vesicle was noted. Hundred and fifty intersections were examined per samples under compound microscope at 200× magnifications and root colonization of AM fungi was expressed in percentage [34].

4.6. Assay of root phosphatase enzyme activity

This experiment comprised of two sets of observations for each type of assay variety^{-1} flowering stage^{-1}. Thus, the study comprised of two repetitions^{-1} variety^{-1} flowering stage and each repetition had five replications. One gram of root tissue was weighed for each repetition. Activities were assayed separately for acid phosphatase and alkaline phosphatase. In all 40 readings each were recorded for acid and alkaline phosphatase separately flowering stage^{-1}. In total 160 readings were recorded for each acid and alkaline root phosphatase activity separately for four flowering stages of male *C. papaya* plants. Methods for quantification of root phosphatase activities were standardized for *C. papaya* based on procedures provided by Kapoor et al. [25] and Sukhada [50].

4.7. Extraction of enzyme

Enzyme was extracted by macerating 1 g of detached root tissue at 4 °C using 20 ml of phosphate (0.1 M KH_2PO_4, pH 6.6) buffer. The homogenate was filtered through muslin cloth and the filtrate was centrifuged at 5000 rev min^{-1} for 15 min using a cooling centrifuge

(Remy C-24, India). The supernatant was stored at 4 °C and further employed for protein estimation of enzyme and assay of root phosphatases [34].

4.8. Protein estimation

Protein content of the enzyme extract was estimated by Lowry et al. [38] method using bovine serum albumin as standard.

4.9. Assay of root phosphatases

For alkaline root phosphatase activity, 5 μg protein equivalent enzyme extract was incubated with 2 ml of 15 mM p-nitrophenyl phosphate (p-NPP) and 0.8 ml 0.25 M Tris HCl (pH 9.8). For acid root phosphatase activity, 5 μg protein equivalent enzyme extract was incubated with 2 ml of 15 mM p-nitrophenyl phosphate (p-NPP) and 0.8 ml 0.25 M Sodium acetate (pH 6.0) [34].

The reactions in both the above mentioned cases were terminated by adding 2 ml of 1N NaOH. For each repetition, T'_0 (zero time of incubation) and T'_{30} (30 min after incubation) were taken separately for alkaline and acid root phosphatase activity. Optical density (O.D) was read at 410 ηm using uv-1201 Shimadzu spectrophotometer (Japan) against a blank solvent (distilled water). Root phosphatase activity was expressed in terms of *n* moles of p-nitrophenol released^{-1} min^{-1} μg protein [34].

4.10. Quantification of spore density of AM fungi

Only, spores and sporocarps of AM fungi from original rhizospheric field samples were isolated by wet sieving and decanting method [15] and quantification of spore density of AM fungi was carried out using method described by using Gaur and Adholeya [14]. Here, only healthy spores, which generally comprised of 75% of extracted spores, were used for quantification of spore density of AM fungi.

4.11. Identification of AM fungi

Diagnostic slides containing intact and crushed spores and sporocarps of AM fungi were prepared in polyvinyl alcohol lactoglycerol (PVLG) [35]. Spore morphology and wall characteristics were considered for the identification of AM fungi and these characteristics were ascertained using compound microscope, Leica WILD MP3 and Nikon E 800. The AM fungi were identified to species level by using Manual of Identification Arbuscular Mycorrhizal Fungi by Schenck and Perez [46]. Taxonomic identification of spores was also carried out by matching the descriptions provided by International Collection of Vesicular Arbuscular Mycorrhizal fungi (http://invam.caf.wvu.edu) and Oehl et al. [42].

4.12. Diversity indices

Species richness is the mean number of AM fungal species recovered at any particular sampling period [2].

4.13. Frequency of occurrence

Frequency of occurrence of AM fungi was calculated using the following formula [2].

$$\text{Frequency}\ (\%) = \frac{\text{Number of soil sample that possess spores of particular species}}{\text{Total number of soil samples analyzed.}} \times 100$$

4.14. Statistical analysis

Data on root colonization and spore density of AM fungi and root phosphatase activity was subjected to analysis of variance in order to investigate the variations in these parameters with respect to four varieties of male *C. papaya* at each flowering stage. Prior to analysis of variance, root colonization values were subjected to arcsine transformations. Additionally, multiple linear regression analysis was carried out to determine the relationship between root colonization and root phosphatase activity at each flowering stage. Data was statistically analyzed using mstac package and WASP 1.0. Standard error was calculated for each parameter undertaken for the study.

