Evaluation, grafting success and field establishment of cashew rootstock as influenced by VAM fungi

R Lakshmipathy¹, A N Balakrishna¹, D J Bagyaraj¹², D A Sumana¹ & D P Kumar²

¹Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK Campus, Bangalore 560 065, India
²College of Horticulture, University of Agricultural Sciences, Mudigere 577 632, India

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Seven isolates of vesicular arbuscular mycorrhizal (VAM) fungi were isolated from cashew rhizosphere soil of different cashew growing regions of South India. These seven isolates along with two more VAM fungi namely Acaulospora laevis and Glomus mossea, which were found to be better symbionts for cashew during our earlier study were used to study their effectiveness on the growth and nutrition of cashew rootstock Ullal-1. Four promising VAM fungi were selected based on this study. Rootstocks inoculated with these four fungi were evaluated for their vigour through grafting success, using Ullal-3 cashew variety as scion. Grafting success was more in rootstocks inoculated with A. laevis and one of local isolates Glomus etunicatum. Grafts with rootstock treated with G. etunicatum and A. laevis survived and performed better when planted in the field compared to the uninoculated and other VAM fungal treatments.

Keywords: Cashew, Graft, Rootstock establishment, VAM fungi

Vesicular arbuscular mycorrhizal (VAM) fungi, a unique group of soil fungi forming symbiotic association with higher plants, which facilitates uptake of plant nutrients such as P, S, Zn, and Cu is well documented¹. VAM fungi have been found to occur in more than 70% of the plant species growing in tropics and subtropics². They are distributed over a broad ecological range from aquatic to desert environments³. VAM fungi with their extramatricular hyphal network beyond the root zone enhances P uptake especially in soils with low available P status, thus improving plant growth through P nutrition. There are some reports, which have shown that the stunted growth in citrus seedlings is due to elimination of VAM fungi by fumigation of nursery soil to eradicate root pathogens. VAM inoculation of such seedlings reversed these ill effects⁴. The benefits accrued by the host plant due to mycorrhizal inoculation have been much more clearly studied in field crops than in plantation crops⁵.

Cashew is an important plantation and cash crop in India, Africa and other tropical countries. It is generally grown in wastelands and leached soils with low fertility status, where probably no other commercial crops grow well. It is under such conditions that mycorrhizal dependency of the host plant becomes more evident and pronounced. Cashew planting operations are usually taken up during the onset of monsoon. The rootstocks are usually grafted with a suitable scion and the grafting success is low ranging from 30-40%. Cashew graft when planted in the field have very low rate of survival, because of poor root system development⁶. Further, heavy rains in the season favour root pathogens. VAM fungi play an important role in protecting the plants against root pathogens and also allowing the plants to withstand transplant shock, thus enabling better establishment when planted in the field site⁷⁸. Earlier studies have brought out the importance of screening and selecting efficient VAM fungi for inoculating a particular host⁹¹⁰. In the present study, an attempt was made to screen and select effective VAM fungi that could be used for inoculating the cashew root stock in order to enhance growth rate and grafting success, and make them establish better when planted in the field.

Materials and Methods

Screening of VAM fungi—Different VAM isolates used in this study were maintained in a glasshouse on Rhodes grass as the host in sterilized sand : soil mix (1:1; v/v) in plastic pots. The sand soil mix along with
hyphae, spores and root segments were used for inoculating the root stocks. The inoculum potential (infective propagules/g of inoculum) of the cultures was estimated using the most probable number (MPN) method with ten-fold dilution, as outlined earlier.

Healthy seeds of cashew variety Ullal-1 (as rootstock) were sown in polyethylene bags of size 25x15 cm, holding 2 kg of unsterilized sand: soil: compost in the ratio of 1:1:0.25, as practiced by farmers in this region. The pH of the substrate used was 5.8 having an organic matter content of 3.10% and available phosphorus of 35 kg/Ha. VAM inoculum containing 12,500 infective propagules (IP) was added 5 cm below the seed to each poly bag as per the treatment and one seed per bag was sown. There were 9 treatments (9 VAM fungi + 1 uninoculated control) and each treatment was replicated 40 times. The experiment was taken up in the monsoon season of the year 2000. Plants were maintained for 120 days in a poly house.

Out of 40 plants in each treatment, 10 plants were used to study the growth parameters and the remaining 30 plants in each treatment were used for studying grafting success. The plant height, number of leaves, stem girth, root and shoot biomass were determined after 120 days of sowing. Stem girth was measured using Vernier calipers. The root and shoot samples were oven dried at 60°C and dry biomass determined separately.

