Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; SP2: 204-209

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National Conference on Conservation Agriculture
(ITM University, Gwalior on 22-23 February, 2018)

Improving performance of cutting derived pomegranate plants using *Arbuscular mycorrhizal* Fungi

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Abstract
Food and Agricultural Organization has characterized Conservation Agriculture as a practice which maintains a permanent or semi-permanent organic soil cover. The soil micro-organisms and soil fauna take over the tillage function and soil nutrient balancing. Thus, soil microbes can be considered as an important functionary of conservation agriculture. To confirm the utility of microbes and to quantify the influence of arbuscular mycorrhizal fungi (AMF) on performance of pomegranate plants derived from hard wood cuttings (*Punica granatum* L.), a pot culture experiment was conducted using hard wood cuttings of pomegranate cv. 'G-137'. Four different AMF strains were used and their effect on hardwood cuttings of pomegranate cv. 'G-137' was studied with respect to cutting success, growth, physiological and biochemical attributes of cutting derived plants. Amongst the AMF strains used for inoculation, *Funneliformis (Glomus) mosseae, Rhizophagus (Glomus) manihotis* and mixed AMF strain were found effective in improving most of the growth, physiological and biochemical attributes. *Funneliformis mosseae* inoculated cuttings registered the highest cutting success (82.59 % at 180 days after inoculation) and the maximum number of primary roots per cutting (13.20 and 15.80 at 90 and 180 DAI, respectively). However, cuttings inoculated with mixed AMF strain synthesized the maximum total phenols (30.53 mg Catechol equivalent /100g fresh weight at 90) and total chlorophyll (3.96 and 3.97 mg/g FW at 90 and 180 DAI, respectively). Inoculation with the arbuscular mycorrhizal fungi helped to improve cutting success by reducing drying of sprouted cuttings due to their certain role in increasing soil exploring capacity of roots and enhancing root biomass. Arbuscular mycorrhizal fungi infection had led to the improved water relations and better physiological and biochemical functioning of cutting derived plants.

Keywords: *Arbuscular mycorrhizal* fungi, cutting success, pomegranate

Introduction
Pomegranate (*Punica granatum* L.), which belongs to the family Lythraceae is widely grown in tropical and subtropical regions of the world[1]. Because of its versatile adaptability, hardy nature and low cost maintenance, it grows well in resource-poor arid and semi arid regions across the globe. It is an ideal candidate fruit crop for utilizing sub-marginal and degraded lands having pH as high as 8.5. It is grown for its sweet acidic fruits and also valued for its immense neutrceutical and therapeutic properties [2, 3]. In India except Deccan Plateau, pomegranate is commercially propagated through stem cutting especially hard wood cutting. Success rate of propagation through hardwood cutting varies depending upon the variety, type of wood, planting medium and season. Feasibility of pretreating the cuttings with fungicides, antibiotics and surface sterilizing agents to produce healthy planting material are some of the advantages of cutting over air layering- another commercial method of propagation practiced in Deccan Plateau region of India. Moreover, propagation through cutting is low cost, less labour intensive and easy, all these attributes make this technology more farmer friendly. Utilization of arbuscular mycorrhizal fungi at nursery stage can play a crucial role in production of quality planting material with high propagation success through cuttings.
Food and Agricultural Organization has characterized Conservation Agriculture as a practice which maintains a permanent or semi-permanent organic soil cover. The soil micro-organisms and soil fauna take over the tillage function and soil nutrient balancing. Thus, soil microbes including Arbuscularmycorrhizal fungi (AMF) can be considered as an important functionary of conservation agriculture.
Fruit crops, like most horticultural plants commonly develop AMF relationships and exhibit a

high degree of dependence on this symbiosis for normal development and improved field performance [4]. Arbuscular mycorrhizal fungi (AMF) helps in improving nutrient acquisition capacity of the host plant, enhances biomass productivity and act as bioprotectant against pathogens [5]. Efficient inoculation of AMF offers the scope for improving performance of plants raised through hardwood cutting and reducing transplant stress by changing the morphology of the root system favouring the establishment of plants [6]. Therefore, the present study was undertaken to evaluate the impact of different AMF strains on growth, physiological and biochemical attributes of plants raised through hardwood cutting.

Materials and Methods

Plant material

The studies were carried out at the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi and ICAR-NRC on Pomegranate, Solapur. Hardwood cuttings were collected from healthy grown mature plants of cultivar G-137; a superior clone of cv. Ganesh, maintained in the Experimental Orchard of IARI for AMF inoculation. Cuttings of 23-25 cm long and 0.8-1.2 cm diameter were prepared for planting.