Acknowledgements

Shri. Waman M. Khade, Ex- director of Agriculture Department and Directorate of Agriculture, State Government of Goa are thanked for their assistance to carry out research work.

References

[1] R. Azcón, F. Borie, J.M. Barea, Exocellular acid phosphatase activity of lavender and wheat roots as affected by phytate and mycorrhizal inoculation. Les Mycorrhizae: Biologie et Utilisation. INRA Publication (Les Colloques de 1'INRA, no13), Dijon, 1982, pp. 5–6.
[2] K.R. Beena, N.S. Raviraja, A.D. Arun, K.R. Sridhar, Diversity of arbuscular mycorrhizal fungi on coastal sand dunes of the West Coast of India, Curr. Sci. 79 (10) (2000) 1459–1465.
[3] I. Bertrand, P. Hinsinger, B. Jaillard, J.C. Arrieu, Dynamics of phosphorus in the rhizosphere of maize and rape grown on synthetic, phosphated calcite and goethite, Plant Soil 211 (1999) 111–119.
[4] B. Bhadraiah, V.V. Kankadurga, P. Ramarao, C. Manoharachary, Effect of VAM fungi and rock phosphate on phosphatase activities in *Terminalia arjuna*, Physiol. Biochem. (1999) 5–7 National Conference on Mycorrhiza. Section 3 (Poster).
[5] N.S. Bolan, A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants, Plant Soil 134 (1991) 189–207.
[6] P. Bonfante-Fasalo, Anatomy and morphology. in: C.L. Powell, D.J. Bagyaraj (Eds.), VA Mycorrhiza. CRC Press Inc, Boca Raton: Florida, 1984, pp. 5–33.
[7] R.H. Bray, L.T. Kurtz, Determination of total organic carbon and available forms of phosphorus in soils, Soil Sci. 59 (1945) 39–45.
[8] M. Clarholm, U. Rosengren-Brinck, Phosphorous and nitrogen fertilization of a Norway spruce forest- effect on needle concentrations and acid phosphatase activity in the humus layer, Plant Soil 175 (1995) 239–249.
[9] E.A. Davis, J.L. Young, S.L. Rose, Detection of high-phosphorous tolerant VAM-fungi colonizing hops and peppermint, Plant Soil 81 (1984) 29–36.
[10] D.D. Douds Jr., L. Galvez, M. Frankesnyder, C. Reider, L.E. Drinkwater, Effect of compost addition and crop rotation point upon vesicular-arbuscular mycorrhizal fungi, Agric. Ecosyst. Environ. 65 (1997) 257–266.
[11] S.M.G. Duff, G. Sarath, W.C. Plaxton, The role of acid phosphatase in plant phosphorus metabolism, Physiol. Plant 90 (1994) 791–800.
[12] M.J. Dunne, A.H. Fitter, The phosphorous budget of a field-grown strawberry (*Fragaria* × *ananassa* cv. Hapil) crop: evidence for a mycorrhizal contribution, Ass. Appl. Biol. 114 (1989) 185–193.
[13] J.R. Ellis, Post flood syndrome and vesicular-arbuscular mycorrhizal fungi, J. Prod. Agric. 11 (1998) 200–204.
[14] A. Gaur, A. Adholeya, Estimation of VAM spores in the soil – a modified method, Mycorrhiza News 6 (1994) 10–11.
[15] J.W. Gerdemann, T.H. Nicolson, Spore density of *Endogone* species extracted from soil wet sieving and decanting, Trans. Brit. Mycol. Soc. 46 (1963) 235–244.

