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EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI AND MULTI-COMBINATION OF BIOINOCULANTS ON REGENERATED SEEDLINGS OF COTTON

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Cotton, referred as "The white gold" is an important commercial crop in India and stands third in the world by means of area of cultivation. Cotton plant regeneration from callus by somatic embryogenesis and its efficiency has been improved significantly in recent times. Our primary investigation was on regenerative studies and multiple shoot induction system focusing mainly on meristematic tissues like seedling cotyledonary nodal explants in RAH-9750 cotton cultivar. An attempt has been made to improve the rate of surveillance and growth of regenerated cotton seedlings by bio-inoculant (mainly AMF) treatment under greenhouse conditions. Out of a total seven pure cultures of AMF fungi, R1-R2 have shown maximum mycorrhizal colonization with RAH-9750 (R) and was identified as *Glomus mosseae*. This variety was also tested with three different bioinoculants i.e., *Rhizobium sp*. RHPU-7, *Azospirillum sp.* PPK-27, *Bacillus sp.* PU-1, apart from AMF R1-R2 in different combinations. The cotton seedlings have shown the best results in single, dual, triple and multiple combinations i.e R+R1-R2, R+R1-R2+*Rhizobium*, R+R1-R2+*Rhizobium*+*Azospirillum* and R+R1-R2+*Rhizobium*+*Azospirillum*+*Bacillus* respectively. The growth of cotton plants (RAH-9750) generated from meristematic tissue culture was found to be increasing significantly when compared with the normal seeds. Similar results were noticed when the same experiment was subjected to the different soil types of Mahabubnagar district. The investigation clearly infers that better yield of cotton RAH-9750 (R) could be achieved by treating the regenerated cotton seedlings with bioinoculants in different combination in various soil types of Mahabubnagar district.

Keywords: Regeneration, AMF pure cultures, *Rhizobium sp.*, *Azospirillum sp.* and *Bacillus sp*.

***Abbreviations*:** BAP- Benzyl Aminopurine, BA- Benzyl Adenine, Kn- Kanamycin, NAA- Naphthalene Acetic Acid, AMF- Arbuscular Mycorrhizal Fungi

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is an important agricultural and commercial crop for fiber, feed, and edible oil in the world and occupies a major source of foreign exchange in India. The growth of cotton plant will be better, when the seeds will be grown in the presence of Arbuscular mycorrhizal fungi (AMF) along with bioinoculants, (Vazquez *et al*., 2000) because the seeds with AMF & bioinoculants have better adaptability to critical sites because of their tolerance to harsh conditions. Cotton plants are capable of assimilating water and nutrition elements, even if they are in small amount of soil, due to their extensive root system (Shabbir *et al*., 2012*)*. Biochemical changes in cotton such as Protein, sugar, aminoacid and phenol levels are also influenced by the increased mycorrhization (Damodaran *et al*., 2010). Plant tissue culture plays an important role in modern cotton breeding and genetic engineering; it has been showing a significant impact on crop production. To take advantage of this promising technology, a reliable and genotype-independent regeneration system is essential for *in vitro* studies and genetic manipulation for crop improvement. Cotton plants can be regenerated from callus by somatic embryogenesis (Tanveer *et al*., 2006) and the efficiency of regeneration via somatic embryogenesis has been improved significantly in recent years.

Earlier many scientists reported and used meristematic tissues and axilary buds as explants which helped in root-shoot regeneration (Morre *et al*., 1998). Cotton is a recalcient species and its very difficult to manipulate *in vitro* response. Very limited number of cultivars are in regenerating manner (Trolinder and Goudin, 1987; Cousins *et al*., 1991). An *in vitro* culture of meristamatic tissues and shoot tips has been reported to give single or sporadically, a few shoots (Bajaj and Gill, 1986; Gould *et al*., 1991). *In vitro* shoot regeneration from the meristematic tissue shoot tip, axillary buds and cotyledonary nodal explants are direct, relatively simple and are not prone to somaclonal variations and chromosomal aberrations. *In vitro* regeneration and genetic engineering studies of Indian local cotton cultivars lag far behind with only few regenerated varieties which are

reported and also the direct regeneration has been attempted in so many species of cotton but the result expected is very limited (Agarwal *et al.,* 1997; Gupta *et al*., 1997; Kumar and Pental, 1998; Sathyavathi *et al*., 2002; Khan *et al*., 2006).

The role of Arbuscular mycorrhizal (AM) fungi has been described as a link between plant and soil (Bethlenfalvay and Linderman, 1992; Miller and Jastrow, 1994). Mycorrhizas are highly evolved associations between soil fungi and plant roots and symbiosis means they are living together (Bagyaraj and Varma, 1995). Many crops depend on mycorrhizal symbiosis and this is especially true for cotton (Dimitris Bilalis *et al*., 2011) because the fungus is the primary means by which it obtains important elements, such as phosphorus from the soil. The fungi increase the volume of soil from which the root system can extract these elements and in return the plant provides the fungus with sugars produced by photosynthesis. 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 counterparts.

In present investigation an attempt has been made on regeneration and multiple shoot induction from meristamatic tissues like seedling cotyledonary nodal explants in RAH-9750 and an attempt has been made to assess the effect of inoculations of AM alone & in combination with other bioinoculants such as *Rhizobium sp.* RHPU-7 (Barbara *et al*., 2008), *Azospirillum sp.* PPK-27 and *Bacillus sp.* PU-1. These bioinoculants are beneficial bacteria, colonizing in the rhizosphere region (Dhale *et al*., 2010) and has the ability to protect the plant from plant pathogens, fixes the nitrogen, solubilizes P and stimulate plant growth. Some of the studies have shown that a relationship exists between chemotactic behavior and *Azotobacter's* influence on plant growth where they play an important role in plant protection and growth promotion (Berg *et al*., 2005; Bell *et al*., 1995).