Microbial inoculum source

Pure cultures of AMF were procured from the Division of Microbiology, IARI. Four AMF strains namely, *Funneliformis (Glomus) mosseae* (T_1), *Acaulospora laevis* (T_2), *Rhizophagus (Glomus) manihotis* (T_3) and mixed AMF strain (T_4) were used along with non-inoculated control (T_0). Pure AMF cultures were maintained under glasshouse conditions in plastic pots filled with sterile potting mixture comprising of sand, soil and Farm Yard Manure (FYM) in 2:1:1 (v/v). Microbial treatments consisted of approximately 20 g rhizosphere soil containing spores and root segments of host plant, i.e. Bahia grass, *Paspalum notatum* Fluegge **(**Bogdan**)**. The spore density of soil based carrier ranged between 80 and 120 spores per g soil.

AMF inoculation procedure and root colonization

Hardwood cuttings of pomegranate cv. G-137, were planted in plastic pots filled with sterile sand, soil and FYM (2:1:1) (medium autoclaved for 2 h at 121 ºC three times). Cuttings were planted after treatment with 1000 ppm IBA solution for 5 minutes and transferred to plastic pots containing the mixture of sterile sand, soil and FYM (2:1:1, v/v) in plastic pots. Approximately 20g of different soil based AMF inocula were spread in a layer at the base of the cuttings. The cuttings were watered immediately and maintained under controlled glasshouse conditions (temperature ranging from 27± 2ºC and humidity was maintained at 85 %)

Rootlets were assessed for root colonization by staining method [7]. Washed root segments from the B-region (elongation zone) were collected for root colonization study. Extent of root colonization was assessed in ten segments, averaged and expressed as a percentage of root length.

Growth, physiological and biochemical attributes of AMF inoculated cutting raised pomegranate plants

Among growth attributes plant height and cutting success, number of primary roots, shoot length, leaf area, shoot dry weight and root dry weight were studied. Plantlets were sampled at 90 and 180 days after inoculation (DAI). Relative Water Content (RWC), photosynthesis rate and respiration rate were recorded. The RWC of the recently matured leaf was determined [8]. Photosynthetic rate and respiratory rates were measured using Infra Red Gas Analyser (LI-COR 6200, chamber size 6.5cm^2) from five mature leaves on each plant and expressed as µmolCO_2/m^2/s. Among biochemical parameters total phenols, reducing sugar, total chlorophyll and polyphenol oxidase activity were quantified.

Phenols: Total phenols were quantified using shoot tips along with a pair of freshly emerged leaves by using methodology as suggested by Malik & Singh [9]. Foliar samples (approximately 500 mg of FW) was homogenized in a mortar by adding 80 per cent ethanol. It was then centrifuged at 10,000 rpm for 20 min and the supernatant was filtered through Whatman No. 42 filter paper. The residue on the filter paper was re-extracted (5 times) with 80 % ethanol and the supernatant collected were evaporated to dryness. Residues were dissolved in 5 ml of distilled water, from which 0.2 ml was taken and total volume was made upto 3 ml with distilled water. To this fresh Folin-Ciocalteau reagent (0.5 ml) was added. After 3 min, 2 ml of 20% Na_2CO_3 (sodium carbonate) solution was added in each tube, mixed thoroughly and the tubes were placed in a hot water bath (58ºC) exactly for one min. It was then cooled to room temperature and then absorbance (650 nm) was measured against the blank. From the standard curve, amount of total phenols present in the sample was calculated using catechol.

Reducing sugars: Reducing sugars were estimated at 90 and 180 DAI [10]. Composite leaf samples (100mg of FW) were weighed and extracted with hot 80 % ethanol twice (5 ml each time). The supernatant were collected and evaporated to dryness at 80˚C. Then, 10 ml distilled water was added to dissolve the sugars. From this, 0.2 ml aliquot was pipetted out to separate test tubes and volume was made to 2 ml with distilled water, 1 ml of alkaline copper tartarate was added followed by keeping in water bath at 100˚C for 10 minutes. Tubes were cooled and 1 ml of arseno-molybdic acid reagent was added to all the tubes. The volume was then made to 10 ml with distilled water and absorbance of blue color was recorded at 620 nm after 10 minutes. From the standard curve, amount of reducing sugars present in the sample was calculated.