[16] A.E. Gilmore, Phycomycetous mycorrhizal organisms collected by open pot cultures, Hilgardia 39 (1968) 87–105.
[17] M. Giovannetti, B. Mosse, An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots, New Phytol. 84 (1980) 489–500.
[18] H. Graham, R.T. Leonard, J.A. Menge, Membrane mediated decrease in root exudation responsible for phosphorous inhibition of vesicular–arbuscular mycorrhiza formation, Plant Physiol. 68 (1981) 549–552.
[19] J.J. Hanway, H. Heidal, Soil analysis method as used in Iowa State College soil testing laboratory, Iowa Agri. 57 (1952) 1–31.
[20] H.M. Helal, Varietals difference in the root phosphatase activity as related to the utilization of organic phosphates, Plant Soil 123 (1990) 161–163.
[21] M.L. Jackson, Soil Chemical Analysis. Prentice Hall, New Delhi, 1971.
[22] N.C. Johnson, F.L. Pfleger, Vesicular-arbuscular mycorrhizae and cultural stresses. in: G.J. Bethlenfalvay, R.G. Linderman (Eds.), Mycorrhizae in Sustainable Agriculture. ASA, CSSA, and SSSA, Madison, WI, 1992, pp. 71–99 ASA Spec. Publ. 54.
[23] E.J. Joner, I.M. Van Aarle, M. Vosatka, Phosphatase activity of extra- radical arbuscular mycorrhizal hyphae: a review, Plant Soil 226 (2000) 199–210.
[24] S.M. Kaeppler, J.L. Parke, S.M. Mueller, L. Senior, C. Stuber, W.F. Tracy, Variation among maize inbred lines and detection of quantitative trait loci for growth at low phosphorus and responsiveness to arbuscular mycorrhizal fungi, Crop Sci. 40 (2000) 358–364.
[25] A. Kapoor, V.P. Singh, K.G. Mukerji, Studies on the phosphatase of mycorrhizal and non mycorrhizal *Trigonella* roots. in: A. Mahadeva, A. Raman, K. Natarajan (Eds.), Mycorrhizae for Green Asia. CAS, Madras: India, 1989, pp. pp.125–127.
[26] S.W. Khade, Studies on arbuscular mycorrhizal status of *Carica papaya* L. in agro-based ecosystem of Goa, Ph.D thesis, Goa University, India, 2003, pp. 1–235.
[27] S.W. Khade, A. Adholeya, Arbuscular mycorrhizae in natural ecosystem: incentives in diversity and ecology. in: T. Satyanarayana, B.N. Johri (Eds.), Current Perspectives and Potential Applications. I. K International Pvt. Ltd., New Delhi: India, 2005, pp. 225–258.
[28] S.W. Khade, B.F. Rodrigues, Ecology of arbuscular mycorrhizal fungi associated with *Carica papaya* L. in agro-based ecosystem of Goa, India, Trop. Subtrop. Agroecosys. 8 (2008) 265–278.
[29] S.W. Khade, B.F. Rodrigues, Spatial variations in arbuscular mycorrhizal (AM) fungi associated with *Carica papaya* L. in a tropical agro-based ecosystem, Biol. Agri. Horti. 26 (2008) 149–174.
[30] S.W. Khade, B.F. Rodrigues, Arbuscular mycorrhizal fungi associated with varieties of *Carica papaya* L. in tropical agro-based ecosystem of Goa, India, Trop. Subtrop. Agroecosys. 10 (2009) 369–381.
[31] S.W. Khade, B.F. Rodrigues, Studies on arbuscular mycorrhisation of papaya, Afr. Crop Sci. J. 17 (2009) 155–165.
[32] S.W. Khade, B.F. Rodrigues, Studies on effects of arbuscular mycorrhizal (AM) fungi on mineral nutrition of *Carica papaya* L, Not. Bot. Hort. Agrobot. Cluj 37 (2009) 183–186.
[33] S.W. Khade, B.F. Rodrigues, Spatio-temporal variations of arbuscular mycorrhizal (AM) fungi associated with *Carica papaya* L. in agro-based ecosystem of Goa, India, Arch. Agron. Soil Sci. 56 (2010) 237–263.
[34] S.W. Khade, B.F. Rodrigues, P.K. Sharma, Arbuscular mycorrhizal status and root phosphatase activities in vegetative *Carica papaya* L. varieties, Acta Physiol. Plant 32 (2010) 565–574.
[35] R.E. Koske, B. Tessier, A convenient permanent slide mounting medium, Mycol. Soc. Am. News Let 34 (1983) 59.
