Effect of the Arbuscular Mycorrhizae *Glomus fasciculatum* and *Acaulospora laevis* on Two Varieties of *Triticum aestivum* L.

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SUMMARY

The bread wheat (*Triticum aestivum* L.) var. DWR-195 and DWR-162 are commonly grown in Dharwad district. To understand the efficacy, indigenous AM fungi, *Glomus fasciculatum* and *Aculospora leavis* were selected and Glass house studies conducted by inoculating these two fungi. The results revealed that the increased plant parameters were recorded on DWR-195 and DWR-162 which were inoculated with *Glomus fasciculatum*. However, the effect on shoot growth, phosphorous content in DWR-162 var. of *Triticum aestivum* L. was not influenced much with inoculation of *Aculospora leavis* compared to *Glomus fasciculatum*.

Key words : AMF (Arbuscular mycorrhizal fungi), Biomass, *Glomus fascieulatum, Aculospora leavis*

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AMF (Arbuscular mycorrhizal fungi) form symbiotic association with 80% of tropical crop plants. They play very important role in plant mineral nutrition and plant health (Allen 1991; Cucenca *et al.*, 2007). The symbiotic association with AM fungi allows the plant to access phosphorus beyond the depletion zone through the extraradical fungal hyphae, in addition to the root uptake (Pearson and Jackobsen, 1993). The host growth benefit resulting from the mycorrhiza is generally quantified as mycorrhizal responsiveness.

Wheat (*Triticum aestivum* L.) is an important food crop next only to paddy. It is known that, 70% of population is depending on food products prepared from wheat. Very limited studies on wheat inoculated with arbuscular mycorrhizae have been carried out. In view of this, investigation is undertaken to evaluate the efficiency of two AMF species towards biomass and growth enhancement in DWR 195 and DWR 162 varieties of *Triticum aestivum* L. (wheat).

MATERIALS AND METHODS

Pot experiment:

The soil physico-chemical characteristics used for pot experiments were estimated as per Jackson (1973). Per cent of organic matter was determined according to Piper (1950). Electric conductivity was measured using Bridge meter and pH by 1:1 (w/v) soil to water ratio (Table 1). The pure cultures of two AM species - *Acaulospora laevis* and *Glomus fasciculatum* were maintained in poly house using jowar (*Sorghum vulgare*) as host for mass multiplication in the 30 cm diameter pots containing sterilized sand - soil mix (1:1) and used as inoculum. 15 cm diameter pots amended with air dried sterilized soil - sand mix (3:1) ratio was used for the experiments. About l0g of inoculum consisting of 3g roots and 7g soil containing 200-250 spores of *Acaulospora laevis* and *Glomus fasciculatum* was inoculated 2cm below the soil surface in the earthen pots.

Later, one week old healthy germinated seedlings of DWR 195 and DWR 162 *Triticum aestvium* L. varieties grown in small plastic cups containing sterilized soil were transferred to the pots containing the inoculum. Uninoculated (non-mycorrhizal) plants received similar amounts of autoclaved sterile soil. The experiment was completely randomized with three replications per treatment of the two varieties. All the pots were maintained under poly house condition. To maintain moisture, pots

Table l: Physico-chemical characteristics of the soil used for pot experiments

Characteristics	Garden soil
pH	5.80(5.77)
Soil moisture (%)	28.36 (0.32)
Organic matter (%)	0.84(1.15)
E.C.mmho/em	10.17(3.33)
Nitrogen (mg/kg)	1.41(5.77)
Phosphorus (mg/kg)	0.22(8.81)
Potassium (mg/kg)	2.41(5.77)
Zinc (mg/kg)*	2.02(8.81)
Copper (mg/kg)	1.04(3.33)
Magnesium (mg/kg)	1.42(3.33)

*Values in parentheses represent standard errors of mea

were watered on every alternate day. Five ml of Hogland solution minus phosphorus was added to all the pots once in fifteen days.