Total chlorophyll: Leaf total chlorophyll was determined at 90 and 180 DAI [11] and calculated according to the following formula:

$$\text{Total chlorophyll (mg/g)} = \frac{(20.20 \times OD645) + (8.02 \times OD663) \times \text{Volume} \times \text{Dilution}}{1000 \times \text{Weight of sample}}$$

Polyphenol oxidase: Samples consisting of 200 mg of shoot tips along with six newly emerged fully open leaflets were chosen instead of 1.0 g of shoot tips alone due to scarcity of plant material. Polyphenol oxidase was extracted at 4˚C by macerating 200 mg of chopped sample in pre-chilled pestle and mortar with 10.0 ml of 100 mM phosphate buffer of pH 7.3 containing 10 mM sodium ascorbate. Thereafter, the extract was filtered through four layers of muslin cloth. The filtrate was treated for 20 minutes with 2.0 ml of 1.5 per cent Triton X-100 solution prepared in 100 mM phosphate buffer (pH 7.3). Final volume of extract was made upto 25.0 ml with 100 mM phosphate buffer of pH 7.3 containing 10.0 mM sodium ascorbate. It was then centrifuged at 15,000 rpm for one hour at 4˚C. The aliquot so obtained was used as an enzyme source. Subsequently, the final volume of extract was reduced to 5.0 ml.

Polyphenol oxidase assay: Both catecholase and cresolase activities were measured 90 and 180 days after inoculation as per the methodology reported by Valero *et al.* [12] with some modifications.

Catecholase activity was determined by using 30 mM 4-methyl catechol, in 10.0 mM sodium acetate buffer (pH 4.5) as substrate. To this substrate 0.5 ml of crude polyphenol oxidase extract and 4.0 ml of 100 mM phosphate buffer (pH 7.3) were added. Then 0.5 ml of 30 mM 4-methyl catechol made in 10 mM sodium acetate buffer of (pH 4.5) was added to the resultant mixture at zero time. The increase in absorbance owing to the formation of4-methyl-0-benzoquinone was measured spectrophotometrically at 400 nm at 30°C. Then absorbance was recorded after 8 min. The enzyme activity was represented as Δ400/g/min (change in absorbance per gram of fresh weight per minute).

For measuring cresolase activity, 0.5 mM 4-methylphenol (p-cresol) made in 10.0 mM phosphate buffer of pH 7.0, was used as substrate. To this substrate 0.5 ml of crude enzyme extract and 4.0 ml of phosphate buffer (pH 7.0) were added. To this reaction mixture, 0.5 ml of 0.5 mM p-cresol made in 10 mM phosphate buffer (pH 7.0) was added at zero time. Cresolase activity was measured spectrophotometrically at 400 nm after 8 min. The cresolase activity was represented as Δ400 /g/min.

Statistical analysis

The experiment was set up in a completely randomized design with five replications in each treatment having fifteen units per replication. The analysis of variance was carried out to detect the significant differences among the treatment means and Pearson product moment correlations between each pair of variables was done by using SAS 9.2 software at the IASRI, New Delhi and ICAR-National Research Centre on Pomegranate, Solapur, Maharashtra, India during 2014-15.

Results and Discussion

Effect of AMF strains on cutting success and growth related attributes of cuttings

Data presented in Table 1 reveal significant improvement in cutting success due to AMF inoculation. All the mycorrhized cuttings (root colonization ranged from 78.80 to 85.95 percent at 180 DAI) gave higher cutting success as compared to non-inoculated control (8.30 percent root colonization). Hardwood cuttings inoculated with different strains of AMF strain registered cutting success ranging from 79.00 to 82.59 per cent at 180 DAI and the results are significantly superior to control (Table 1). At 180 DAI, the cutting success with *F. mossae*, *A. laevis* and mixed AMF were at par and significantly better than cutting success with *F. manihotis*. The higher survival rates of mycorrhiza inoculated cuttings might be due to an increased rhizosphere exploring volume through the differentiation of extra-radical mycorrhiza mycelia which initially improves water uptake and consequently nutrients uptake [13, 14, 15]. The higher cutting success could also be attributed to the increased root biomass, branching and rapid development of functional root system due to AMF infection [16], though, many cuttings initiate rooting but roots for some of the cuttings either dry up or rot due to fungal infections. In the Pearson product moment correlations (Table 4) also cutting success has been found positively and significantly correlated with number of primary roots indicating availability of more roots for microbial infection and ultimately higher soil exploration capacity of the cutting derived plants.

The cuttings inoculated with *Funneliformis mosseae* produced the maximum number of primary roots (13.20 and 15.80 at 90 and 180 DAI, respectively) and longest shoots (64.60 and 81.23 cm at 90 and 180 DAI, respectively), these results were significantly higher than other treatments and control (Table 1). These results were in agreement with the findings of Rupnawar & Navale [17] and Aseri *et al,* [18].

It was found that cuttings inoculated with AMF produced higher shoot and root dry weights than non mycorrhized control. The interaction between cutting success and days after inoculation has been found significant indicating the increase in root biomass due to mycorrhizal infection with progressing time duration (Table 5). Root colonization by AMF found to be positively and significantly correlated with leaf area, shoot dry weight and root dry weight. The higher biomass production could be attributed to the enhanced photosynthetic rate due to more uptake of water and nutrients by plants [19]. Earlier, Rupnawar & Navale **17** also observed increased biomass and dry matter formation in mycorrhized plants of pomegranate.