[36] K.R. Krishna, D.J. Bagyaraj, K.G. Papavinashasundaram, Acid and alkaline phosphatase activities in mycorrhizal and uninfected roots of *Arachis hypogaea* L, Ann. Bot. 51 (1983) 551–553.
[37] W.L. Lindsay, W.A. Norvel, Development of DPTA soil test for zinc, iron, manganese and copper, Soil Sci. Soc. Am. J. 42 (1978) 421–488.
[38] O.H. Lowry, N.J. Rosebrough, A.L. Fan, R.J. Randall, Protein measurement with the Folin–Phenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[39] T.P. Mcgonigle, M.H. Miller, D.G. Evans, G.L. Fairchild, J.A. Swan, A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi, New Phytol. 115 (1990) 495–501.
[40] D.D. Miller, P.A. Domoto, C. Walkar, Colonization and efficacy of different endomycorrhyzal fungi with apple seedlings at two phosphorus levels, New Phytol. 100 (1985) 393–402.
[41] C. Morel, P. Hinsinger, Root induced modifications of the exchange of phosphate ion between soil solution and soil solid phase, Plant Soil 211 (1999) 103–110.
[42] F. Oehl, F.A. de Souza, E. Sieverding, Revision of *Scutellospora* and description of five new genera and three new families in the arbuscular mycorrhiza-forming *Glomeromycetes*, Mycotaxon 106 (2008) 311–360.
[43] J.M. Phillips, D.S. Hayman, Improved procedure for clearing roots and staining of mycorrhizal fungi for rapid assessment of infection, Trans. Brit. Mycol. Soc. 55 (1970) 158–161.
[44] R. Rubio, E. Moraga, F. Borie, Acid phosphatase activity and vesicular-arbuscular mycorrhizal infection associated with roots of 4 wheat cultivars, J. Plant Nut. 13 (1990) 585–598.
[45] F.E. Sanders, P.B. Tinker, Mechanism of absorption of phosphate from model substrates and P compounds in soil by *Endogone* mycorrhizas, Nature 133 (1971) 278–279.
[46] N.C. Schenck, Y. Perez, Manual for Identification of VA Mycorrhizal Fungi. INVAM, University of Florida, Gainesville: USA, 1990, pp. 1–283.
[47] K. Schneider, M.B. Turrion, P.F. Grierson, J.F. Gallardo, Phosphatase activity, microbial phosphorous and fine root growth in forest soils in the Sierra de Gata, West Central Spain, Biol. Fertil. Soils 34 (2001) 151–155.
[48] S.E. Smith, N.A. Walker, A qualitative study of mycorrhizal plants in *Trifolium*: separate determination of the rates of infection and of mycelial growth, New Phytol. 89 (1981) 225–240.
[49] T.W. Speir, D.J. Ross, Effect of storage on the activity of protease, urease, phosphatase and sulphates in the three soils under pasture. New Zeal, J. Sci. 18 (1975) 231–237.
[50] M. Sukhada, Effect of VAM inoculation on plant growth, nutrient level and root phosphatase activity in papaya (*Carica papaya* cv. Coorg Honey Dew), Fert. Res. 31 (1992) 263–267.
[51] D.M. Sylvia, S.E. Williams, Vesicular-arbuscular mycorrhizae and environmental stresses. in: G.J. Bethlenfalvay, R.G. Linderman (Eds.), Mycorrhizae in Sustainable Agriculture. ASA, CSSA, and SSSA, Madison, WI, 1992, pp. 101–124 ASA Spec. Publ. 54.
[52] T. Tadano, K. Ozawa, H. Sakai, M. Oaski, H. Matsui, Secretion of acid phosphatase by the roots of crop plants under phosphorous deficient conditions and some properties of the enzyme secreted by lupin roots, Plant Soil 156 (1993) 95–98.
[53] H.S.L. Tandon, Fertilizer Guide, second ed. Fertilizer Development and Consultation Organization, New Delhi, 1994, pp. 156.
[54] L.L. Tews, R.E. Koske, Towards a sampling strategy for vesicular arbuscular mycorrhizas, Trans. Brit. Mycol. Soc. 87 (1986) 353–358.
[55] R.S. Yadav, J.C. Tarafdar, Influence of organic and inorganic phosphorous supply on the maximum secretion of acid phosphatase by plants, Biol. Fertil. Soils 34 (2001) 140–143.