British Microbiology Research Journal
2(3): 123-130, 2012

SCIENCEDOMAIN *international*
www.sciencedomain.org

Role of Arbuscular Mycorrhizae (AM) Fungi and Multi Bioinoculants in Cotton Plant Growth

Tasleem Sultana[1] and Pavan Kumar Pindi[1*]

[1]Department of Microbiology, Palamuru University, Mahabubnagar, P.O. Box 509001, Andhra Pradesh, India.

Authors' contributions

This work was carried out in collaboration between both the authors. PKP designed the study, wrote the protocol and managed the analyses of the study and wrote the first draft of the manuscript. TS carried out the designed research work, managed the literature searches, performed the statistical analysis, and. Both the authors read and approved the final manuscript.

Research Article

Received 16th March 2012
Accepted 28th June 2012
Published 15th September 2012

ABSTRACT

Aim: The present study reflects the effect of Arbuscular Mycorrhizae (AM) fungi and other bioinoculants on the growth of cotton seedlings.
Study Design: Screening of efficient biofertilizers from undisturbed forest soils to improve the crop yield of cotton in barren lands of Mahabubnagar District.
Place and Duration of Study: Department of Microbiology, Palamuru University, Mahabubnagar, Andhra Pradesh, India, between May 2011 and February 2012.
Methodology: Mahyco hybrid variety, the most widely cultivated variety in Mahabubnagar District, Andhra Pradesh, India was selected among the sixteen varieties of cotton seeds for this study. Soil samples were analyzed for physicochemical characteristics. Seven different isolates of AM fungi were maintained as pure cultures in laboratory, which were isolated from different Agroforestry tree rhizosphere soils. Among these pure cultures, R1-R2 has shown maximum colonization with Mahyco variety and this isolate was identified as *Glomus mosseae*. Mahyco hybrid variety was also tested with three different bioinoculants (*Rhizobium* sp., *Azospirillum* sp., *Bacillus* sp.) along with the combination of AM pure culture of R1-R2. These three potential bioinoculants were identified based on 16S rRNA gene sequence. Preliminarily Mahyco hybrid variety was investigated with individual pure cultures of AM fungi and with other bioinoculants. R1-R2 was taken in single, dual, triple and multi combinations with other three bioinoculants.

**Corresponding author: Email: pavankumarpindi@gmail.com;*

Results: In single combination M+R1-R2 showed the best growth by M+*Rhizobium*, followed by M+*Bacillus* and in dual combination M+R1-R2+*Rhizobium* and in triple combination M+R1-R2+*Rhizobium+Azospirillum* and in multicombination i.e. M+R1-R2+*Rhizobium+Azospirillum+Bacillus* showed the best growth among all the combinations.
Conclusion: The multicombination mediates increased the cotton growth characteristics. The effect of multicombination was not significantly different in treatment affected by various varieties. Inoculation of multicombination along with AM pure culture resulted significant increase in shoot and root length of cotton plant. So multicombination was proved to be superior.

Keywords: Agroforestry trees; cotton seedlings; AM fungi pure cultures; Rhizobium; Azospirillum; Bacillus.

1. INTRODUCTION

Cotton (White gold) is an important commercial crop of Mahabubnagar District. The quantity and quality of cotton yield are related to its variety. The growth of cotton plant will be the better, when the seeds will be grown in the presence of AM along with bioinoculant (Vazquez et al., 2000). This is because seeds with AM and bioinoculant have better adaptability to critical sites since they have better tolerance to harsh conditions. In mycorrhizal associations, the hyphae of the fungal species invade plant roots and form arbuscles, which facilitate ready exchange of nutrients between the host and the fungus, resulting in the association known as AM (Arbuscular Mycorrhizae) (Linderman, 1998). This association may be parasitic, benign or beneficial (Siqueira, 1986), but it is commonly mutualistic with the fungus receiving energy from the plant. The plant in turn, may receive several benefits from the association (Anne et al., 2009; Sutton, 1973).

