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Interactive effect with AM fungi and *Azotobacter* inoculated seed on germination, plant growth and yield in cotton (*Gossypium hirsutum*)

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ABSTRACT

Studies were carried out to evaluate the response of cotton to inoculation with IAA producing diazotroph, *Azotobacter chroococcum*. The effect of *A. chroococcum* inoculation on cotton seed germination and seedling development was observed to be beneficial for both parameters. A field experiment was also conducted to ascertain the effects of dual inoculation of *A. chroococcum* (CBD-15, M-4, BA-l and OA-4) as seed application and AM fungi as soil inoculant on cotton (*Gossypium hirsutum* L.) var. Pusa 8-6. There was significant improvement in plant height, number of flowers and bolls formed, boll weight and seed cotton yield in most of the treatments as compared to uninoculated control. Synergistic effects of dual inoculation of all the *A. chroococcum* strains with AM fungi were observed for boll weight and seed cotton yield. A significant positive correlation between population of *Azotobacter* sp. in rhizosphere of cotton with weight of bolls and seed cotton yield was observed.

Key words: AM fungi, *Azotobacter chroococcum,* Dual inoculation, IAA production, Seed-cotton, Seed germination, Seedling development

Cotton (*Gossypium hirsutum* L.) is an important fibre crop cultivated in nearly 9 million ha of land in India. It constitutes for more than 70% of fibre consumption in the textile sector. Nitrogen is frequently the most expensive plant nutrient applied to cotton for enhancing seed cotton yield. Plant growth-promoting rhizobacteria (PGPRs) are root colonizing bacteria which play a pivotal role in improving plant growth and yield. *Azotobacter* sp. a well known PGPR has been observed to improve plant growth by production of IAA and fixation of atmospheric nitrogen (Jackson *et al.* 1964, Barea and Brown 1974, Anjum *et al.* 2007). Plants inoculated with *Azotobacter* sp. have shown improved seed germination, increase in shoot, root biomass and yield. The beneficial effects have been observed in wheat, rice, maize, millets, mustard, sunflower, cotton and vegetable crops (Malik *et al.* 2005, Malik *et al.* 2009, Paul and Paul 2009). Arbuscular mycorrhizal (AM) fungi commonly occur in the roots of agricultural crops. The positive effect of AM fungi on plant growth is through improved nutrients and water uptake by root and increased defenses against soil pathogens (Marulanda *et al.* 2003). Widespread inoculation trials conducted on millets, groundnut, oilseeds and cereals with AM fungi showed beneficial effects on plant growth and yield (Singh and Rana 2005). Individually effect of both the bio-inoculants has been investigated in cotton, however, effects of dual inoculation of *Azotobacter* sp. and AM fungi is lacking. Reports by many workers indicate that beneficial effects are usually enhanced when PGPRs are coinoculated with AM fungi (Vivas *et al.* 2006). Hence, the objective of the present work was to evaluate the effect of different *A. chroococcum* strains in combination with AM fungi on plant growth, flower and boll formation and yield of cotton so as to identify the most effective combination for exploitation as bioinoculants for enhancing of cotton growth and yield.

MATERIALS AND METHODS

Four *Azotobacter chroococcum* strains, viz CBD 15, M 4, BA 1 and OA 4 and one arbuscular mycorrhizal fungus, *Glomus fasciculatum* obtained from the culture collection of Division of Microbiology were used in the present study.

All the *A. chroococcum* strains were inoculated on Jensen's N-free medium (Jensen 1951) slants. Acetylene reduction activity (ARA) of *A. chroococcum* strains was determined by the method of Hardy *et al.* (1971) using Gas chromatograph with flame ionization detector having Porapak N column. Three replications for each treatment and control were maintained. ARA activity was expressed as nmoles of ethylene produced/mg protein/hr. Growth of the cultures

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present on the slant was carefully scrapped and used for estimation of soluble protein by Lowry's method (Lowry *et al.* 1951).

A. chroococcum strains were inoculated in test tubes containing Jensen's N-free broth (Jensen 1951) supplemented with filter sterilized tryptophan (@ 100µg/ml). Indole acetic acid production by *A. chroococcum* strains was determined by the method Hartmann *et al.* (1983). Three replications for each treatment and uninoculated control were maintained. Indole acetic acid (IAA) production was expressed as µg IAA produced/mg protein. Bacterial growth was determined by estimating the soluble protein content by Lowry's method (Lowry *et al.* 1951).

