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Screening of efficient Drought Tolerant Arbuscular Mycorrhizal Fungi for Chilli (*Capsicum annuum* L.)

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ABSTRACT: Abiotic stresses on crops are a result of the long-term changes in climate. The principal factor limiting crop growth, development and production is drought stress. Eighty percent of all known terrestrial plants have symbiotic relationships with Arbuscular Mycorrhizal Fungi (AMF). It has a positive effect on plant development, water uptake and abiotic stress defence. In this perspective, AM fungal isolates were obtained from Karnataka's drought-prone regions. The MPN technique was used to count the infectious propagules in each isolate. The effective isolates were tested in chilli at various field capacity (FC) levels (25, 50, 75, and 100% FC) for drought tolerance. Plants were grown in polythene bags for 30 days under greenhouse condition and based on performance of mycorrhizal parameters and plant parameters, the efficient drought tolerant isolates (AMC23 and AMR1) were selected.

Keywords: AM fungi, Chilli, drought stress.

INTRODUCTION

Abiotic stresses have been caused by global climate change. One of the key issues preventing crop growth and development is soil droughtstress. drought is one of the serious and adverse abiotic stress steadily increasing in arid and semi-arid regions, affecting adversely worldwide, limiting the productivity of most of the crops (Barnawal *et al*., 2014). Plant physiology is significantly impacted by drought stress. It disrupts photosynthesis, the state of the plant's water supply, the structure of its enzymes, the distribution of its biomass, lowers nutrient intake and creates a nutritional imbalance in the plant (Kumar and Verma 2018; Xie *et al*., 2018). Recently, there has been a lot of interest in using beneficial microorganisms to mitigate the effects of drought stress. To withstand varied environmental challenges, plants rely on microorganisms that are connected with their roots. 80% of terrestrial plants have symbiotic relationships with arbuscular mycorrhizal fungus (AMF), which is the most prevalent kind. Through a variety of pathways, the symbiosis between AMF and plants improves plant growth, nutrient absorption and stress resistance.

Chilli (*Capsicum annuum* L.) is an important horticultural crop and it has huge diversity and cultivated widely for its pungent fruits. It is considered as a significant commercial crop due to its enriched antioxidants, high pungency, rich flavour, and vitamins (Lee and Kader 2000). India ranks first in the world with an area of 7.75 lakh ha with an average yield of 1.6 metric tonne/ha (FAOSTAT). India contributes about 36 per cent of the total world's production and in terms of international trade, Chilli is vulnerable to drought stress as multiple studies indicate that water stress significantly decreased chilli productivity (Sezen *et al.*, 2006). In this context, present study was aimed to screen the efficient drought tolerant AM fungi for chilli.

MATERIAL AND METHODS

AM fungal spores were extracted from soil by using wet sieving and decantation method and sucrose centrifugation method. The collected AMF spores were mass produced by funnel technique. The isolates were mass produced by using maize as host plant**.** MPN method was carried out to check the potentiality of AM fungal isolates (Mythra and Krishna Naik 2023). Efficient isolates from MPN technique were screened for drought tolerance in chilli.

Screening of AM fungi for drought tolerance in crop plants. AM fungal isolates were evaluated based on the ability of mycorrhizal fungi to colonize the plant roots and enhances the growth under drought stress in green house condition. To evaluate drought stress tolerance, polythene bags were filled with soil (500 g) and were inoculated with AM fungal isolates @ 20 g per polythene bag prior to sowing of seeds. Surface sterilized chilli seeds were sown and different field capacity levels (25 %, 50 %, 75 % and 100 % FC) were maintained during plant growth (Fig. 1). The treatment details were encompassing the AMF isolates and a control without AMF. These treatments were laid out in a factorial CRD design and replicated three times. Ability of AM fungal isolates to perform under drought stress conditions were recorded after 30 days of sowing. The AMF isolates were screened based on per cent root colonization in roots, plant height, dry biomass of plant and total antioxidant activity in plants. Top two promising AMF isolates which showed highest root colonization, higher antioxidant activity and biomass were taken for further validation under pot culture studies.