Growth parameters:

Plants were uprooted periodically after 30, 60 and 90 days. Shoot-root fresh weight and dry weight was recorded. Shoot length, root length and number of grains were also counted. AMF colonization and spore count were made in roots and rhizosphere soil.

Association of AM fungal colonization was studied. The roots were carefully dug out and gently washed with tap water. The 1 cm segmented fresh roots were preserved in FAA. Later, the root pieces were washed in distilled water, transferred to test tubes containing 10% KOH and autoclaved for thirty minutes. KOH was drained out and roots were carefully washed with distilled water, stained with 0.05% Trypan blue (Philips and Hayman, 1970). Per cent AMF colonization was calculated using simple formula of (Nicolson, 1955).

$$\text{Per cent colonization} = \frac{\text{Number of segments colonized}}{\text{Total number of segments observed}} \times 100$$

The AM fungal spores were enumerated by isolating from the rhizospheric soil of each plant by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Phosphorus content in the shoots was determined in terms of percentage in plant sample (Jackson, 1973).

One way analysis of variance test was carried out to study the significant variation between the control and other treatments. Further, Post Hoc Dunette test was applied to know which treatment significantly differed with the control.

RESULTS AND DISCUSSION

Pot experiments showed that AM fungi substantially

Table 2: Effect of treatment with *Acaulospora laevis* and *Glomus fasciculatum* on different parameters in DWR-195 variety after 30, 60 and 90 days of inoculation

		CN	AL	GF	F
SL	30	27 ± 0.2886	25.5 ±0.2877	39.5 ± 0.2877	2.736
	60	55 ± 0.2787	95 ± 0.4774	113 ± 0.5874	
	90	76 ± 0.5884	108 ± 0.0000	120 ± 5. 6646	
FWS	30	3.588 ± 0.1996	5.825 ± 0.4384	6.415 ± 0.2853	1.84
	60	16.693 ± 0.5747	34.637 ± 0.0886	53.155±0.3057	
	90	31.717 ± 0.0473	39.39 ± 0.731	63.522 ± 0.0844	
DWS	30	0.336 ± 0.0078	0.492 ± 0.0091	0.332 ± 0.0304	1.47
	60	3.15 ± 0.0216	5.001 ± 0.0000	8.170 ± 0.0047	
	90	2.979 ± 0.0102	9.100 ± 0.0685	10.532 ± 0.084	
RL	30	4 ± 0.4665	5.7 ± 0.5727	5.7 ± 0.4407	7.112**
	60	8 ± 0.3778	10 ± 0.2258	13 ± 0.6527	
	90	7.6 ± 0.0488	13.5 ± 0.0688	13.5 ± 0.40805	
FWR	30	0.473 ± 0.0653	0.544 ± 0.0528	0.412 ± 0.0047	1.712
	60	1.407 ± 0.268	5.021 ± 0.0071	4.181±0.002	
	90	3.644 ± 0.4587	0.211 ± 0.0005	6.013 ± 0.0050	
DWR	30	0.052 ± 0.0400	0.538 ± 0.0047	0.156 ± 0.00	1.157
	60	0.467 ± 0.02	0.635 ± 0.0005	0.0788 ± 0.00	
	90	0.552 ± 0. 0224	0 ± 0.0000	0.868 ± 0.8847	
PC	30	0 ± 0.0000	21.5 ± 0.2244	34 ± 0.6310	3.921
	60	0 ± 0.0000	19 ± 0.3116	46.13 ± 0.5001	
	90	0 ± 0.0000	19.7 ± 0.1238	100 ± 0.444	
SN	30	0 ± 0.0000	97.75 ± 0. 2554	31 ± 0.1336	4.925*
	60	0 ± 0.0000	20 ± 2.9227	16 ± 0.4763	
	90	0 ± 0.0000	23 ± 1.0759	71 ± 0	
P	30	0.06 ± 0.0102	62 ± 0.3112	0.13 ± 0.00367	8.823
	60	0.16 ± 0.0000	0.25 ± 0.0069	0.20 ± 0.0114	
	90	0.14 ± 0.0046	0.27 ± 0.0069	0.30 ± 0.0047	

