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Effects of Arbuscular Mycorrhizal Fungi on Root Phosphatase Activity of *Carica papaya* L.

By

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With 2 Figures

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Key words: *Carica papaya* L. cv. Surya, *Glomus intraradices* SCHENCK & SMITH, *Glomus mosseae* (NICOL. & GERD.) GERD. & TRAPPE, mixed inoculum, root phosphatase activity.

Summary

KHADE S. W., RODRIGUES B. F. & SHARMA P. K. 2012. Effects of arbuscular mycorrhizal fungi on root phosphatase activity of *Carica papaya* L. – *Phyton* (Horn, Austria) 52 (2): 291-300, with 2 figures.

Studies were carried out on physiological responses of *Carica papaya* L. cv. Surya to inoculation with arbuscular mycorrhizal fungi. The experiment dealt with un-inoculated seedlings, seedlings inoculated with *Glomus intraradices* SCHENCK & SMITH, seedlings inoculated with *Glomus mosseae* (NICOL. & GERD.) GERD. & TRAPPE and seedlings inoculated with mixed inoculum (*G. intraradices* + *G. mosseae*). The arbuscular mycorrhizal fungus, *G. mosseae*, significantly enhanced the root phosphatase activity (acid and alkaline) in papaya plants as compared to control plants. It was followed in descending order of effectiveness by mixed inoculum and *G. intraradices*.

Zusammenfassung

KHADE S. W., RODRIGUES B. F. & SHARMA P. K. 2012. Effects of arbuscular mycorrhizal fungi on root phosphatase activity of *Carica papaya* L. [Effekte arbusku-

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lärer Mikorrhizapilze auf die Wurzel-Phosphatase-Aktivität von *Carica papaya* L.J. – *Phyton* (Horn, Austria) 52 (2): 291–300, mit 2 Abbildungen.

In dieser Arbeit wurden die physiologischen Reaktionen von *Carica papaya* L. cv. Surya auf eine Beimpfung mit arbuskulären Mikorrhizapilzen untersucht. Als Versuchsmaterial dienten nicht überimpfte Samen, ferner Samen, die mit *Glomus intraradices* SCHENCK & SMITH inokuliert waren, Samen, mit *Glomus mosseae* (NICOL. & GERD.) GERD. & TRAPPE beimpft und schließlich Samen mit einem gemischten Inokulum (*G. intraradices* + *G. mosseae*). Gegenüber den Kontrollpflanzen war die Wurzel-Phosphatase-Aktivität (sowohl saure, wie auch basische) von den Papaya-Pflanzen durch den arbuskulären Mycorrhizapilz, *G. mosseae* signifikant erhöht. Eine geringere Wirkung war beim Mischinokulum und bei *G. intraradices* zu beobachten.

Introduction

Arbuscular mycorrhizal (AM) fungi are the most widespread type of symbiotic association. They are ecologically important root fungal symbionts associated with more than 90% of higher plant species, including crop and fruit tree species. These micro-symbionts occur widely under various environmental conditions with beneficial effects on soil structure improvement (MILLER & JASTROW 2000) and have great importance due to their higher capacity to increase growth and yield through efficient nutrient uptake in infertile soils (GUISOU 2009). They also enhance water uptake (KHALAFALLAH & ABO-GHALIA 2008) and drought resistance (MARULANDA & al. 2003) in plants (GUISOU 2009). These mycorrhizal attributes are of interest for increasing crop productivity. Thus the selection of the most specific appropriate plant-fungus association for each specific environmental and ecological situation is one of the main challenges in current research on AM fungi (KHANAM 2007).