RESULTS AND DISCUSSION

The present study, with an objective of screening for an effective AMF for inoculating mung bean and chilli plants, has shown varied percent colonization, plant growth, plant biomass and physiological responses to different AMF. In general, mycorrhizal inoculation resulted in a significant increase in plant height, plant biomass and physiological parameters in many crops. Previous researchers have found that different AM fungi have varying levels of symbiotic efficiency for a certain host (Soram *et al*., 2012; Ashwin *et al*., 2019; Ranadev *et al*., 2022). Therefore, it has been emphasized that choosing effective AM fungus that could be used for inoculating plants is essential (Bagyaraj and Kehri 2012; Sale, 2021). All the 28 AM fungal isolates from soil of different dry regions were screened for their ability to colonize to the plants, increased dry matter accumulation and enhanced antioxidant system in chilli plants at four different levels of field capacity *viz.*, 25, 50, 75 and 100 per cent FC at 30DAS has been reported.

From the screening study conducted for mung bean, out of the 28 AM fungal isolates, at all the FC levels, AMC23 and AMR1 isolates recorded highest percentage of root colonization with an average of 66.97 and 67.92 per cent respectively. AMC23 isolate exhibited the highest percentage mycorrhizal colonization (79.29 %) at 75 per cent FC. AMR1 isolate revealed the highest percentage mycorrhizal colonization (35.5, 68.08 and 90.08 %) at 25, 50 and 100 per cent FC levels respectively (Table 1). This was may be due the ability of plant species to be colonized by specific group of AM fungi (Grman, 2012).

The isolate AMC23showed the highest plant height of chilli (17.0 21.2 23.9 and 24.4 cm) when exposed to 25, 50, 75 and 100 per cent FC levels respectively. When chilli plantswere subjected to 25, 50, 75, and 100% FC levels, the AMR1 isolate recorded the highest height of 18.2, 21.6, 23.4, and 24.6 cm respectively. At all the FC levels in chilli, AMC23 and AMR1 isolates were the best among the 28 AM fungal isolates, with an average plant height of 21.8 and 21.9 cm, respectively (Fig. 2).

The chilli plants which received AMC23 isolate recorded the highest plant biomass (0.68 and 1.19 g/plant) at 25 and 75 per cent FC levels respectively. The isolate AMR1showed highest plant biomass (0.88 and 1.52 g/plant) at 50 and 100 per cent FC levels respectively. The highest biomass of chilli at all the FC levels was found in the isolates AMC23 and AMR1 (Fig. 3). Among all the AM fungal isolates, AMC23 and AMR1 recorded highest antioxidant activity of chilli plants with an average of 63.15 and 62.58 per cent respectively (Table 2).

In the present screening study, AMB2 and AMC3 isolates showed the highest mycorrhizal colonization, plant height, total biomass of plant and total antioxidant activity, among the 28 AM fungal isolates screened in mung bean. Whereas, in chilli, the highest mycorrhizal colonization, plant height, total biomass of plant and total antioxidant activity was observed in AMC23 and AMR1 isolates. AMB2 and AMC3 isolates were selected for mung bean to validate under pot culture experiment. AMC23 and AMR1 isolates were selected for chilli to validate under pot culture experiment. The chlaymdospore and root colonization images of best isolates were represented in Fig. 4a and 4b.

These results were in support with the results of Ashwin *et al.* (2019) where they screened 10 different species of AMF for drought tolerance in two soybean varieties *viz.*, DSR 2 and DSR 12. Plant parameters like plant height, stem diameter, bio-volume index, total leaf area, dry biomass, P concentration in plants and mycorrhizal parameters like root colonization, spore number in the root zone soil were recorded according to the standard procedures. Based on the improved plant parameters *viz.*, plant dry biomass, pod and seed yield, total leaf area and P uptake, they concluded that *Rhizophagus fasciculatus* was the best AMF for inoculating DSR 2 variety and *Ambispora leptoticha* was the best for inoculating DSR 12 variety.

Thilagar and Bhagyaraj (2015) screened 11 different species of arbuscular mycorrhizal fungi (*Acaulospora laevis, Gigaspora margarita, Glomus bagyarajii, G. etunicatum, G. fasciculatum, G. intraradices, G. leptotichum, G. macrocarpum, G. monosporum, F. mosseae and Scutellospora calospora*) for drought tolerance in chilli. Based on the improved plant parameters like bio-volume index, plant biomass, fruit yield and phosphorus uptake, they concluded that *Glomus mosseae* was the best arbuscular mycorrhizal funguzs for inoculating chilli in the nursery.