SL – shoot length; FWS-fresh weight of shoot; DWS-dry weight of shoot; RL-root length; FWR-fresh weight of root; DWR-dry weight of root; PC-percentage of colonization; SN-spore number; P-phosphorous uptake; CN-control; AL- *Acaulospora laevis*, GF-*Glomus fasciculatum*; F-Fischer test; ** - P<0.01 at 1% significance; * - P<0.05 at 5% significance; Mean ± Standard error

improved the plant growth compared to uninoculated. All the inoculated plants of both the *Triticum aestium* varieties showed improvement in the mycorrhizal colonization, phosphorus uptake, shoot length, root length, fresh and dry weight of root and shoot over the uninoculated control. The effect of two different AM fungal species on shoot and root length, fresh weight of shoot and root, dry weight of shoot and root, number of spikelets, per cent colonization, spore number/50 g soil and phosphorus uptake of DWR-195 and DWR-162 varieties are shown in Table 2 and 3, respectively. In DWR-195 variety root length, per cent colonization, spore number and phosphorus uptake were significantly higher with *Glomus fasciculatum* than uninoculated and other AM species (Table 2). Analysis of variance for root length, P uptake showed 1% ($P< 0.01$) level of significance and 5% ($P< 0.05$) level of significance to per cent colonization, spore number in DWR-195 variety. The value of F was significant for root length, per cent colonization, spore number and P uptake. Later, the analysis of the Dunette test showed *Glomus fasciculatum* was significant treatment in per cent colonization, spore number and P uptake with the control. But *Acaulospora laevis* was significant in root length and in per cent colonization with control. In general *Glomus fasciculatum* was significant with control in DWR195 variety.

In DWR-162 variety analysis of variance for root length, spore number, P uptake showed 1% ($P< 0.01$) level of significance and 5% ($P< 0.05$) level of significance to per cent colonization (Table 3). The value of F was significant for root length, per cent colonization, spore number, P uptake. The analysis of Dunette test showed that *Acaulospora laevis* was significant treatment in root length, per cent colonization, spore number, P uptake with

Table 3: Effect of treatment with *Acaulospora laevis* and *Glomus fasciculatum* on different parameters in DWR-162 variety after 30, 60 and 90 days of inoculation

		CN	AL	GF	F
SL	30	21.5 ± 0.5742	34 ±0.6552	26 ± 0.6256	2.934
	60	35.73 ± 0.4703	95.6 ± 0.4774	66.33 ± 0.5874	
	90	76 ± 0.2992	121 ± 0.0565	81.01 ± 0.0217	
FWS	30	2.735 ± 0.1688	6.535 ± 0.0491	2.691 ± 0.579	1.328
	60	25.298 ± 0.2266	49.980 ± 0.0886	29.478±0.0688	
	90	39.655 ± 0.0689	59.271 ± 0.087	38.521 ± 0.0638	
DWS	30	0.18 ± 0.0065	0.338 ± 0.008	0.197 ± 0.0057	1.47
	60	2.472 ± 0.1101	6.342 ± 0.1	4.977 ± 0.0746	
	90	3.972 ± 0.0489	6.545 ± 0.2	6.033 ± 0.0005	
RL	30	5 ± 0.4665	7.2 ± 0.6727	6.1 ± 1.0428	10.627**
	60	7 ± 0.6773	9 ± 0.0678	5.8 ± 0.0001	
	90	7.1 ± 0.0633	12.7 ± 0.2	7.5 ± 0.0644	
FWR	30	0.22± 0.0045	0.455 ± 0.03	0.352 ± 0.0067	1.627
	60	1.43 ± 0.0545	5.542 ± 0.0671	4.241±0.0047	
	90	3.102 ± 0.0488	5.227 ± 0.0442	5.476 ± 0.0047	
DWR	30	0.04 ± 0.0048	0.052 ± 0.0048	0.07 ± 0.0047	0.653
	60	0.372 ± 0.0005	0.41 ± 0.00049	0.042 ± 0.0007	
	90	0.422 ± 0.0067	0.52 ± 0.0000	0.65 ± 0.00665	
PC	30	0 ± 0.0000	45 ± 0.6554	16 ± 1.6311	3.834
	60	0 ± 0.0000	59 ± 10.6732	19 ± 6.6632	
	90	0 ± 0.0000	91 ± 0.6552	89 ± 0.3886	
SN	30	0 ± 0.0000	28 ± 4.4444	15 ± 0.4886	8.924**
	60	0 ± 0.0000	41 ± 0.6445	17 ± 0.4787	
	90	0 ± 0.0000	48 ± 1.3456	42 ± 1.3678	
P	30	0.04 ± 0.0488	0.33 ± 0.02	0.25 ± 0.0067	23.134**
	60	0.06 ± 0.0067	0.29 ± 0.002	0.12 ± 0.0066	
	90	0.05 ± 0.0049	0.26 ± 0.0277	0.34 ± 0.02	

