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Short communication

Adaptation of arbuscular mycorrhizal fungi inoculated *Jatti khatti* (*Citrus jambhiri*) seedlings under water deficit stress conditions

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ABSTRACT

The effect of arbuscular mycorrhiza fungi (AMF), on physiological and biochemical parameters of plants under well watered (WW) and water deficit stress (WDS) conditions were studied under glasshouse conditions. *Jatti khatti* (*Citrus jambhiri*) seedlings were inoculated with two species of AMF, *viz*., *Glomus fasciculatum* and *Glomus intraradices* along with non-mychorrhizal control. The plants were allowed to grow normally upto 120 days after inoculation, thereafter, the plants were subjected to well watered (WW) and water deficit (WDS) treatments for 25 days. *G. fasciculatum* inoculated plants showed maximum root colonization in *Jatti khatti* seedlings under both WW (49.38%) and WDS (49.10%) conditions. Our findings clearly revealed that AMF colonization during WDS improved the growth, physiological, biochemical parameters and had a positive effect on adaptation and water stress mitigation of *Jatti khatti* seedlings.

Key words: Arbuscular mycorrhizal fungi, *Citrus jambhiri*, water deficit stress.

Arbuscular mycorrhizal fungi (AMF) symbiosis can protect host plants against detrimental effects caused by drought stress. Knowledge concerning specific relationships between citrus plants and fungi is important for successful utilization of AM fungi under water deficit stress conditions. *Jatti khatti* is predominantly used as rootstock for Kinnow mandarin in the north western plains of India. It is a preferred rootstock in the region because of its good adaptability to light deep sandy soils. The rootstock is moderately tolerant to salinity and it also induces good fruit quality such as increased fruit size, total soluble solids (TSS) and overall productivity of the trees budded on this rootstock. Keeping in view the importance of AMF in drought stress, an attempt was made to evaluate the effectiveness of AMF strains on *Jatti khatti* seedlings for increasing the drought stress tolerance in citrus. The mechanism of enhancing tolerance to drought stress by AMF strains were studied by observing physiological and biochemical parameters.

Eight-month-old seedlings of citrus rootstock *Jatti khatti* were maintained in a glasshouse with day-night temperatures ranging from 27 ± 1°C. A day length of 16/8 h was maintained at 630 µmol $m^{-2}s^{1}$ by cool-white fluorescent lamps and humidity was maintained at 80-85% using a humidifier. Seedlings were watered on alternate days with sterile tap water. The soil based pure cultures of arbuscular mycorhizal fungi were maintained under glasshouse conditions in plastic pots containing sterile potting mixture of soil: sand: vermicompost (2:2:1). Microbial treatment consisted of 20 g inocula per replication which contained approximately 2400 spores. Seedlings were transferred to plastic pots containing 5.5 kg of autoclaved (0.11 MPa, 121°C, 2 h) soil mixture comprising of soil, vermiculite and sphagnum moss (4:3:1, v/v/v), the characteristics of which were: pH 5.4, 1.1% organic matter, 26.14 mg/kg of available phosphorus, 130.18 mg/kg of alkali hydrolysable nitrogen and 152.26 mg/kg of available potassium. The three treatments were AMF species *Glomus fasiculatum* (S1), *Glomus intraradices* (S2) and a non-mychorrhizal control (NM). Treatment combinations used were AMF inoculated plants subjected to WDS, WW AMF inoculated plants without subjecting to WDS and NM plants with and without WDS. Four replications were used for each treatment. Water stress treatments were started 120 days after transplanting where pots with WW and WDS seedlings were maintained everyday at 75% (corresponding to -0.09 MPa) and 55% (corresponding to -0.40 MPa) relative soil-water content by gravimetric methods, respectively.

The root samples were assessed for colonization after 25 days of WDS treatment by staining method as described by Phillips and Hayman (9). Leaf photosynthesis was measured on five mature leaves from each plant using an Infrared Gas Analyzer (LiCor 6200). Relative water content was determined in leaves by the method suggested by Weatherley (10). The rapid colorimetric method as advocated by Bates *et al.* (2) was followed for the estimation of proline. Total phenols in leaf samples was assayed by the method proposed by Malik and Singh (5). Superoxide dismutase (SOD) assay was based method described by Dhindsa *et al*. (3). Catalase assay was based on

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the decrease in the absorbance over a time period as described by Aebi (1). Ascorbate peroxidase (APX) assay was based on the method described by Nakano and Asada (7). Protein content of the enzyme extract was estimated by Lowry's method.

The experiment was laid out in factorial completely randomized design having two levels of water treatment (WW and WDS) and three levels of mycorrhizal strains (S-1, S-2 and NM), with 4 replications of each. The data were analysed using two factor analysis of variance (ANOVA), using the SPSS software (SPSS System for Windows, version 10.0). Valid conclusions were drawn only on significant differences between treatment means at the $P \leq 0.05$ level of probability.

Maximum root colonization was observed in S-1 inoculated seedlings both under WW and WDS conditions (Table 1). There was no significant difference in the colonization between the two strains both under WW and WDS conditions but colonization decreased with water stress treatment while no colonization was observed in NM seedlings as confirmed microscopically. The AM colonization under WW conditions was higher than that under WDS conditions suggesting a negative effect of arid or semiarid environment for the AM development of host plants. Roots were shorter in WDS seedlings than in WW seedlings (Table 1). However, AM seedlings under WW and WDS conditions had significantly higher root length than corresponding NM seedlings (Table 1). Under WDS root length of NM plants were 24.61 and 21.30% shorter than S-1 and S-2 inoculated plants. Similar results have also been reported for other plant species (Wu and Xia, 11). WDS decreased photosynthetic rate in NM seedlings as compared to WDS AM seedlings and WW seedlings (Table 1). Photosynthetic rate was significantly higher in S-1 (7.9% higher) and S-2 (12.81% higher) plants than NM plants under WDS condition. Our study confirmed that AM citrus seedlings had higher photosynthetic activity then NM plants.