Papaya (*Carica papaya* L.) is a widely consumed fruit in tropical areas and known for its nutritive values. The utilization of AM fungi for papaya has been suggested (ROSALIND PADMA & KANDASWAMY 1990, SUKHADA 1992). Recent studies have confirmed these findings and have demonstrated the importance of AM symbiosis to papaya in low fertility soils (MARTINS & al. 2000). MAMATHA & al. 2002 have observed that papaya trees respond to inoculation with efficient AM fungi reducing the need for P fertilization at least until the start of production. On the other hand, KHADE & RODRIGUES 2009 reported increased in elemental P and K in papaya plants inoculated with AM fungi. In view of the above, the present study was undertaken to evaluate the influence of selective AM fungi on one more parameter i.e. root phosphatase activity of papaya.

Material and Methods

Plant and Fungus Material

Seeds of *Carica papaya* L. cv. Surya were procured from the Indian Institute of Horticultural Research (IIHR), Bangalore. Cv. Surya is gynodioecious, high yielding

(50–65 kg/ plant) variety and is a progeny from the cross between cv. Sunrise Solo and cv. Pink Flesh Sweet released by IIHR, Bangalore. Sand based pure cultures of *Glomus mosseae* (NICOL. & GERD.) GERD. & TRAPPE and *Glomus intraradices* SCHENCK & SMITH containing extramatrical chlamydospores, colonized root segments containing mycelium and vesicles were obtained from, The Energy Research Institute (TERI), New Delhi. The inoculum of each culture consisted of 30 spores/2g moist soil.

Growing Conditions

The experiment was conducted for the period of four months (May 2002 – August 2002) in a poly-house on agriculture farm located at Mapusa, North Goa, India. The relative humidity during the study period ranged from 84 % to 93 %. Maximum and minimum temperatures ranged from 29.3°C to 31.6°C and 24.1°C to 25.4°C, respectively. Throughout the experimental period, papaya plants were watered thrice a week and fertilized with Hoagland's nutrient solution without phosphorus (HOAGLAND & ARNON 1939), at an interval of fifteen days.

Raising of Seedlings

Seeds of papaya were sown in plastic trays with sterilized nursery soil (sand soil mixture, 1:1). The soil used for the experiment was low (6 kg/ha) in available phosphorus of pH 6.1 (pH meter, LI – 120, Elico) and electrical conductivity 0.06 mS/cm (conductivity meter, CM-180, Elico). Total nitrogen and available potassium was 0.4 % and 80 kg/ha respectively. Organic carbon content of the soil was also low (0.42 %). Available Zn, Cu, Fe and Mn concentrations were 2.67, 3.84, 2.63, and 19.5 ppm respectively. Seedlings were maintained in plastic trays for one month.

Inoculation with AM Fungi

Seedlings of uniform length (10 cm) were selected for inoculation with AM fungi at the end of one month. The experiment comprised of following four treatments and each treatment had five replicates. Treatment 1 (C) – Un-inoculated papaya seedlings. Treatment 2 (GI) – Papaya seedlings inoculated with *G. intraradices*. Treatment 3 (GM) – Papaya seedlings inoculated with *G. mosseae*. Treatment 4 (MI) – Papaya seedlings inoculated with mixed inoculum (*G. intraradices* + *G. mosseae*).

Nursery bags of 0.5 kg capacity were filled with sterilized soil up to three-fourth of its volume. A small pit was made into soil and 5 g of inoculum was placed at a depth of 5 cm. Papaya seedling was then placed in this pit and layered with sterilized soil. One seedling was planted per bag. In case of MI inoculation, equal quantity (75 g each) of inoculum of *G. intraradices* and *G. mosseae* were mixed thoroughly and then 5 g of inoculum was added to each nursery bag. Control plants were inoculated with autoclaved inoculum. The plants were maintained for one and half month (45 days) in sterilized soil.

Transplantation of Seedlings in Unsterilized Soil

After a period of two and a half months of growth (76 days), papaya seedlings were transferred to nursery bags of 3 kg capacity containing unsterilized nursery soil with low nutrient status as mentioned earlier. Papaya seedlings were further maintained for a period of 45 days. The experiment was terminated at end of 123 days of

growth (four months) and papaya plants were subjected to analysis of various parameters.