Effect of AMF strains on physiological attributes of cutting derived plants

Effect of AMF inoculation on physiological parameters of plantlets was very visible and inoculation with microbes considerably improved the recorded physiological activities. Amongst various AMF treatments, *R. manihotis and A. laevis* treated plants registered at par and high relative water content (RWC) of leaves at 90 DAI and both of these along with *F. mossae* produced similar results at 180 DAI (Table 2). All the mycorrhizal treatments were significantly superior over control. The increased RWC could be attributed to improved water uptake through greater exploration of soil volume, improved plant nutrition and regulation of stomatal opening through hormonal biosynthesis as the result of mycorrhiza inoculation [14]. Improved water relations of plants with microbial inoculation may be the key reason for better cutting success. Leaf relative water content had been found significantly correlated with most of the growth, physiological and biochemical attributes of the cutting derived plants (Table 4). AMF inoculation also had significant impact on photosynthesis and respiration. Among the AMF strains tried, *Funneliformis mosseae* inoculated plantlets registered the maximum photosynthetic rate (4.31 and 4.53μmol $CO_2/m^2/s$ at 90 and 180 DAI, respectively). The result was in conformity with the earlier reports [19]. The increased photosynthetic rate could be attributed to increased leaf area and increased synthesis of chlorophyll [19]. Root colonization by AMF found to be positively and significantly correlated with leaf relative water content and photosynthetic rate (Table 4). Interaction studies also revealed significant interaction between net photosynthetic rate and days after inoculation (Table 5).

Effect of AMF strains on biochemical attributes of cutting derived plants

The maximum total phenols production was found (30.53 and 31.33 mg/100 g FW at 90 and 180 DAI, respectively) in the mixed AMF inoculated cuttings (Table 3). However, at 180 DAI, *Funneliformis mossae* inoculated cutting also produced at par total phenols (29.33 mg/100 g FW). The result was in conformity with the earlier findings of Nelsen & Achar [20]. Microbial inoculated plantlets recorded higher leaf reducing sugars (2.27 and 2.13and 180 DAI, respectively) as compared to control (1.14 and 1.10 mg/g at 90 and 180 DAI,

respectively). The level of reducing sugars was found to decrease with progressing time period in all the AMF treatments and control (Table 3). This indicates that reducing sugars get converted into structural components with time resulted in enhanced vegetative growth of plants. Higher reducing sugar in mycorrhizal plants as compared to control may be attributed to low respiratory rates together with higher photosynthetic rates in mycorrhizal plants (Table 2). It is also evident from the correlation table where negative and significant correlation had been found between net respiration rate and reducing sugar content of the leaves (Table 4). Irrespective of duration, the maximum chlorophyll content was observed (3.96 and 3.97 mg/g at 90 and 180 DAI, respectively) in mixed AMF strain inoculated plantlets (Table 3).Similar results were also obtained by earlier workers [19]. The increased chlorophyll content of leaves could be attributed to enhanced uptake of different nutrients, i.e. Mg, Fe, and Cu which are essential for synthesis of chlorophyll in leaves [21]. This fact is also supported by the positive and significant correlation between total leaf chlorophyll content and dry root weight and more root dry weight means higher uptake of water and nutrients for chlorophyll synthesis (Table 4).

Polyphenol oxidase is one of the important components of plant defence mechanism against rot causing pathogens. There was an increase in polyphenol oxidase activity in the foliar parts of AMF inoculated plants (Figure 1).The activity of cresolase was much lower than the catecholase, which could probably be due to its lag period and greater instability [12]. The increase in polyphenol oxidase activity was due to stimulation resulting from the increased root colonization and symbiotic association between host plants and microbes with the passage of time [22].

Symbiotic association of mycorrhiza with pomegranate roots positively stimulated the growth and development of cutting derived plants. These AMF also perform soil nutrient balancing and help plants in exploring greater volume of soil by their roots thus, improved absorption without tillage. Thus, soil microbes including AMF can be considered as an important functionary of conservation agriculture. For most of the growth, physiological and biochemical attributes of cutting derived plants, *Funneliformis (Glomus) mosseae*, *Rhizophagus (Glomus) manihotis* and mixed AMF strain performed significantly better which may have far reaching consequences in producing the large number of high performing saplings through cuttings and reducing the transplantation shock while transferring these saplings to the field.