The differences of relative water content were significant (P≤5) between WDS AM (89.25% for S-1 and 89.62% for S-2) and NM (86.49%) seedlings (Table 1). The greater stomatal conductance of AM plants, compared with non-AM plants, implied a lower resistance to vapour transfer from inside the leaves to the atmosphere when exposed to the same water conditions. Similarly, the higher RWC and the lower leaf temperature of AM plants, compared with those of non-AM plants, were propitious to moving liquid water through the plants to the evaporating surfaces in the leaves (Nelsen and Safir, 8). High RWC and stomatal conductance are the important reason for higher survival of AM plants post transplantation when they are experiencing extreme moisture stress.

Comparing the proline content of NM seedlings under WW and WDS conditions, the proline content of AM seedlings were reduced under WW condition (Table 2). The proline content was significantly reduced by AM colonization under WDS (30.66 µg g^{-1} Fw in S-1 and 32.52 µg g^{-1} Fw in S-2) compared to NM (39.33 µg g^{-1} Fw). In the present study, leaves of AM plants had lower proline levels than NM leaves when exposed to WW and WDS conditions, which may be attributed to either greater drought resistance of AM seedlings or less injury in AM seedlings grown under WDS conditions. The result agrees with previous reports obtained from *Citrus tangerina* (Wu and Xia, 11).

The total phenol of WW and WDS AM seedlings was higher than that of WW and WDS NM seedlings. Under WDS condition both S-1(38.12 µg g^{-1} Fresh weight) and S-2 (36.73 µg g^{-1} Fw) showed significantly higher (P≤0.05) total phenols compared with that in NM (30.60 µg g^{-1} Fw) seedlings (Table 2). The increased phenolic content might be attributed to enhanced polyphenol oxidase activity in plants (Mathur and Vyas, 6).

The WS AM (15.19 and 15.43 U mg^{-1} protein) seedlings showed significantly higher unit of SOD than the NM (13.94 U mg^{-1} protein) seedlings (Table 2).

Table 1. Effect of AMF species on root colonisation, root length, photosynthetic rate and relative water content of *Jatti khatti* seedlings grown under well watered and water deficit stress conditions.

Stress	AMF species	Root colonisation (%)	Root length (cm)	Photosynthetic rate (µmol $m^{-2}s^{-1}$)	Relative water content (RWC) (%)
Well watered	*Glomus fasciculatum* (S-1)	49.38	20.58	9.41	92.51
	Glomus intraradices (S-2)	47.28	19.73	9.91	92.15
	Non-mycorrhizal (NM)	0	15.79	8.83	92.13
Water deficit	*Glomus fasciculatum* (S-1)	49.10	19.30	9.01	89.25
	Glomus intraradices (S-2)	45.71	18.49	9.42	89.62
	Non-mycorrhizal (NM)	0	14.55	8.35	86.49
$CD_{0.05}$		4.12	1.11	0.65	1.30

Table 2. Effect of AMF species on proline, total phenols, super oxide dismutase, catalase and ascorbate peroxidase of *Jatti khatti* seedlings grown under well watered and water deficit stress conditions.

Stress	AMF species	Proline (µg g^{-1} Fw)	Total phenols (µg g^{-1} Fw)	Super oxide dismutase (U mg^{-1} protein)	Catalase (U mg^{-1} protein)	Ascorbate peroxidase (U mg^{-1} protein)
Well watered	*Glomus fasciculatum* (S-1)	27.95	35.52	11.87	6.55	6.24
	Glomus intraradices (S-2)	27.13	33.97	11.86	7.05	6.18
	Non-mycorrhizal (NM)	30.09	31.41	10.34	6.52	5.74
Water deficit	*Glomus fasciculatum* (S-1)	30.66	38.12	15.19	10.75	13.46
	Glomus intraradices (S-2)	32.52	36.73	15.43	10.69	13.41
	Non-mycorrhizal (NM)	39.33	30.60	13.94	9.72	11.27
$CD_{0.05}$		2.89	3.86	0.96	0.77	0.67

More SOD was related with greater scavenging of free radicals. Further, the WS AM (10.75 and 10.69 U mg^{-1} protein) seedlings showed significantly higher unit of catalase than the NM (9.72 U mg^{-1} protein) seedlings. Similarly, WS AM (13.46 and 13.41 U mg^{-1} protein) seedlings showed significantly higher unit of ascorbate peroxidase than the NM (11.27 U mg^{-1} protein) seedlings. This result is in conformity with the earlier findings of Kohler *et al.* (4).

This study suggests that mychorrhizal inoculation brought about significant improvement in root length, physiological and biochemical status of plants like enhancement in the levels of photosynthetic rate, phenol and antioxidant enzymes, which are necessary to mitigate adverse effects of WDS and enhancing survival upon transplanting. Inoculation of host specific suitable AM fungal isolates in nursery plants will increase their survival upon transplanting and are potentially important for maintaining and restoring the plant-soil equilibrium for realization of sustainability in agriculture.

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