Rich and Bird (1974) reported that early-season root and shoot growth of cotton was increased in the presence of mycorrhizal fungi and that these plants flowered and matured bolls earlier. Zak et al. (1998) suggested that the fungus forms a hyphal network in the soil that can serve as an extension of the plant root system. Thus a seedling that is colonized early can explore a much greater soil volume than is possible with an uncolonized newly developing root system. Inorganic ions such as Phosphorus (P) and Zinc (Zn) are absorbed by the fungus and transferred to the plant (Kumar et al., 2001; Qureshi et al., 2012). This improvement of P nutrition is a critical factor in soils with low P content. In turn, this can lead to reduced fertilizer requirements and more efficient use of soil nutrients (Marschner and Dell, 1994). Such seedlings are likely to be more persistent in adverse conditions than non mycorrhizal associated seedlings counterparts.

In the present investigation an attempt has been made to assess the effect of inoculations of AM alone and in combination with other bioinoculants such as *Rhizobium sp RHPU-7, Azospirillum sp PPK-27* and *Bacillus sp PU-17.* These bioinoculants are beneficial bacteria, colonizing in the rhizosphere region (Dhale et al., 2010) and has the ability to fix the nitrogen, solubilizes P and stimulate plant growth.

2. MATERIALS AND METHODS

All experiments were conducted in a greenhouse of the campus, Palamuru University, Mahabubnagar, Andhra Pradesh, India.

2.1 Step 1

Sixteen different varieties of cotton seeds were collected and sown in polythene bags (30x11 cm) containing nursery soil under natural condition without providing any chemical or biological fertilizers. These bags were routinely watered and all the routine nursery precautions were taken. After 65 days the seedlings were carefully extricated and the different parameters were recorded. Philips and Hayman (1970) procedure was employed for clearing and staining the roots. Percentage of infection was calculated by the formula of Giovannetti and Mosse (1980). Among all the sixteen seedlings Mahyco hybrid variety was grown best in all parameters. Hence it was selected for further investigation. The results of Step 1 are presented in (Table 1).

Table 1. AM colonization of 65 days old cotton variety seedlings

Sl. no	Seed Variety	Mycorrhizal Colonization (%)	Height of the plant (cm)		Plant fresh weight (gm)		Plant dry weight (gm)	
			Shoot	Root	Shoot	Root	Shoot	Root
1	Mahyco	80.0	25.1	10.2	2.90	1.21	0.507	0.085
2	Rashi	78.9	20.2	9.5	2.10	1.00	0.502	0.081
3	Super seeds non bt	76.2	19.3	19.1	2.01	0.90	0..499	0.088
4	Obama hybrid	70.4	19.2	8.9	1.90	0.81	0.459	0.072
5	Super nova	69.2	19.1	8.7	1.82	0.70	0.410	0.069
6	Brahmaputra non bt	69.0	18.5	7.9	1.53	0.61	0.392	0.060
7	Ajeet-155 hybrid	65.9	18.1	7.5	1.40	0.51	0.350	0.052
8	Kaveri non bt	64.5	17.5	6.2	1.30	0.40	0.340	0.050
9	S99bt	62.8	17.1	6.0	1.20	0.47	0.339	0.049
10	Raj seeds	60.9	16.2	5.9	0.91	0.42	0.329	0.051
11	Veda-2Bg-ll hybrid	60.5	16.1	5.7	0.89	0.40	0.322	0.050
12	Nusun seeds	55.9	14.2	5.5	0.69	0.39	0.189	0.051
13	Marvel bt-ll	55.5	13.8	5.1	0.61	0.31	0.185	0.045
14	PCH-125	50.2	12.9	4.8	0.59	0.29	0.182	0.043
15	Bunny seeds	49.8	12.8	4.5	0.58	0.25	0.177	0.041
16	Sunny NCS-108	48.9	11.9	4.1	0.52	0.22	0.161	0.035
	F-Values		0.0058	0.0316	0.1481	0.2944	0.0370	0.1443

2.2 Step 2

Mahyco hybrid variety along with the seven different pure cultures of AM were sown in polythene bags (20x15cm) containing sterilized soil.

2.2.1 Maintenance of pure culture

AM pure cultures are single spore cultures isolated from Agro forestry tree rhizosphere soil. These pure cultures are always maintained in active stage by sowing Sorghum seeds at regular time intervals.