Quantification of Root Phosphatase Enzyme Activity

Quantification of activity of root phosphatases for papaya seedlings were carried out based on procedure given by KAPOOR & al. 1989, KHADE & al. 2010, SUKHADA 1992. Two plants per treatment were considered for assay of root phosphatases in papaya plants. Root samples were harvested, collected in polyethylene bags and transported to the laboratory on ice. Root samples were washed in ice cold distilled water to remove soil debris and were dried with blotting paper. One gram of root tissue was weighed per plant per treatment. The study comprised of two repetitions per treatment and each repetition had five replications. Activities were assayed separately for acid root phosphatases and alkaline root phosphatases. In all, 10 readings were recorded per treatment per type of assay.

Extraction of enzyme – Enzyme was extracted by macerating 1g 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 r. p. m 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.

Protein estimation – Protein content of the enzyme extract was estimated by Lowry's method (LOWRY & al. 1951) using bovine serum albumin as standard.

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 (pNPP) and 0.8 ml 0.25 M Tris HCl (buffer 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 (pNPP) and 0.8 ml 0.25 M Sodium acetate (buffer pH 6.0). The reactions in both the above mentioned cases were terminated by adding 2 ml of 1N NaOH. For each set, T'_0 (zero time of incubation) and T'_{30} (30 minutes after incubation) were taken separately for alkaline and acid root phosphatase activity. Optical density (O.D) was read at 410 nm 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/min/µg protein.

Statistical Analysis

Data on acid and alkaline root phosphatase activity for all the treatments was compared by analysis of variance (ANOVA) using statistical package mstac. Standard error was calculated for each parameter undertaken for the study.

Results and Discussion

Many fruit tree species are dependent on AM fungal colonization for survival and growth (POWELL & SANTHANAKRISHNAN 1986). The present study was carried out in poly house under nutrient poor and acidic soil conditions. Here, at the end of four months, maximum linear growth was recorded in papaya plants inoculated with *G. mosseae* followed by papaya plants inoculated with mixed inoculum (MI) and *G. intraradices* as com-

pared to un-inoculated controls (Fig. 1) (Data not shown). These observations support the earlier findings of REDDY & al. 1996 who reported that *G. mosseae* (ICRISAT) was the most efficient fungus for improving plant biomass of papaya plants. JAIZME-VEGA & AZCON 1995 reported *G. fasciculatum* (Thaxter) GERD. & TRAPPE emend. WALKER & KOSKE to be the most efficient fungus in improving growth of papaya, pineapple and banana under green house and field conditions. ROSALIND PADMA & KANDASWAMY 1990 reported increase in plant biomass of papaya after 90 days of growth by application of 75% of recommended dose of phosphorus along with mixed inoculum (*G. mosseae* + *G. fasciculatum* + *Gi. margarita* BECKER & HALL) than control plants. These inter-specific variation in the efficiency or growth promoting abilities of AM fungi could be attributed to the mechanism of mycorrhizal colonization development (SANDERS & al. 1977), the physiological differences between the fungi in rate of nutrient uptake, translocation and release (GIANINAZZI-PEARSON & GIANINAZZI 1976).

Phosphatase activity may be detected in several ways, however the use of P-nitrophenyl phosphate (pNPP) as a substrate in quantitative measurement of endogenous soil phosphatase and extracellular phosphatase of plant and micro-organism has dominated due to its convenience and is incorporated in the present study. Phosphatases (P-ases), classified either as acid or alkaline, constitute an enzyme group which is presumed to catalyze the hydrolysis of several organic phosphate-monoesters liberating available inorganic phosphorous, and occurring scattered in all tissue cells of plant organs (JUMA & TABATABAI 1988). Plant species differ in secretion ability and enzyme activity (YAN & al. 2001). Therefore the present study was carried out in yet another newly released variety of papaya Surya under low P- conditions to study the effects of AM fungi on root phosphatase activity.