Staining of root segments was carried out as per the procedure proposed by Philips and Hayman and per cent mycorrhizal root colonization was determined by the gridline intersect method proposed by Giovanetti and Mosse. Soil samples (50 g) were collected from each polyethylene bag and subjected to wet sieving and decantation as outlined by Gerdemann and Nicolson to estimate the population of VAM spores. Phosphorus content of the plant samples was estimated by vanadomolybdate phosphoric acid yellow colour method as described earlier.

**Grafting success**—Four VAM fungal isolates which were found to be promising in the screening trial viz. *Acaulospora laevis*, isolates from Kerekatte, Ullal and Thrissur were tested for their influence on grafting success. Root stocks inoculated with these four fungi separately were grafted at different intervals viz., 30, 60 and 90 days after sowing to see the per cent success of grafting. Each treatment had 10 replications. The scion used for grafting was the cashew variety ullal-3. The grafting method adopted was epicotyl grafting. Vigour of the root stocks was calculated multiplying germination percentage by total dry matter produced.

**Field trial**—Field establishment of the grafts inoculated with different VAM fungi versus uninoculated grafts was studied. Grafts on 90 day old root stocks, with higher grafting success, were used in this field trial. The experiment was taken up at Cashew Research Station, University of Agricultural Sciences (Bangalore), Ullal. The cashew grafts were planted in pits of size 0.6 m² following Randomised Complete Block Design (RCBD) with spacing of 3x3 m. There were 10 replications in each of the treatments i.e. successful grafts with their rootstocks inoculated with i) *A. laevis*; ii) *Glomus etunicatum*; iii) Ullal isolate; iv) Thrissur isolate; and v) grafts with no VAM inoculation to the rootstock. Survival rate, plant height, stem girth and number of branches per graft were monitored at 30 days interval up to 180 days but only the final observations are presented in this paper.

Identification of VAM isolates—The isolate obtained from Kerekatte which proved to be the best among the VAM fungi studied, was mass multiplied and the spores were collected by wet sieving and decantation method. Spores were surface sterilized using 200 ppm of streptomycin sulphate for 5 min and with chloramidine-T (0.2%) for 5 min. These sterilized spores were then mounted on a glass slide with a drop of lacto-glycerol. Spore shape, colour, surface characters and hyphal attachment were observed under an Olympus binocular microscope. The spore size, spore wall thickness and hyphal length were measured using an ocular micrometer. Identification was done using the manual for the identification of VAM fungi by Schenck and Perez.

Data generated were subjected to statistical analysis of completely randomized block design.

**Results and Discussion**

**Symbiotic response of cashew root stock to different VAM fungi**—Inoculation with VAM fungi improved the growth of cashew root stock. Inoculation of *A. laevis* resulted in maximum plant height (42.8 cm), number of leaves/plant (18), stem girth (0.76 cm), total biomass (10.60 g/plant) and P uptake (1.37 mg/plant). Next best treatment was VAM isolate...
from Kerekatte and the least was in case of uninoculated plants (plant height (37.10 cm), number of leaves/plant (15), stem girth (0.68 cm), total biomass/plant (5.58 g/plant) and P uptake/plant (0.44 mg/plant)). Several workers have reported that species and strains of VAM fungi differ to the extent by which they increase nutrient uptake and plant growth, and host preference among VAM fungi.

Inoculation with VAM fungi increased the P-content of the shoot and root compared to uninoculated plants. Various mechanisms have been suggested for increased P-uptake by mycorrhizal plants viz., (a) the external hyphae of VAM fungi exploring greater volume of soil for phosphorus away from the root; (b) smaller radii of hyphae related to roots enabling them to exploit smaller pores and adding surface area to the absorptive system; and (c) effective phosphorus acquisition by external hyphae by production of extra cellular acid phosphatases which catalyze the release of phosphorus from organic complexes in the soil. However, maximum plant P-content was observed in plants inoculated with A. laevis and G. etunicatum which was significantly more compared to most of the other treatments. The treatments in which P uptake was significantly more also recorded a significant growth improvement. Similar observations have been made earlier in citrus and teak. Per cent mycorrhizal root colonization and spore numbers per 50 g root zone soil was also highest in the plants inoculated with A. laevis (75.87 % and 341 spores 50 g soil) and least in the uninoculated plants (37.87 % and 107 spores 50 g soil). The VAM root colonization as well as spore numbers were maximum in A. laevis and G. etunicatum inoculated plants thus bringing out a positive relation between intensity of mycorrhization and plant growth response. This is in accordance with the observations made by earlier workers.