2.2.2 Preparation of sterilized soil

1:1 ratio of sand and red soil were mixed properly and sterilized in autoclave at 121ºC, 15 lbs pressure. After sterilization the soil was filled in polythene bags (20x15) and AM pure cultures were placed just below the seeds in order to make it available to the germinating seeds. The following treatments were used:

1. M + Control
2. M + V1
3. M + V2
4. M + V3
5. M + S1
6. M + S2
7. M + P1-P2
8. M + R1-R2

These bags were given sterilized water regularly and important precautions were taken. After 50 days the seedlings were carefully extricated and the different parameters were determined. Philips and Hayman's (1970) procedure was employed for clearing and staining the roots. Percentage of infection was calculated by the formula of Giovannetti and Mosse (1980). Among all 7 pure cultures R1-R2 pure culture showed the best growth in all parameters and was identified as *Glomus mossae*. So it was taken for further investigations. The results of Step 2 are presented in (Table 2).

Table 2. Assessment of the AM fungi effect on Mahyco hybrid variety cotton seedlings after 50 days of growth

Sl. no	Treatment of AM Pure culture	Mycorrhizal Colonization (%)	Height of the plant (cm)		Plant fresh weight (gm)		Plant dry weight (gm)	
			Shoot	Root	Shoot	Root	Shoot	Root
1	M-Control	-	10.1	7.10	0.52	0.41	0.27	0.79
2	M+V1	71.0	17.5	10.4	0.95	0.87	0.69	0.59
3	M+V2	75.1	18.3	11.5	1.00	0.95	0.82	0.66
4	M+V3	42.8	12.1	10.5	0.81	0.83	0.67	0.53
5	M+S1	57.0	12.3	8.10	0.77	0.74	0.58	0.51
6	M+P1-P2	69.3	12.9	10.3	0.89	0.86	0.69	0.59
8	M+R1-R2	85.0	30.3	12.1	2.00	1.24	1.20	0.79
	F-Values		0.00001	0.0010	0.9432	0.0215	0.0417	0.0518

Key; M- Mahyco, Rhi- Rhizobium, Azo- Azospirillum, Bac- Bacillus.

2.3 Step 3

2.3.1 Funnel experiment

Mahyco hybrid variety was sown along with R1-R2 AMF and three different bioinoculants (*Rhizobium sp RHPU-7, Azospirillum* sp *PPK-27, and Bacillus* sp *PU-1*) in funnels containing sterilized soil.

2.3.2 Preparation of standard inoculum

The standard inoculum was prepared by inoculating log phase cultures of bioinoculant *Rhizobium* sp. *RHPU*-7, *Azospirillum* sp. *PPK-27 and Bacillus* sp. *PU-1* in nutrient broth. *Rhizobium RHPU-7* was isolated from Kuthagudem soil, *Azospirillum PPK-27* was isolated from Khammam soil and *Bacillus* sp *PU-1* was isolated from Achampet soil and maintained as pure cultures in laboratory of Microbiology, Palamuru University.

2.3.3 Treatment of seeds

Rhizobium sp. *RHPU*-7, *Azospirillum* sp. *PPK-27, Bacillus* sp. *PU-1* are novel species which were identified based on the 16s r-RNA genomic sequence and maintained as pure cultures in the laboratory. One µl of each bioinoculant was added over the seed at the time of sowing. The following treatments were used in Single, Dual, Triple and Multi combinations.

1. Single combination : M+R1-R2, M+Rhi, M+Bac, M+Azo.
2. Dual combination : M+R1-R2+Rhi, M+R1-R2+Bac, M+R1-R2+Azo.
3. Triple combination : M+R1-R2+Rhi+Azo, M+R1-R2+Azo+Bac, M+R1-R2+Rhi+Bac.
4. Control : For every combination one control was maintained.

After germination of seeds in funnels, the seedlings were transferred to the pots containing sterilized soil. The pots were given sterilized water regularly and all the important precautions were taken. After 60 days the seedlings were carefully extricated and the different parameters were recorded. Philips and Hayman (1970) procedure was employed for clearing and staining the roots. Percentage of infection was calculated by the formula of Giovannetti and Mosse (1980). The results of Step 3 are presented in (Table 3).