Observations revealed enhanced activity of root phosphatases in mycorrhizal papaya plants as compared to control plants (Fig. 2). Acid root phosphatase activity was higher than alkaline root phosphatase activity in papaya plants of all the treatments. This is because plants usually secrete acid root phosphatases when P availability is low (YAN & al. 2001). Similarly, GARCÍA-GÓMEZ & al. 2002 reported increased acid root phosphatase activity in papaya plants inoculated with *G. claroideum* SCHENCK & SMITH emend. WALKER & VESTBERG as compared to non- mycorrhizal plants. Further, the findings of the present study is in accordance with SUKHADA 1992 who reported higher acid phosphatase activity in comparison to alkaline phosphatase activity on root surface of five months old papayas inoculated with *G. mosseae* and *G. fasciculatum* in acidic soil conditions.

In the present study, acid root phosphatase activity varied significantly within the treatments (C. D = 3.35; P= 0.05) and ranged from 3.02 – 35.97 n moles of p-nitro-phenol released/min/ μ g of protein in mycorrhizal plants. Control plants recorded a minimum acid root phosphatase

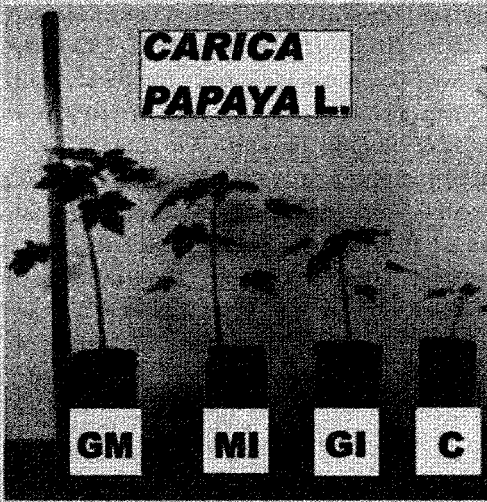


Fig. 1. Response of *Carica papaya* L. cv. Surya plants to inoculation with arbuscular mycorrhizal fungi [C – Un-inoculated seedlings. GI – Seedlings inoculated with *Glomus intraradices*; GM – Seedlings inoculated with *Glomus mosseae*; MI – Seedlings inoculated with mixed inoculum (GI+ GM)].

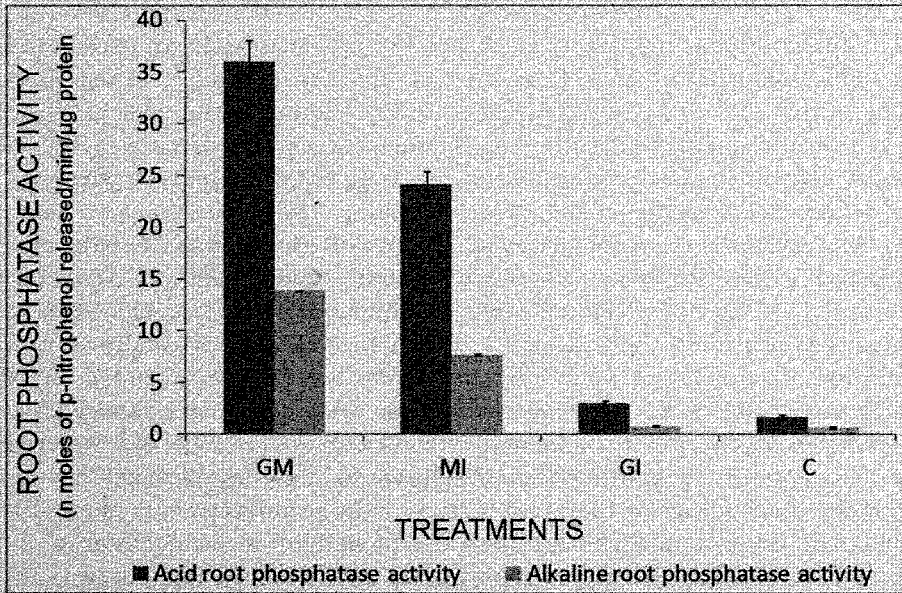


Fig. 2. Effect of arbuscular mycorrhizal fungi on acid root phosphatase activity and alkaline root phosphatase activity in *Carica papaya* L. cv. Surya plants. Error bar indicates \pm SE (n=10). [C – Un-inoculated seedlings. GI – Seedlings inoculated with *Glomus intraradices*; GM – Seedlings inoculated with *Glomus mosseae*; MI – Seedlings inoculated with mixed inoculum (GI+ GM)].