A. chroococcum strains were bioassayed for their ability to promote seed germination and seedling growth using the method as described by Paul *et al.* (2002). Surface sterilized cotton seeds were inoculated with log phase broth cultures of *A. chroococcum* strains (approx. 1×10^8 colony forming units(cfu)/ml) and were kept for germination on 0.8% agar. Ten seeds/Petri plate were maintained. Three replications for each treatment and control were maintained. Data on percent seed germination and radicle length was recorded after 72 hr.

The experiments were conducted for two years in summer-*kharif* season (2007–08 and 2008–09) at IARI research farm. *Gossypium hirsutum* (L.) var. Pusa 8-6 was used in the present study. The plot size was 4.5m x 4.5m with 6 lines/plot with spacing of 0.75m between the lines. In each line 12 plants were maintained. The soil belonged to Indo-Gangetic typical Ustochrept plains having high clay and silt content. The organic carbon of soil was 0.8%, total nitrogen 0.058%, available phosphorus 28.8 kg/ha, available potassium 302 kg/ha, EC 0.47 dS/meter at 25^0C and *p*H 7.8. Only basal application of NPK @ 30 kg/ha each was given at the time of field preparation.

Four solid sterile carrier (charcoal:soil)-based formulations of *A. chroococcum* strains (CBD-15, M-4, BA-l and OA-4) containing approx. 10^8 cfu/g carrier and one soil based AM fungi formulation (*Glomus fasciculatum*) containing 1 600 spores/100 g were used. *Azotobacter* sp. was inoculated as seed treatment @ 500 g for 15 kg seeds using 10% solution of jaggery as sticker (approx. 1×10^5 cfu/seed). AM fungi was inoculated as soil application @ 7.5 kg/ha.

Sampling was carried out at 45 and 90 days after sowing (DAS) to determine the rhizospheric population of *Azotobacter* sp. Root adhering soils were collected, serially diluted and plated for determining rhizospheric counts of *Azotobacter* sp. Five plants were randomly selected for each replication, from each treatment and data on plant height, number of flowers, number of bolls/plant and weight of 20 bolls was recorded. Data on seed cotton yield/plot was recorded. Sampling for flowering was carried out after every three day interval and a total of five samplings were done. The data was analyzed using randomized block design (Cochran and Cox 1958).

RESULTS AND DISCUSSION

Plant growth-promoting activities and effect on seed germination and seedling development

All the *A. chroococcum* strains used in the present investigation possessed IAA production ability which ranged between 5.36 and 82.62 µg/mg protein (Table 1). *A. chroococcum* strain OA-4 produced significantly higher IAA than other strains. The nitrogen fixation ability of *A. chroococcum* strains as determined by ARA activity ranged between 783.1 and 3539.4 nmoles/mg protein/hr (Table 1). Highest ARA activity was observed in case of *A. chroococcum* strain M-4. Both IAA production and N_2 fixation under free-living conditions are well reported plant growth promoting activities of *Azotobacter* sp. (Anjum *et al.* 2007).

Seed germination is a problem in cotton with a low germination percentage of about 70%. Seedling development is an important attribute that determines the overall performance of a crop (Hafeez *et al.* 2004). Both per cent seed germination and radicle length were significantly improved over control treatment in seeds treated with all the *A. chroococcum* strains (Table 1). Highest germination as well as maximum increase in radicle length was observed in seeds inoculated with *A. chroococcum* strains M 4 and OA 4. Inoculation with *A. chroococcum* strains significantly enhanced seed germination as well as seedling development in other plants also, viz wheat, onion and maize (Paul *et al.* 2002). Bacteria derived IAA may have stimulatory effect on seed germination, plant growth and development (Egamberdieva 2009). Asymbiotic nitrogen fixers enhance N uptake by plant and hence stimulate root growth (Zaidi and Khan 2005).