Table 1: Effect of AMF strains on growth parameters of pomegranate hardwood cuttings

Treatment	Root Colonization (%)		Cutting Success (%)		No. of primary roots per plant		Shoot length (cm)		Leaf area (cm^2)		Shoot dry wt (g)		Root dry wt. (g)	
	90 DAI	180 DAI	90 DAI*	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
T_0	4.50^{c}	8.30^{b}	75.60^{b}	70.27^{c}	8.2^{c}	11.2^{d}	44.70^{e}	54.23^{e}	1.96^{b}	2.02^{c}	7.51^{b}	11.81^{b}	2.55^{c}	4.58^{b}
T_1	43.70^{a}	85.04^{a}	83.84^{a}	82.59^{a}	13.2^{a}	15.80^{a}	64.60^{a}	81.23^{a}	2.40^{a}	2.26^{b}	9.56^{a}	14.53^{a}	3.23^{a}	5.75^{a}
T_2	40.30^{ab}	78.80^{a}	81.92^{a}	80.62^{ab}	11.0^{b}	13.80^{b}	52.10^{c}	70.46^{c}	2.26^{a}	2.26^{b}	8.94^{a}	13.66^{a}	3.10^{a}	5.65^{a}
T_3	37.00^{b}	80.10^{a}	81.59^{a}	79.00^{b}	11.2^{b}	13.80^{b}	54.90^{b}	72.77^{b}	2.40^{a}	2.70^{a}	9.47^{a}	14.70^{a}	2.64^{bc}	5.71^{a}
T_4	42.15^{ab}	85.95^{a}	81.92^{a}	80.62^{ab}	10.6^{b}	13.0^{c}	50.70^{d}	68.32^{d}	2.30^{a}	2.32^{b}	9.77^{a}	15.18^{a}	3.04^{ab}	6.18^{a}
CD at 5%	6.52	10.14	2.75	2.85	1.00	0.79	1.35	0.76	0.26	0.20	0.99	1.58	0.41	0.66

Values with different letters differ significantly

T0 = AMF free soil; T1 = Funneliformis mosseae; T2 = Acaulospora laevis; T3 = Rhizophagus manihotis; T4 = Mixed (IARI) strain

DAI* = Days after inoculation

Table 2: Effect of AMF strains on physiological attributes of hardwood cutting derived pomegranate plants

Treatment	Leaf relative water content (%)		Net photosynthetic rate (μmol CO_2 /m^2/s)		Net respiration rate (μmol CO_2 /m^2/s)	
	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
T_0	83.00^{c}	84.67^{c}	3.35^{e}	3.36^{d}	4.73^{a}	4.83^{a}
T_1	91.33^{b}	92.67^{ab}	4.31^{a}	4.53^{a}	3.76^{b}	3.56^{d}
T_2	92.67^{a}	93.00^{ab}	4.23^{b}	4.26^{b}	3.96^{b}	3.95^{c}
T_3	92.67^{a}	93.33^{a}	4.01^{d}	4.23^{b}	3.93^{b}	4.03^{bc}
T_4	90.67^{b}	92.00^{b}	4.08^{c}	4.11^{c}	4.36^{a}	4.19^{b}
CD at 5%	1.19	1.18	0.02	0.04	0.39	0.24

Values with different letters differ significantly

Table 3: Effect of AMF strains on biochemical attributes of hardwood cutting derived pomegranate plants

Treatment	Total phenols (mg Catechol equivalent/100g FW)		Reducing sugars (%)		Total chlorophyll (mg/g)	
	90 DAI	180 DAI	90 DAI	180 DAI	90 DAI	180 DAI
T_0	22.23^{d}	23.00^{c}	1.14^{d}	1.10^{d}	2.91^{e}	3.01^{d}
T_1	28.63^{b}	29.33^{ab}	2.21^{b}	2.03^{b}	3.04^{d}	3.19^{c}
T_2	26.37^{c}	28.33^{b}	2.16^{b}	2.01^{b}	3.22^{b}	3.28^{b}
T_3	26.53^{c}	28.67^{b}	2.27^{a}	2.13^{a}	3.15^{c}	3.19^{c}
T_4	30.53^{a}	31.33^{a}	2.09^{c}	1.90^{c}	3.96^{a}	3.97^{a}
CD at 5%	0.99	2.64	0.05	0.04	0.05	0.03

Values with different letters differ significantly

Table 4: Pearson product moment correlations between each pair of variables at 180 DAI