SL – shoot length; FWS-fresh weight of shoot; DWS-dry weight of shoot; RL-root length; FWR-fresh weight of root; DWR-dry weight of root; PC-percentage of colonization; SN-spore number; P-phosphorous uptake; CN-control; AL- *Acaulospora laevis*, GF-*Glomus fasciculatum*; F-Fischer test; ** - P<0.01 at 1% significance; * - P<0.05 at 5% significance; Mean ± Standard error

control. But *Glomus fasciculatum* was significant in spore number and P uptake. In general *Acaulospora laevis* was significant treatment with the control. Performance of *Glomus fasciculatum* as inoculant on DWR-195 variety was significantly superior to species and *Acaulospora laevis* on DWR-162 variety. This showed that mycorrhizal efficiency with a particular AM fungal species *i.e.*, AM fungi have host preference but they are not host specific.

The physico-chemical characteristic of the soil used for pot experiments were estimated in triplicates as shown in Table 1. In general, mycorrhizal inoculation results in an increased growth and biomass production. The results clearly shows that AMF species isolate would vary in interaction with a host plant and soil. The fungus utilizes nutrients from the host for the establishment which might affect the growth of roots (Smith and Harley, 1997; Lakshman and Patil, 2003; Bhagyraj, 2006). It has been demonstrated that many tropical crops do not grow well in low P soil without an effective mycorrhizal association. Since mycotrophic plants depend on specific AMF colonization when they grow under low external P condition, their yield can be enhanced by inoculating efficient Arbuscular mycorrhizal fungi (Patil and Lakshman, 2005; Cucenca *et al*., 2007). Krishna *et al*. (1987) have pointed out that rapid colonization of roots by AM fungi is an essential factor for good host response. It is noted that the most dominant species of AM fungi may not be the most benefitial mutualists. Johnson *et al*. (1992) showed that crop monocultures selected for AM fungi were inferior mutualists. There is not only a difference between crop species in the degree to which they form mycorrhizal, there is also a difference between cultivars of the same species. Cultivars of wheat (Young *et al.*, 1985; Manske, 1990) and corn (Toth *et al.*, 1984) have been shown to vary in levels of colonization by AM fungi. Thus, *Glomus fasciculatum* responded favourably and significant to DWR-195 and *Acaulospora laevis* showed efficient colonizer to DWR-162 variety. Benefitial responses in terms of physical growth parameters to AM inoculation have been obtained with peanut (Krishna and Bhagyaraj, 1982) and soybean (Ross and Harper, 1970).

Thus, there are excellent opportunities to incorporate AMF as biofertilizer to enhance crop productivity and reduce fertilizer inputs. Therefore, the use of arbuscular mycorrhizal fungi as biofertilizer helps in establishment of plants in a stressed soil conditions and it also helps in increasing the fertility of soil by making available phosphorus to plants.