Rhizospheric azotobacter counts

Rhizospheric population determined on 45 days after sowing (DAS) showed higher counts in inoculated treatments over control, indicating increase in *Azotobacter* sp. population in cotton rhizosphere due to seed bacterization (Table 2). In most of the treatments, with the exception of CBD 15+AM

Table 1 Plant growth-promoting activities of *Azotobacter chroococcum* strains and effect of *A. chroococcum* strains on per cent seed germination and seedling vigour

Strain	µg IAA produced/mg protein	nmoles of ethylene produced/mg protein/hr	Per cent seed germination	Radicle length (cm)
Control			77	4.13
CBD 15	5.36	783.1	80	4.24
M 4	51.23	3539.4	85	4.77
BA 1	49.71	2071.3	81	4.24
OA 4	82.62	1881.1	89	4.78
SEm±	8.68	75.11	1.13	0.03
CD (*P*=0.05)	3.55	244.96	3.55	0.09

Table 2 Effect of combined inoculation of *A. chroococcum* and AM fungi on *Azotobacter* population in cotton rhizosphere (pooled data)

Treatment	*Azotobacter* counts (log values)	
	45 days after sowing	90 days after sowing
Control	2.11	2.40
Azo-CBD 15	2.41	2.59
Azo-M 4	2.72	2.72
Azo-BA 1	2.55	3.16
Azo-OA 4	2.62	2.79
AMF	2.49	2.78
Azo-CBD 15+AMF	2.70	3.88
Azo-M 4+AMF	2.52	3.03
Azo-BA 1+AMF	2.54	3.03
Azo-OA 4+AMF	2.53	2.87
SEm±	0.016	0.012
CD (*P*=0.05)	0.048	0.037

fungi, a depressive effect of co-inoculation with AM fungi on rhizospheric population of *Azotobacter* sp. was observed. There was an increase in *Azotobacter* sp. population over time as indicated by counts obtained at 90 (DAS) (Table 2). At this stage AM fungi appeared to have a beneficial effect on *Azotobacter* sp. population in cotton rhizosphere since in most of the co-inoculation treatments significantly higher cfu/gm was obtained. Survival, establishment and proliferation in rhizosphere are essential for a bioinoculant to stimulate plant growth. An increase in *Azotobacter* sp. population over time indicated that this microbe could establish and proliferate in the rhizosphere of cotton as reported earlier (Kumar *et al.* 2006).

Effect of Azotobacter inoculation on plant growth

All the treatments, with the exception of *A. chroococcum* strain CBD 15, significantly enhanced plant height over control (Table 3). Number of flowers/plant was significantly enhanced due to seed bacterization with all *A. chroococcum* strains. Highest number of flowers was obtained in case of inoculation with *A. chroococcum* strain M 4. All the treatments, with the exception of *A. chroococcum* strain OA 4, significantly enhanced number of bolls/plant over control. Maximum number of bolls was obtained in case of inoculation with *A. chroococcum* strains M 4 and CBD 15. All treatments significantly improved boll weight and seed cotton yield over uninoculated control. Maximum boll weight and average seed-cotton (3.54 tonnes /ha) was obtained due to inoculation with *A. chroococcum* strain OA 4. Such improvement in plant growth and increase in the yield of fibre crops due to *Azotobacter* sp. inoculation in cotton has been reported earlier also (Anjum *et al.* 2007). *Azotobacter* is known to improve growth of plant through production of IAA and biologically fixed nitrogen (Hafeez *et al.* 2004).

Effect of co-inoculation of Azotobacter and AM fungi on plant growth

Inoculation with AM fungi improved plant growth, flower and boll number and boll weight and also enhanced yield (Table 3). Growth of cotton seedlings was promoted, the number of flowers and bolls were increased due to mycorrhizal inoculation and this resulted in an increase in yield of seed-cotton (Sridevi and Ramakrishnan 2010). Dual inoculation of *A. chroococcum* strains and AM fungi had a synergistic effect on plant height, boll weight and seed cotton yield. Maximum height was obtained in case of dual inoculation of *A. chroococcum* strain OA 4 and AM fungi. In most of the treatments dual inoculation of *A. chroococcum* strains and AM fungi did not appear to have a synergistic effect on number of flowers and bolls/plant. Only in case of *A. chroococcum* strains CBD 15 and OA 4, co-inoculation with AM fungi had a synergistic effect on number of flowers and