Table 1: Effect of AM fungal isolates on mycorrhizal colonization in chilli roots grown under different field capacity levels (30 DAS).

Mycorrhizal colonization (%)					
Isolates	**25 % FC**	**50 % FC**	**75 % FC**	**100 % FC**	**Mean of I**
AMC1	23.99^{g}	57.78^{i}	66.87^{jk}	84.67^{efgh}	58.33^{fg}
AMC2	27.90^{d}	60.91^{hi}	67.33^{ij}	83.77^{ghij}	59.98^{cde}
AMC3	31.15^{c}	63.33^{cd}	74.00^{cd}	87.00^{b}	63.87^{b}
AMC4	20.73^{hi}	55.26^{k}	66.99^{jk}	80.87^{k}	55.96^{h}
AMC5	24.35^{g}	57.92^{i}	69.90^{fg}	82.92^{j}	58.77^{efg}
AMC7	21.93^{h}	54.95^{kl}	64.33^{l}	80.22^{k}	55.36^{h}
AMC8	30.80^{c}	61.42^{fghi}	73.86^{cd}	86.25^{bcd}	63.08^{b}
AMC10	24.60^{fg}	56.01^{k}	65.93^{k}	84.42^{fghi}	57.74^{g}
AMC12	25.73^{ef}	58.78^{j}	68.36^{hi}	83.42^{hij}	59.07^{efg}
AMC13	28.85^{d}	61.89^{efgh}	71.01^{f}	82.95^{j}	61.18^{c}
AMC14	26.35^{e}	60.28^{i}	70.43^{fg}	85.40^{cdef}	60.62^{cd}
AMC15	31.22^{c}	63.76^{cd}	73.83^{cd}	86.00^{bcde}	63.70^{b}
AMC16	21.85^{h}	53.93^{l}	60.18^{mn}	78.42^{lm}	53.60^{i}
AMC17	31.23^{c}	62.43^{defg}	75.00^{c}	86.00^{bcde}	63.67^{b}
AMC19	26.00^{e}	58.42^{j}	68.18^{hij}	84.95^{defg}	59.39^{def}
AMC20	30.13^{c}	63.11^{cde}	74.18^{cd}	86.90^{0}	63.58^{b}
AMC21	30.87^{c}	63.89^{c}	72.99^{de}	86.48^{bc}	63.56^{b}
AMC22	31.50^{c}	62.69^{cdef}	72.43^{e}	86.11^{kbcd}	63.18^{b}
AMC23	33.65^{b}	65.42^{b}	79.29^{a}	89.50^{a}	66.97^{a}
AMT1	20.90^{hi}	52.59^{m}	61.33^{m}	80.9^{2k}	53.94^{i}
AMT2	19.76^{ij}	48.99^{pq}	60.50^{mn}	77.82^{m}	51.77^{j}
AMT4	19.29^{j}	51.0^{no}	58.99^{n}	76.23^{n}	51.38^{j}
AMB1	20.95^{hi}	51.48^{mn}	61.45^{m}	79.60^{kl}	53.37^{i}
AMB2	28.10^{d}	61.21^{ghi}	69.38^{gh}	83.21^{ij}	60.48^{i}
AMB3	18.29^{j}	47.99^{q}	60.23^{mn}	77.25^{mn}	50.94^{cd}
AMB5	20.86^{hi}	49.99^{op}	60.20^{mn}	76.20^{n}	51.81^{i}
AMR1	35.53^{a}	68.08^{a}	77.99^{b}	90.08^{a}	67.92^{a}
AMR2	18.56^{j}	47.53^{q}	59.70^{n}	77.50^{mn}	50.82^{j}
Mean of II	26.00^{D}	57.97^{C}	68.03^{B}	83.04^{A}	