	Cutting Success	No. of Primary Roots	Shoot Length	Leaf Area	Shoot Dry Wt.	Root Dry Wt.	Relative Water Content	Net Photosynthetic Rate	Net Respiration Rate	Total Phenols	Reducing Sugar	Total Chlorophyll	Root Colonization
Cutting Success		0.4571	0.6103	0.31	0.3852	0.145	0.1469	0.2047	-0.1925	0.1849	0.207	0.2828	0.2976
		0.0216	0.0012	0.1316	0.0572	0.4892	0.4833	0.3262	0.3567	0.3763	0.3209	0.1707	0.1485
No. of Primary Roots	0.4571		0.9414	0.1894	-0.0771	-0.1446	-0.1498	-0.0791	0.0978	-0.1508	-0.129	-0.2996	-0.138
	0.0216		0	0.3644	0.7141	0.4903	0.4747	0.7069	0.6418	0.4719	0.539	0.1456	0.5107
Shoot Length	0.6103	0.9414		0.2109	0.1261	0.0486	-0.0662	-0.0205	0.0571	0.0326	-0.0566	-0.0503	0.0039
	0.0012	0		0.3115	0.5482	0.8174	0.7532	0.9223	0.7862	0.8769	0.7881	0.8114	0.9853
Leaf Area	0.31	0.1894	0.2109		0.4185	0.4549	0.5803	0.4732	-0.4105	0.3651	0.6493	0.1212	0.5493
	0.1316	0.3644	0.3115		0.0373	0.0223	0.0024	0.0169	0.0415	0.0727	0.0004	0.564	0.0045
Shoot Dry Wt.	0.3852	-0.0771	0.1261	0.4185		0.6338	0.6066	0.5965	-0.468	0.5912	0.6259	0.493	0.6897
	0.0572	0.7141	0.5482	0.0373		0.0007	0.0013	0.0016	0.0183	0.0019	0.0008	0.0123	0.0001
Root Dry Wt.	0.145	-0.1446	0.0486	0.4549	0.6338		0.6388	0.6105	-0.5378	0.7247	0.6495	0.5678	0.7319
	0.4892	0.4903	0.8174	0.0223	0.0007		0.0006	0.0012	0.0056	0	0.0004	0.0031	0
Relative Water Content	0.1469	-0.1498	-0.0662	0.5803	0.6066	0.6388		0.906	-0.7773	0.7073	0.9656	0.3585	0.9285
	0.4833	0.4747	0.7532	0.0024	0.0013	0.0006		0	0	0.0001	0	0.0784	0
Net Photosynthetic Rate	0.2047	-0.0791	-0.0205	0.4732	0.5965	0.6105	0.906		-0.9232	0.6743	0.9386	0.2385	0.9093
	0.3262	0.7069	0.9223	0.0169	0.0016	0.0012	0		0	0.0002	0	0.2508	0
Net Respiration Rate	-0.1925	0.0978	0.0571	-0.4105	-0.468	-0.5378	-0.7773	-0.9232		-0.5317	-0.8186	-0.1105	-0.7846
	0.3567	0.6418	0.7862	0.0415	0.0183	0.0056	0	0		0.0062	0	0.5991	0
Total Phenols	0.1849	-0.1508	0.0326	0.3651	0.5912	0.7247	0.7073	0.6743	-0.5317		0.708	0.6292	0.793
	0.3763	0.4719	0.8769	0.0727	0.0019	0	0.0001	0.0002	0.0062		0.0001	0.0008	0
Reducing Sugar	0.207	-0.129	-0.0566	0.6493	0.6259	0.6495	0.9656	0.9386	-0.8186	0.708		0.3186	0.9359
	0.3209	0.539	0.7881	0.0004	0.0008	0.0004	0	0	0	0.0001		0.1206	0
Total Chlorophyll	0.2828	-0.2996	-0.0503	0.1212	0.493	0.5678	0.3585	0.2385	-0.1105	0.6292	0.3186		0.5105
	0.1707	0.1456	0.8114	0.564	0.0123	0.0031	0.0784	0.2508	0.5991	0.0008	0.1206		0.0091
Root Colonization	0.2976	-0.138	0.0039	0.5493	0.6897	0.7319	0.9285	0.9093	-0.7846	0.793	0.9359	0.5105	
	0.1485	0.5107	0.9853	0.0045	0.0001	0	0	0	0	0	0	0.0091	

The second number in each location of the table is a P-value which tests the statistical significance of the estimated correlations. P-values below 0.05 indicate statistically significant non-zero correlations at the 95.0% confidence level.