Table 3 Effect of combined inoculation of *A. chroococcum* and AM fungi on plant parameters and seed cotton yield (pooled data)

Treatment	Plant height (cm)	No. of flowers/ plant*	No. of bolls/ plant*	Weight of 20 bolls (g)	Seed cotton yield (tonnes/ha)
Control	101.3	158	17	86.0	2.86
Azo-CBD 15	106.6	212	25	95.0	3.36
Azo-M 4	111.9	230	25	94.0	3.51
Azo-BA 1	112.1	201	24	93.0	3.42
Azo-OA 4	112.6	217	18	97.0	3.54
AMF	113.5	179	23	91.0	3.36
Azo-CBD 15+AMF	113.3	227	22	107.0	3.86
Azo-M 4+AMF	120.1	219	21	101.0	3.73
Azo-BA 1+AMF	121.5	172	19	97.0	3.69
Azo-OA 4+AMF	123.0	209	21	103.0	3.74
SEm±	2.42	2.62	0.77	1.2	0.03
CD (*P*=0.05)	5.65	7.73	2.27	3.55	0.10

Azo, *Azotobacter chroococcum;* AMF, AM fungi, * Average of five plants

Table 4 Correlation between population of *Azotobacter* sp. in rhizosphere of cotton and plant parameters[a]

Parameter	Plant height (cm)	No. of flowers/ plant	No. of bolls/ plant	Weight of 20 bolls (g)	Seed cotton yield (tonnes/ha)
Population of *Azotobacter* sp.					
45 DAS	0.537	0.756	0.420	0.642	0.810
90 DAS	0.400	0.417	0.150	0.752	0.726
Plant parameters					
Plant height	1.00	0.234	-0.074	0.658	0.823
No. of flowers /plant		1.00	0.488	0.656	0.631
No. of bolls/plant			1.00	0.041	0.155
Weight of 20 bolls				1.00	0.886
Seed cotton yield					1.00

[a]Pearson correlation; r > 0.444 means significant at $P = 0.005$; DAS, days after sowing

bolls/plant respectively. Dual inoculation had a synergistic effect on boll weight and seed cotton yield in all of the cases. The combination of CBD-15 and AM fungi was found to be the best over rest of the combinations in improving both boll weight and seed cotton yield. Co-inoculation trials conducted on various field crops with AM fungi and nitrogen fixers or PGPRs showed beneficial effects on growth and yield (Zaidi and Khan 2005). Dual inoculation of *A. chroococcum* and *G. fasciculatum* enhanced root infection of AM fungi, stimulated plant growth and increased shoot N, Ca, Mg and K in luxor tomatoes (Saxena *et al.* 2006).

Correlation between population of Azotobacter *sp. in rhizosphere of cotton and various plant parameters*

A significant positive correlation was obtained between population of inoculated strains at 45 days after sowing and plant height, number of flowers, weight of 20 bolls and seed cotton yield (Table 4) indicating significant contribution by *Azotobacter* sp. in improving growth and yield of cotton at this stage. A significantly positive correlation was obtained between population of inoculated strains at 90 days after sowing and weight of bolls and seed cotton yield indicating that at this stage of plant growth, only these parameters were significantly influenced by rhizospheric population of *Azotobacter* sp.

A significant positive correlation was obtained between plant height and weight of bolls and seed cotton yield; between number of flowers/plant and number of bolls/plant, weight of bolls and seed cotton yield. Weight of bolls and seed cotton yield was also positively correlated.

Inoculation with *A. chroococcum* strains appeared to promote blooming of the flowers and boll formation at an earlier period by a few days than uninoculated treatments, both singly and in combination with AM fungi (Fig 1 a, b). Maximum number of flowers was present on the plant during the 2nd sampling as compared to uninoculated control where highest number of flowers was observed on 4th sampling date. A higher ratio of boll weight was obtained between 1st and 2nd picking of cotton in inoculated plants as compared to uninoculated control (Fig 2). Highest ratio was obtained for plants inoculated with M 4. IAA in low amounts as produced by PGPRs has a positive and direct influence on blooming of