Evaluation of vigour of the root stock through grafting success—VAM fungal inoculation significantly improved the vigour of rootstocks as compared to uninoculated plants. The vigour was highest in plants inoculated with A. laevis. The next best treatments were the isolates from Kerekatte, Ullal and Thrissur (Table 1).

The percentage grafting success improved in rootstocks when inoculated with VAM fungi. However, maximum grafting success was encountered when inoculated with A. laevis and the isolate from Kerekatte. Grafting success was also more when 90 days old rootstock was used compared to 60 days old rootstock. Thirty days old rootstock resulted in the least grafting success. Grafting success was significantly more in rootstocks inoculated with A. laevis and G. etunicatum. This could be mainly because of the increased stem girth and vigour of the rootstocks due to VAM fungal inoculation. Earlier workers have observed increased stem girth and vigour in VAM fungal inoculated trifoliate orange rootstocks which were ready for budding 5-6 months early compared to uninoculated plants.

**Identification of VA mycorrhizal isolate**—The isolate obtained from Kerekatte proved to be the best among the isolates obtained from different cashew growing regions of Southern India in enhancing growth of cashew rootstock. Spores of this isolate were globose, yellowish brown with a diameter ranging from 88-128 µm. Spore surface was smooth to dull roughened with a single subtending hypha. Sporocarps were absent. These characters confirmed that it belonged to the genus *Glomus*.

The spores were formed singly, with spore wall thickness 10-15 µm, composed of an ephemeral outer wall up to 5 µm thick and a persistent yellow to brown laminated inner wall 2-8 µm thick. Spores with single subtending hypha thickened by the extension of the inner spine wall up to 30 µm. Spore contents separated from hypha by a thin curved septum. Based on the above characters the fungus was identified as *Glomus etunicatum*.

**Evaluation of the performance of VAM inoculated cashew grafts in the main field**—The establishment of grafts inoculated with *A.laevis* and *G. etunicatum*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vigour</th>
<th>Grafting success (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAS</td>
<td>30</td>
</tr>
<tr>
<td><em>A. laevis</em></td>
<td>1060</td>
<td>40</td>
</tr>
<tr>
<td><em>G. etunicatum</em></td>
<td>1033</td>
<td>70</td>
</tr>
<tr>
<td>Ullal-Isolate</td>
<td>981</td>
<td>30</td>
</tr>
<tr>
<td>Thrissure-Isolate</td>
<td>921</td>
<td>40</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>587</td>
<td>30</td>
</tr>
<tr>
<td>DAS-Days after sowing</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
(Kerekatte isolate) was 100% followed by 75% establishment in grafts inoculated with isolate from Ullal and Thrissur (Table 2). Field establishment was least (63.0%) in case of uninoculated grafts. Plant height was significantly higher in case of grafts inoculated with G. etunicatum followed by those treated with A. laevis and the isolates from Thrissur and Ullal. The height of un inoculated grafts was the least. Stem girth of the established grafts in the main field was significantly more in all the four VAM inoculated grafts compared to the uninoculated grafts, but it was highest in grafts inoculated with G. etunicatum followed by those inoculated with A. laevis. A similar trend was observed in the number of branches/plant. Grafts with rootstock treated with A. laevis and G. etunicatum established and performed better when planted in the field compared to uninoculated control grafts. This is perhaps due to enhanced rooting induced by VAM fungal inoculation which helps to withstand transplant shock better, and establish and perform well when planted in the field site. The simple method of inoculating polythene bags before sowing with small quantities of efficient VAM inoculum may easily be followed by growers.

References

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Survival (%)</th>
<th>Plant height (cm)</th>
<th>Stem girth (cm)</th>
<th>No. of branches graft $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. laevis</td>
<td>100</td>
<td>70.7</td>
<td>1.49</td>
<td>4</td>
</tr>
<tr>
<td>G. etunicatum</td>
<td>90</td>
<td>79.5</td>
<td>1.53</td>
<td>6</td>
</tr>
<tr>
<td>Ullal-Isolate</td>
<td>75</td>
<td>57.7</td>
<td>1.13</td>
<td>2</td>
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<tr>
<td>Thrissur-Isolate</td>
<td>75</td>
<td>65.6</td>
<td>1.29</td>
<td>3</td>
</tr>
<tr>
<td>Uninoculated</td>
<td>63</td>
<td>51.6</td>
<td>0.97</td>
<td>3</td>
</tr>
<tr>
<td>LSD (P&lt;0.05)</td>
<td>11.5</td>
<td>8.2</td>
<td>0.18</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2—Field performance of cashew grafts inoculated with different VAM fungi.