2.3.4 Measurement of growth parameters

The observations on different growth parameters of experimental plants were recorded after 50 days of growth. The length of the shoot was adjusted by taking the physical count of the length of the shoot from color region to apical bud. The length of the root was adjusted by taking the physical count of the length of root from color region to the tip of the tap root. The fresh root and shoot samples were measured physically on the top loading balance and resulting weight were recorded as shoot and root fresh weight in grams. The dry matter accumulation by root and shoot was recorded by subjecting the root and shoot to oven drying at 60ºC till the constant weight and physical weight was recorded.

Table 3. Effect of AM fungi and other bioinoculant on 50days old Mahyco hybrid variety cotton seedlings

Sl. No.	Treatment of AM fungi and other bioinoculants	Mycorrhizal Colonization (%)	Height of the plant (cm)		Plant fresh weight (gm)		Plant dry weight (gm)	
			Shoot	Root	Shoot	Root	Shoot	Root
1	M-Control	-	6.1	0.9	0.800	0.004	0.534	0.029
2	M+R1-R2	83	7.5	1.8	0.927	0.201	0.674	0.100
3	M+Rhi	70	6.5	1.5	0.900	0.100	0.600	0.060
4	M+Bac	65	6.0	1.0	0.807	0.060	0.545	0.030
5	M+Azo	50	5.2	0.5	0.773	0.030	0.409	0.010
6	M+R1-R2+Rhi	75	6.0	1.1	0.806	0.060	0.500	0.039
7	M+R1-R2+Bac	70	5.7	0.9	0.800	0.050	0.490	0.020
8	M+R1-R2+Azo	60	4.7	0.4	0.700	0.020	0.400	0.008
9	M+R1-R2+Rhi+Azo	75	8.0	2.0	0.997	0.220	0.635	0.009
10	M+R1-R2+Azo+Bac	65	6.4	1.2	0.899	0.090	0.515	0.050
11	M+R1-R2+Rhi+Bac	70	5.5	0.7	0.798	0.040	0.400	0.020
12	M+R1-R2+Rhi+Azo+Bac	82	8.2	2.3	1.000	0.300	0.700	0.150
	F-Values		0.1830	0.0782	0.6760	1.5560	0.6934	0.0079

Key; M- Mahyco, Rhi- Rhizobium, Azo- Azospirillum, Bac- Bacillus.

3. RESULTS AND DISCUSSION

3.1 Plant Growth Parameters

Mycorrhizal colonization, height of the plant, fresh and dry weight of the plant was studied and data are presented in Table 1. The data clearly indicated that among all cotton seed varieties examined within the limits of this investigation, Mahyco hybrid showed the best in all parameters. Its mycorrhizal colonization (80.0%) showed maximum shoot length 25.1cm, highest root length 10.2cm, highest dry weight of shoot i.e. 0.507g and highest dry weight of root i.e. 0.085g.

The effect of inoculations of AM pure culture on plant height and root length of cotton was studied and data are presented on Table-2. The data evidently indicated that the plant which was getting R1-R2 AM pure culture showed the maximum mycorrhizal colonization i.e.85.0%. Its shoot length of 30.73cm, root length of 12.1cm, fresh shoot weight of 2.00g, fresh root weight of 1.24 g, dry shoot weight of 1.20g and dry root weight of 0.79g were recorded.

The effect of combined inoculation of AM pure culture i.e. R1-R2 and bioinoculants on plant height and root length of cotton was studied (Saharan and Nehra, 2011) and data are presented in Table-3. The data clearly indicated that plant which was provided with R1-R2 pure culture *Rhizobium* sp. *RHPU*-7, *Azospirillum* sp. *PPK-27 and Bacillus* sp. *PU-1* showed the best growth in all parameters (Sobhan Ardakani et al., 2010). Its mycorrhizal colonization was found to be superior to Single, Dual and Triple combinations i.e. 82.0%. Plant shoot length was maximum i.e. 8.2cm, root length was 2.3cm, shoot fresh weight was 11.0 g , root fresh weight was 0.30 g, dry weight was 0.70 g and root dry weight was 0.15 g, (Table 3). Triple combination M+R1-R2+*Rhizobium+Azospirillum* showed the best growth in all parameters rather than M+R1-R2+*Azospirillum+Bacillus*. The least colonization was found in M+R1-R2+*Rhizobium+Bacillus* (Table 3). In Dual combination best growth was shown by M+R1-R2+*Rhizobium*. Its height, fresh weight, dry weight and mycorrhizal colonization were found to be superior to M+R1-R2+Bac. The least colonization was found in M+R1-R2+Azo. In single combination best growth has shown by M+R1-R2, its height, fresh weight, dry weight and mycorrhizal colonization was found to be superior to M+Rhi, M+Bac and M+Azo.