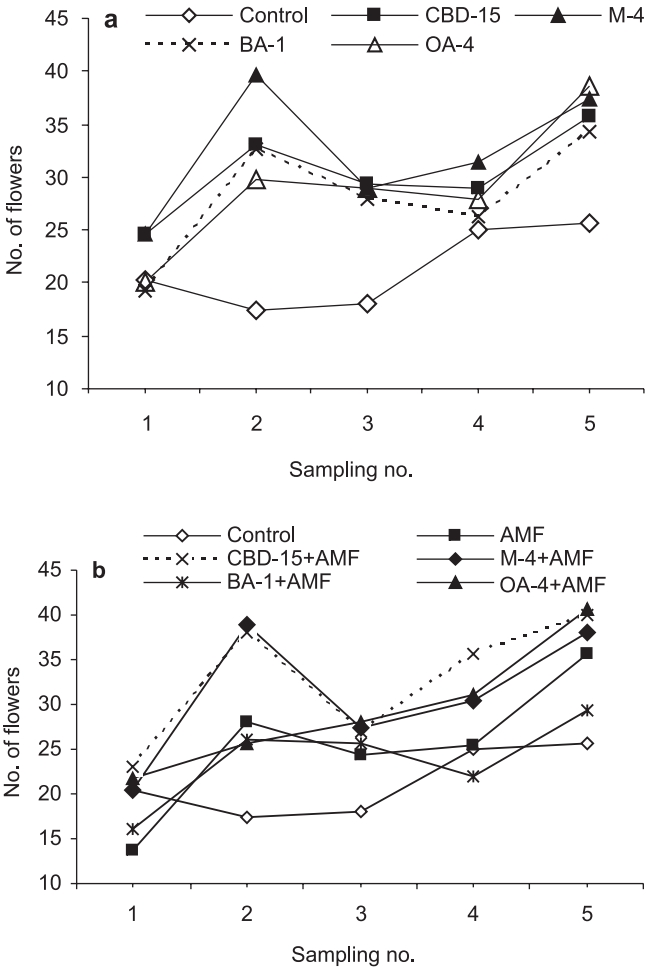

Fig 1 Number of flowers present on plant at different sampling times *(A)* Inoculation of cotton with *A. chroococcum* strains, *(B)* Co-inoculation of cotton with *A. chroococcum* strains and AM fungi.

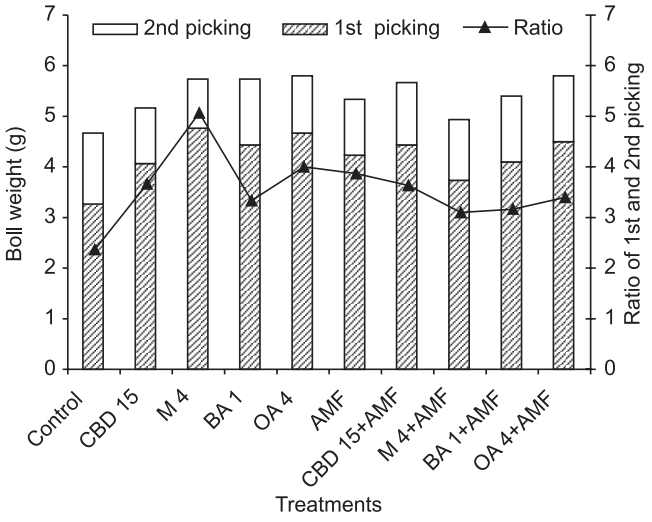

Fig 2 Number of bolls present on plant and the ratio of boll weight obtained during first picking and second picking

flowers and also prevents flower senescence in cotton (El-Saeid *et al.* 2010).

Results indicate that plant growth was stimulated by *A. chroococcum* in cotton as indicated by improvement in plant height, number of flowers and bolls formed, increase in boll weight and yield. Dual inoculation of *A. chroococcum* and AM fungi had a synergistic effect on boll weight and yield. Thus, use of co-inoculation of *Azotobacter* and AM fungi in cotton can be recommended for stimulating plant growth and enhancing yield through bioinoculant application and combination of *A. chroococcum* strain CBD 15+AM fungi can be exploited for maximizing the benefits derived by the plant.

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