activity of 1.65 n moles of pNPP released/min/ μ g of protein (Fig. 2). Further, alkaline root phosphatase activity also varied significantly within the treatments (C. D = 0.77; P = 0.05). Comparatively higher alkaline root phosphatase activity was recorded in mycorrhizal plants (0.72 - 13.90 n moles of pNPP released/min/ μ g of protein) than in control (0.59 n moles of pNPP released/min/ μ g of protein) plants (Fig. 2).

It is reported that enhanced enzymatic activity of root phosphatases in mycorrhizal plants was closely related to increased mycorrhizal colonization (KAPOOR & al. 1989) and increased growth rate (KAPOOR & al. 1989, SUKHADA 1992). Further, improved growth of mycorrhizal plants is often related to more efficient uptake of phosphorus (P) from soil (BOLAN 1991). Higher rates of P uptake and higher tissue P content have been reported in mycorrhizal plants compared to controls (MORIN & al. 1994). Depending on host plant-AM fungus combinations and pedo-climatic conditions, different amounts of P are necessary to obtain growth increments comparable to those observed in mycorrhizal plants (GEDDEDA & al. 1984, JOHNSON 1984). Thus in the present study, enhanced root phosphatase activity can be related to increased acquisition of phosphorus by inoculated papaya plants as compared to un-inoculated control plants. This can be explained by the fact that root phosphatases, by breaking down complex phosphates (penta and hexa phosphate salts of aluminum and calcium) make P available to the plants (SUKHADA 1992), and root colonization in mycorrhizal plants helps to efficiently take up P from the soil thus resulting in improved growth.

In the present study, *G. mosseae* was the most effective AM fungal species in enhancing the root phosphatase activity (acid and alkaline) in Surya variety of papaya and it was followed in descending order of effectiveness by mixed inoculum and *G. intraradices*. This is in accordance with the findings of SUKHADA 1992 who reported higher acid phosphatase activity and alkaline phosphatase activity on root surface of five months old *Coorg Honey* dew variety of papaya inoculated with *G. mosseae* as compared to *G. fasciculatum* in acidic soil conditions. Further, ALARCON & al. 2002 also reported higher soluble and extractable root acid phosphatase activity in the *Red Maradol* variety of papaya inoculated with *G. claroideum* under low P conditions.

Thus, the selection of an efficient AM fungal species and the choice of the genotype which can get maximum benefits of the symbiosis are two important factors which may improve the existing plant - fungus association (MENGE 1983, KRİKUN & al. 1987). However, the widespread occurrence of AM fungi in soils throughout tropical regions has sometimes led to the notion that inoculation of soils with AM fungi is not essential. But, inoculation is necessary where the fungi have been eliminated or their populations are reduced by pesticide application, fumigation, erosion, or other forms of soil disturbance. In some instances, indigenous AM fungi

may either express their symbiotic effectiveness after a prolonged lag phase, or their inherent effectiveness may be too low, and thus this needs to be preempted by more aggressive, highly effective AM fungal inocula (HABTE & FOX 1993). Thus, the results of the present study upholds the view that inoculation with AM fungi helps in increment of root phosphatase activity and growth in new variety of papaya viz., Surya grown in acidic, nutrient poor soils under poly-house conditions. In view of above, use of AM fungi should be part of papaya management programme highlighting its role in increasing the productivity of papaya in the tropics.

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