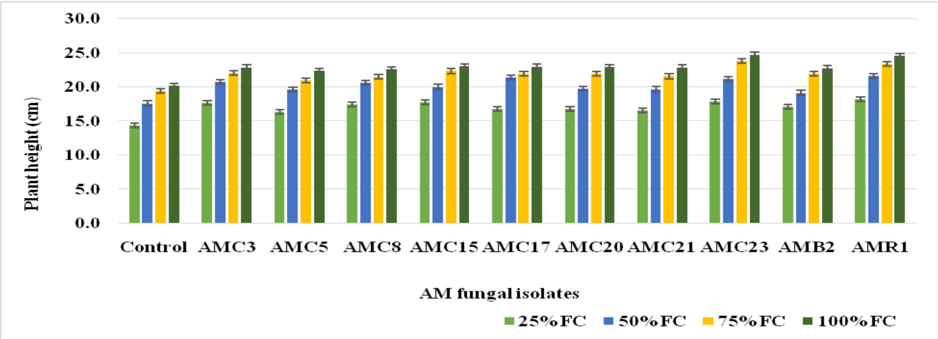
Note: Means with different letter in a column differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT), FC-Field capacity, DAS- Days after sowing. AMC- Arbuscular Mycorrhizae; Chitradurga, AMT-Arbuscular Mycorrhizae Tumkur, AMB- Arbuscular Mycorrhizae, AMR-Arbuscular Mycorrhizae Raichur.

Table 2: Effect of AM fungal isolates on ABTS+ scavenging activity in chilli plants grown under different field capacity levels (30 DAS).

The ABTS+ scavenging activity (%)					
Isolates	**25 % FC**	**50 % FC**	**75 % FC**	**100 % FC**	**Mean of I**
Control	46.34^{o}	44.8^{p}	41.29^{p}	38.01^{p}	42.86^{q}
AMC1	58.78^{h}	56.24^{h}	54.18^{ij}	48.48^{k}	54.42^{j}
AMC2	61.23^{de}	58.46^{e}	54.57^{hij}	49.46^{j}	55.93^{hi}
AMC3	65.43^{b}	60.11^{d}	56.42^{e}	55.14^{b}	59.42^{b}
AMC4	57.32^{i}	55.69^{hi}	52.51^{k}	45.37^{l}	52.72^{k}
AMC5	60.23^{g}	58.11^{ef}	55.8^{efg}	50.68^{i}	56.21^{gh}
AMC7	57.23^{i}	55.14^{ij}	51.57^{l}	45.21^{lmn}	52.29^{kl}
AMC8	60.32^{fg}	61.45^{c}	56.56^{e}	54.37^{bcd}	57.17^{f}
AMC10	57.23^{i}	57.34^{fg}	53.01^{k}	50.79^{hi}	54.59^{j}
AMC12	58.98^{h}	57.1^{g}	53.92^{j}	51.48^{gh}	55.37^{i}
AMC13	61.02^{ef}	59.92^{d}	55.78^{efg}	50.24^{i}	56.74^{fg}
AMC14	58.67^{h}	57.93^{ef}	54.73^{hi}	51.7^{g}	55.76^{hi}
AMC15	61.98^{d}	61.56^{c}	56.17^{ef}	53.41^{ef}	58.28^{cd}
AMC16	56.12^{j}	54.14^{k}	51.1^{lm}	44.54^{mn}	51.47^{m}
AMC17	61.98^{d}	61.66^{c}	58.35^{cd}	55.03^{bc}	59.26^{b}
AMC19	63.02^{c}	61.1^{c}	55.17^{gh}	52.68^{f}	57.99^{de}
AMC20	63.01^{c}	62.43^{b}	57.67^{d}	54.03^{de}	59.29^{b}
AMC21	61.23^{de}	61.32^{c}	58.97^{c}	53.7^{de}	58.81^{bc}
AMC22	62.01^{d}	61.38^{c}	55.37^{fgh}	54.26^{cd}	58.26^{cd}
AMC23	67.46^{a}	65.18^{a}	61.35^{a}	58.59^{a}	63.15^{a}
AMT1	56.87^{i}	55.78^{hi}	51.53^{lm}	43.22^{o}	51.85^{lm}
AMT2	56.98^{i}	54.55^{jk}	50.76^{m}	45.32^{lm}	51.9^{lm}
AMT4	54.34^{l}	52.22^{m}	49.56^{n}	42.95^{o}	49.77^{o}
AMB1	55.22^{k}	53.14^{l}	49.75^{n}	44.52^{mn}	50.66^{n}
AMB2	61.23^{de}	60.22^{d}	55.34^{gh}	52.66^{f}	57.36^{ef}
AMB3	53.21^{m}	51.14^{n}	48.23^{o}	42.87^{o}	48.86^{p}
AMB5	52.11^{n}	51.57^{mn}	48.05^{o}	44.43^{n}	49.04^{op}
AMR1	67.23^{a}	64.87^{a}	60.22^{b}	58.01^{a}	62.58^{a}
AMR2	53.12^{m}	50.23^{o}	49.97^{n}	42.47^{o}	48.95^{p}
Mean of II	58.96^{A}	57.41^{B}	53.72^{C}	49.47^{D}	