The following pairs of variables have P-values below 0.05: cutting success and number of primary roots, cutting success and shoot length, number of primary roots and shoot length, leaf area and shoot dry wt., leaf area and root dry wt., leaf area and relative water content, leaf area and net photosynthetic rate, leaf area and net respiration rate, leaf area and reducing sugar, leaf area and root colonization, shoot dry wt. and root dry wt., shoot dry wt. and relative water content, shoot dry wt. and net photosynthetic rate, shoot dry wt. and net respiration rate, shoot dry wt. and total phenols, shoot dry wt. and reducing sugar, shoot dry wt. and total chlorophyll, shoot dry wt. and root colonization, root dry wt. and relative water content, root dry wt. and net photosynthetic rate, root dry wt. and net respiration rate, root dry wt. and total phenols root dry wt. and reducing sugar, root dry wt. and total chlorophyll, root dry wt. and root colonization, relative water content and net photosynthetic rate, relative water content and net respiration rate, relative water content and total phenols, relative water content and reducing sugar, relative water content and root colonization, net photosynthetic rate and net respiration rate, net photosynthetic rate and total phenols, net photosynthetic rate and reducing sugar, net respiration rate and total phenols, net respiration rate and reducing sugar, net respiration rate and root colonization, net photosynthetic rate and root colonization, total phenols and reducing sugar, total phenols and total chlorophyll, total phenols and root colonization, reducing sugar and root colonization, total chlorophyll and root colonization.

Table 5: Summary of ANOVA table for DAI and treatment interaction

Type of experiment/assays	Degree of freedom	Type III sum of square	Mean Square	F value	P value
Cutting Success*	4	21.4802	5.37005	16.57	<.0001
No. of Primary Roots	4	0.52	0.13	0.28	0.8914
Shoot Length*	4	135.112572	33.778143	48.62	<.0001
Leaf Area	4	0.25520006	0.06380002	2.04	0.1069
Shoot Dry weight	4	1.907294	0.476824	0.48	0.7529
Root Dry weight*	4	2.06769947	0.51692487	2.99	0.0301
Relative Water Content	4	3.0402974	0.7600743	0.94	0.4517
Net Photosynthetic Rate*	4	0.11670021	0.02917505	58.35	<.0001
Net Respiration Rate	4	0.2063003	0.05157508	0.84	0.5064
Total Phenols	4	5.0717961	1.267949	0.56	0.6959
Reducing Sugars*	4	0.03549995	0.00887499	7.1	0.0002
Total Chlorophyll*	4	0.02969978	0.00742494	8.74	<.0001

Above mentioned Table showing summary of ANOVA for days after inoculation (DAI) and treatment interaction analysis of various assays. P-values less than 0.05 indicate the significant interaction between DAI and treatment at 5% level of significance or in other words significant effect of DAI on various treatments. Significant effect of DAI on various treatments has been observed in the case of cutting success, shoot length, root dry weight, net photosynthetic rate, reducing sugar and total chlorophyll. Whereas in the case of number of primary roots, leaf area, shoot dry weight, relative water content, net respiration rate and total phenols, effect of days after inoculation on various treatment is statistically non-significant

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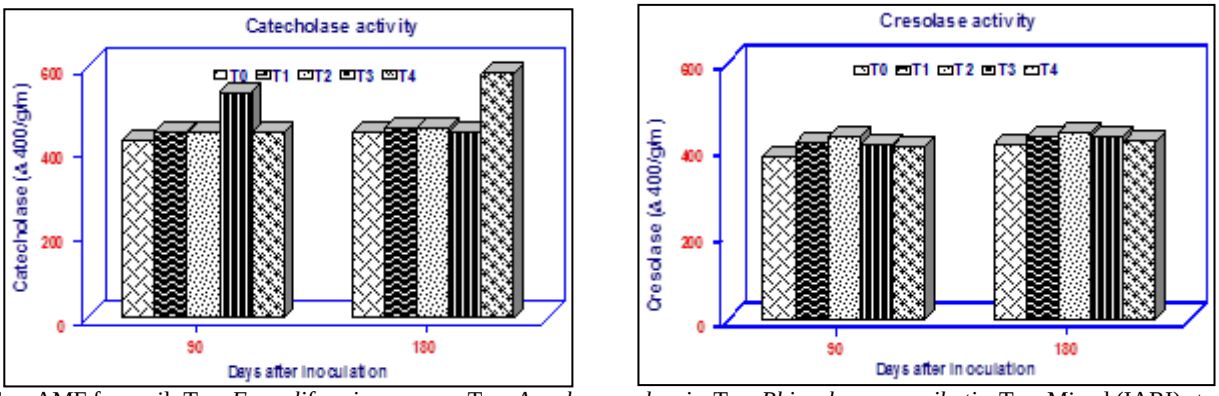

T_0 = AMF free soil; T_1 = *Funneliformis mosseae*; T_2 = *Acaulospora laevis*; T_3 = *Rhizophagus manihotis*; T_4 = Mixed (IARI) strain

Fig 1: Effect of AMF inoculation on polyphenol oxidase activity in cutting derived pomegranate plants