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REFERENCES

Allen, M.F. (1991). *The ecology of mycorrhizae*. Cambridge University Press. Cambridge, 439pp.

Bhagyraj, D.J. (2006). Arbuscular Mycorrhizal fungi in sustainable agriculture, In; *Technique in mycorrhizae*. Eds MJ Bukhari and B.F.Rodrigues. Morgoa. pp 1-8.

Cucenca, G., Caceres, A., Oirdobro, G., Hasmy, Z. and Urdaneta, C. (2007). Arbuscular mycorrhizae as alternative for a sustainable agriculture in tropical areas. *Interciencia,* **32**(1) : 23-29.

Gerdemann, J.W. and Nicolson, T.H. (1963). Spores of mycorrhizal *Endogone* species extracted from the soil by wet sieving and decanting. *Trans. Br. mycol. Soc.*, **46** : 235-244.

Gerdemann, J.W. (1975). Vesicular arbuscular mycorrhizae. In: *The Development and Function of roots.* J.G.Torrey and D.T. Clarkson, eds. Academic Press, San Diego, pp. 575-591.

Jackson, M.L. (1973). *Soil chemica analysis*. Prentice Hall, New Delhi.

Johnson, N.C., Copeland, P.J., Crookston, R.K and Pfleger, F.L. (1992). Mycorrhizae: Possible explanation for yield decline with continuous corn and soybean. *Agronomy J.*, **84**, 387-390.

Krishna, K.R. and Bhagyaraj, D.J. (1982). Influence of VA mycorrhiza on growth and nutrition of *Arachis hypogaea*. *Leg. Res.*, **5** : 18-22.

Krishna, K.R., Bhagyaraj, D.J. and Rai, P.V. (1987). Response of groundnut to VA mycorrhizal inoculation to black clay soil. *Indian J. Microbiol*., **22** : 206-208.

Lakshman, H.C. and Patil, B. Patil (2003). Effect of *Glomus fasciculatum* in relation to nutrient application on *Acacia auriculifolia*. *Microbial Word.*, **6**(1) : 37-40.

Manske, G.G.B. (1990). Genetical analysis of the efficiency of VA mycorrhiza with spring wheat. *Agric. Ecosystem & Environ.*, **29** : 273-280.

Nicolson, T.H. (1955). Taxonomy of endomycorrhizal fungi: In *Advances in Botany*; pp. 212-218; eds. E.G. Mukerji, Binny Mathur. B.P.Chamola and P.Chitralekha (APH Publishing Corporation, New Delhi, India).

Patil, G.B. and Lakshman, H.C. (2005). Variation among the finger millet varieties in root colonization and responsiveness to VAM fungi. *N.E. & P. Technol.*, **4**(2): pp. 83-87.

Pearson, J.N. and Jackobsen, I. (1993). The relative contribution of hyphae and roots to phosphorus uptake by arbuscular mycorrhizal plants, measured by dual labeling with ^P and ^{33}P. *New Phytologist.*, **124** : 489-494.

Philips, J.M. and Hayman, D.S. (1970). Improved procedure for clearing roots and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection; *Trans. Br. mycol. Soc.*, **55** : 158-161.

Piper, C.S. (1950). Soil and plant analysis interscience Publications, New York. 91pp.

Ross, J.P. and Harper, J.A. (1970). Effect of *Endogone* mycorrhiza on soybean yields. *Phytopathol.*, **60** : 1552 - 1556.

Smith, S.E. and Harley, J. L. (1997). *Mycorrhizal symbiosis*. 2nd Ed. Academic press. London. 513 pp.

Toth, R., Page, T. and Castleberry, R. (1984). Differences in mycorrhizal colonization of maize selections for high and low ear leaf phosphorus. *Crop Sci.*, **24** : 994-996.

Young, J. L., Davis, E. A. and Rose, S.L. (1985). Endomycorrhizal fungi in bread wheat and triticale cultivars field-grown on fertile soil. *Agronomy J.*, **77** : 219-224.

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