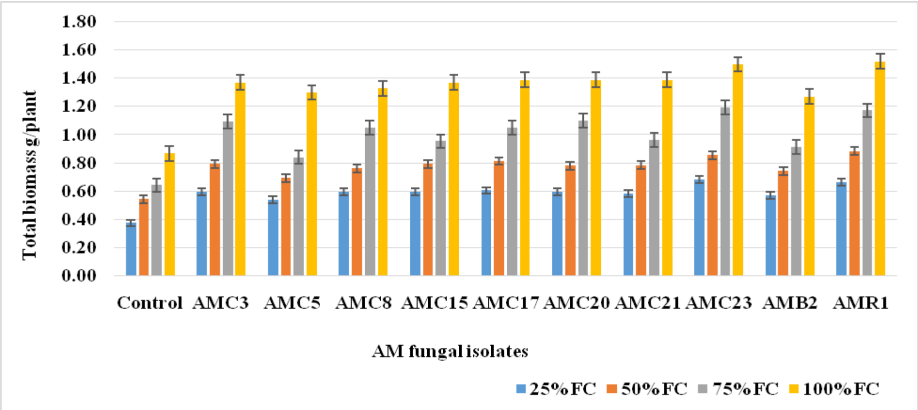
Note: Means with different letter in a column differ significantly at P=<0.05 as per Duncan Multiple Range Test (DMRT), FC- Field capacity, DAS- Days after sowing AMC-Arbuscular Mycorrhizae; Chitradurga, AMT- Arbuscular Mycorrhizae Tumkur, AMB- Arbuscular Mycorrhizae, AMR- Arbuscular Mycorrhizae Raichur

Fig. 1. General view of screeningof AM fungal isolates for drought tolerance in chilli.



Note: AMC- Arbuscular Mycorrhizae Chitradurga, AMT- Arbuscular Mycorrhizae Tumkur, AMB-Arbuscular Mycorrhizae, AMR- Arbuscular Mycorrhizae Raichur, FC-Field capacity.

Fig. 2. Effect of AM fungal isolates on plant height of chilli grown under different field capacity levels at 30 DAS.



Note: AMC- Arbuscular Mycorrhizae Chitradurga, AMT- Arbuscular Mycorrhizae Tumkur, AMB- Arbuscular Mycorrhizae, AMR- Arbuscular Mycorrhizae Raichur, FC-Field capacity.

Fig. 3. Effect of AM fungal isolates on total biomass of mung bean grown under different field capacity levels at 30 DAS.

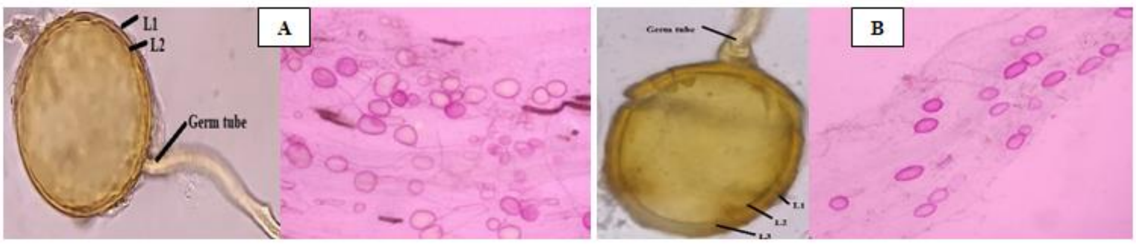


Fig. 4. Chlamydospores and root colonization of A) AMC23 and B) AMR1 AM fungal isolates (At 40X magnification).

CONCLUSIONS

Plants respond to drought stress by adopting different strategies, which allow them to avoid stress and/or enhance drought tolerance. Plant roots have highly plastic traits that can be modulated by AMF to enhance water uptake and/or minimize water loss. This dehydration tolerance is associated with survival and sustained physiological adaptations to improve the performance of plants and enhance the yield under drought stress condition.

FUTURE SCOPE

This study encourages the investigation of AM fungi in order to develop a drought stress resistant AM fungi promoting plant growth under stress environment and to study the genes associated with drought stress tolerance in AM fungal association with plants.

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