References

1. Jalikop SH. Linked dominant alleles of inter-locus interaction results in a major shift in pomegranate fruit acidity of 'Ganesh' and 'Kabul Yellow'. Euphytica. 2007; 158:201-207.
2. Lansky EP, Newman RA. *Punica granatum* (Pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol. 2007; 109:177-206.
3. Opara LU, Al-Ani MR, Al-Shuaibi YS. Physio-chemical properties, vitamin C content and antimicrobial properties of pomegranate fruit (*Punica granatum* L.). Food Bioprocess Technol. 2009; 2:315-321.
4. Nemec, S., VA mycorrhizae in horticultural systems. *In Ecophysiology of VA mycorrhizal plants*. (ed. Safir, G.R.). CRC, Boca Raton, Florida, USA, 1986, 193-212.
5. Janouskova M, Krak K, Wagg C, Storchova H. Caklova P, Vosatka M. Effects of Inoculum Additions in the Presence of a Preestablished Arbuscular Mycorrhizal Fungal Community. J App. Microbiol. 2013; 20:6507-6515.
6. Bompadre MJ, Colombo R, de Molina MR, Godeas A, Arbuscular Mycorrhizal Fungi in the Alleviation of Oxidative Stress under Cutting Propagation Managemen. In *Mycorrhizas: Structure, Development and Functions* (ed. *Warwick, E.*), NOVA Science Publishers, Hauppauge, New York. 2014, 181-202.
7. Phillips JM, Hayman DS. Improved procedure for clearing and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment for infection. Tr. Br. Mycol. Soc. 1970; 55:158-161.
8. Weatherley PE. Studies in water relation of cotton plants I. Field measurement of water deficits in leaves. New Physiol. 1950; 49:81-89.
9. Malik CP, Singh MB. Plant Enzymology and Histo-Enzymology. Kalyani Publishers, New Delhi, India. 1980, 286.
10. Somogyi M. Notes on sugar determination. J Biol. Chem. 1952; 195:19-23.
11. Barnes JD, Balaguer L, Maurigue E, Elvira S, Davison AW. A reappraisal of the use of DMSO for the extraction and determination of chlorophyll 'a' and 'b' in lichens and higher plants. Environ. Exp Bot. 1992; 32:87-99.
12. Valero E, Sanchez-Ferrer A, Varon R, Garcia-Carmona F. Evolution of grape polyphenol oxidase activity and phenolic content during maturation and vitrification. Vitis. 198; 28:85-95.
13. Elmeskaoui A, Damant JP, Paulin MJ, Piche Y, Desjardins Y. A tripartite culture system for endomycorrhyzal inoculation of micropropagated straw berry plantlets *in vitro*. Mycorrhiza. 1995; 5:313-319.
14. Krishna H, Singh SK, Patel VB. Screening of arbuscular-mycorrhizal fungi for enhanced growth and survival of micropropagated grape (*Vitis vinifera*) plantlets. Indian J Agr. Sci. 2006; 76:297-301.
15. Singh NV, Singh SK, Singh AK, Meshram DT, Suroshe SS, Mishra DC. Arbuscular Mycorrhizal Fungi (AMF) induced hardening of micropropagated pomegranate (*Punica granatum* L.) plantlets. Sci. Hort. 2012; 136:122-127.
16. Berta G, Fusconi A, Trotta A. VA Mycorrhizal infection and the morphology and function of root systems. Environ. Exp. Bot. 1993; 33:159-173.
17. Rupnawar BS, Navale AM. Effect of VA-mycorrhizal inoculation on growth of pomegranate layers. J Maharashtra Agr. Univ. 2000; 25:44-46.
18. Aseri GK, Jain N, Panwar J, Rao AV, Meghwal PR, Biofertilizers improve plant growth, fruit yield, nutrition, metabolism and rhizosphere enzyme activities of Pomegranate (*Punica granatum* L.) in Indian Thar Desert. Sci. Hort. 2008; 117:130-135.
19. Mathur N, Vyas A. Influence of VA mycorrhizae on net photosynthesis and transpiration on *Ziziphus mauritiana*. J Plant Physiol. 1995; 147:328-330.
20. Nelsen R, Achar PN. Stimulation of growth and nutrient uptake by VAM fungi in *Brassica oleracea* var. *capitata*. Biol. Plantarum. 2001; 44:277-281.
21. Eftekhari M, Alizadeh M, Mashayekhi K, Asghari H, Kamkar B. Integration of arbuscular mycorrhizal fungi to grape vine (*Vitis vinifera* L.) in nursery stage. J Adv. Lab. Res. Biol. 2010; 1:102-111.
22. Panwar JA, Vyas A. Changes in peroxidase and polyphenol oxidase activities in roots of *Tamarix aphylla* by different AM fungi. J Eco. Physiol. 2002; 3:103-106.