4. CONCLUSION

Inoculation of multicombination along with AM pure culture resulted significant increase in shoot and root length of cotton plant and multicombination was proved to be superior. Hence this combination was tested with different soil types of Mahabubnagar District and it was shown the similar results in all soil types.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. K. Narasimha Reddy, Vice Chancellor and Prof. K. Venkata Chalam, Registrar, Palamuru University for the constant support and facilities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Anne, P., James, D.B., Monique, G., Jeri, L.P., Matthias, C.R., John, N.K. (2009). Mycorrhizal symbioses and plant invasions, annual review of ecology. Evolution and Systematics, 40, 699-715.

Ardakani, S., Heydari, A., Tayebi, L., Mohammadi, M. (2010). Promotion of cotton seedlings growth characteristics by development and use of new bioformulations. International Journal of Botany, 6(2), 95-100.

Dhale, D.A., Chatte, S.N., Jadhav, V.T. (2010). Effect of bioinoculant on the growth, Yield and Fiber quality of cotton under irrigation. Research J. of Agri and Bio. Sci., 6(4), 542-547.

Giovannetti, M., Mosse, B. (1980). An evaluation of techniques for measuring VAM infection in roots. New Phytologist, 84, 489-500.

Kaushik, J.C., Kaushik, N. (1995). Proceedings of the third national conference on Mycorrhiza, 212-215.

Kumar, R., Gupta, P.P., Jalali, B.L. (2001). Impact of VA-Mycorrhiza, *Azotobacter* and *Rhizobium* on the growth and nutrition of cowpea. J. Mycol. Plant. Pathol., 31, 38-41.

Linderman, R.G. (1988). Vesicular arbuscular mycorrhizal symbiosis. Scienntia Ani. Pl. Sci., 1, 183-188.

Marschner, H., Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil, 159, 89-102.

Phillips, J.M., (1970). Hayman's improved procedure for clearing and staining parasitic and vesicular-Arbuscular mycorrhizal fungi for assessment of infection. Transactions of the British Mycological Society, 55, 158.

Qureshi, M.A., Ahmad, Z.A., Akhtar, N., Iqbal, A., Mujeeb, F., Shakir, M.A. (2012). Role of Phosphate solubilizing bacteria (PSB) in enhancing P availability and promoting cotton growth. The Journal of Animal and Plant Sciences, 22(1), 204-210.

Rich, J.R., Bird, G.W. (1974). Association of early-season vesicular-arbuscular mycorrhizae with increased growth and development of cotton. Phytopathology, 64, 1421-1425.

Saharan, B.S., Nehra, V. (2011). Plant growth promoting rhizobacteria; A critical review. Life Sciences and Medicine Research, Vol 2011, LSMR-21, 1-30.

Siqueira, J.Q., Colozzi-Filhu, A., Fairia, F.H.S., Oliverira, E. (1986). Symbiotic effectiveness of vesicular Arbuscular mycorrhizal fungal species in Cotton. Rev. Brasi. De. Cien. Dosolo., 10, 231-218.

Sutton, J.C. (1973). Development of vesicular arbuscular mycorrhizae in crop plants. Can. J. Bot., 51, 2487-2493.

Vazquez, M., Cesar, S., Azcon, R., Barea, J.M. (2000). Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum, Pseudomonas, Trichoderma*) and their effects on microbial populations and enzyme activities in the rhizosphere of Maize plants. Appl. Soil Ecol., 15(3), 261-272.

Zak, J.C., McMichael, B., Dhillion, S., Friese, C. (1998). Arbuscular-mycorrhizal colonization dynamics of cotton (*Gossypium hirsutum* L) growing under several production system on the southern high plains, Texas. Agriculture Ecosystem and Environment, 68(3), 245-254.