MATERIALS AND METHODS

***Plant material*:** The preliminary experiment was conducted *in vivo* for the selection of best three cultivars namely RAH-9750, N-32 and ARB-8901 among the nine Indian cotton cultivars. Again among these three cultivars RAH-9750 was selected for further experimental studies.

***Seed germination and plant material*:** Cotton seeds (*Gossypium hirsutum* L) were collected from Regional Agricultural Research Centre, Warangal, N.G Ranga Agricultural University, Hyderabad, India. The seeds were decoated and sterilized with 0.1% (w/v) aqueous mercuric chloride ($HgCl_2$) solution for 10 min then washed four times subsequently with double distilled water. The sterile seeds were inoculated in 100 ml conical flasks containing 50ml ½ strength MS (Murashige and Skoog, 1962) medium consisting of 15g/l sucrose and 0.8% (w/v) agar, the pH of the medium was adjusted to 5.8 before autoclaving as described by Wu and others (2004). Seeds were incubated at 28°C in dark for 7 days.

***Shoot regeneration from seedling cotyledonary nodal explants*:** Among nine Indian cotton cultivars, three best responsive tissue culture cultivars (RAH-9750, N-32 and ARB-8901) were selected. Among these three cultivars, the best explants responsive tissue culture cultivar is RAH-9750. Seedling cotyledonary nodal explants were excised from aseptically grown seedlings and cut into 5-7 mm size explants placed on the medium containing MSB1 (MS basal salts, B5 vitamins) (Gamborg *et al*., 1968) supplemented with 3% (w/v) glucose with different hormones supplemented with BA (0.5–2.0 mg/l) and NAA (0.1 mg/l) and Kn (0.5–2.0 mg/l) with NAA (0.1 mg/l) for initiation, proliferation and shoot elongation. The viable shoots were sub cultured onto different media i.e., MSB2 (MS basal salts supplemented with the low concentration of BA (1.5 mg/l) combination with NAA (0.1mg/l) for shoot elongation. The explants initiation and elongation of shoots data were recorded after 30 days of the inoculation.

***Grafting of micro shoots*:** Among in vitro grown micro shoots, some of them did not develop roots on the MS medium supplemented with different auxins concentration and also MS ½ strength media. Unrooted micro shoots were grafted (Shuangxia *et al*., 2006) onto the seedling stocks of the same variety grown *in vitro*. These seedlings stocks were the healthy normal plantlets with two primary leaves grown from in vitro grown seedlings of the age between 8–10 days. The first step was to cut the bottom of the scion into a wedge with a scalpel blade then the upper part of the seedling stocks was cut under the first true leaf; and a slit (about 1.0 cm) on the stem was cut vertically. The decapitated end of the root stocks and matching cut ends of the scions. Then the scion was inserted into the slit and the cambiums were lined up. Final step was to bind the grafted parts together with Para film then inoculated in the conical flask with medium supplemented with ½ strength MS basal salts with B5 vitamin and the cultures maintained in an automatically controlled incubation room at 26±2°C under a 16-h (day) / 8-h (night) photoperiod with light provided by cool – white fluorescent lamps at an irradiation of 135 µmol m-2 s-¹ for 30 days.

Next step was to remove fully rooted plant from conical flask then thoroughly washed in running tap water for 5 min to remove the media residues then transferred into pot with sterilized soil and sand (1:1) and kept in incubation chamber maintained with temperature of 26±2°C and humidity of 70-80% for another 15 days before being transferred to the greenhouse. It is important to keep proper humidity in the chambers. Before transferring to the greenhouse, seedlings were inoculated with AMF and different combinations of AMF and other bioinoculants. Then the plantlets were transferred to the greenhouse for further growth and

establishment. 90 days old RAH-9750 cotton plantlets were used for experimental observations.

***AMF pure cultures*:** *AMF* pure cultures are single spore cultures isolated from Agro forestry tree rhizosphere soil. Resting spores of AM fungi were extracted by wet sieving and decanting method of Gerdemann and Nicolson and Pacioni. The collected soil was poured into 500 ml of water and shaked gently and allowed to settle for few seconds. The supernatant liquid was poured through a coarse soil sieve (500 to 800 mm) to remove large pieces of organic matter. The lower collected liquid was passed through different sieves (250, 106 and 45 micropores). This process was continued till all colloidal materials passed through the sieves. All the debris collected on sieves were taken into a petri dish and was observed for resting spores under the stereobinacular microscope. Similar spores were carefully collected with needles and brushes for the preparation of pure cutures and respective spore slide was made in lactophenol for further study and identification. The resting spores were identified by following by key provided by Schenck and Perez (1987).

***Maintenance of AM inoculum*:** AM inoculum is maintained on grass host Cenchrus ciliaris under green house conditions. The pots for the maintenance and multiplication are prepared as follows. Sand soil mixture (1:1) was sterilized at 15lbs/inch pressure for 40 minutes two times on alternate days. The autoclaved soil mixture was filled in small sterilized pots. After that, the inoculum containing root pieces and resting spores present in soil was placed 5 cm below the surface of the soil as a uniform layer in such a way that roots pass through the inoculum layer. Cenchrus ciliaris seeds were placed just below the soil surface. The pots were watered uniformly and now and then with Hoagland nutrient solution without phosphorus. The pots were kept in uniformly lighted condition. After 15 days of plant growth small pieces of roots were taken out from the centre of the pot and examined for mycorrhizal infection after staining with trypan blue. The roots and the rhizosphere soil were taken for counting the number of spores. At the time of inoculation, the upper shoot system was cut off and roots mixed with soil is employed as inoculum. The inoculum thus prepared was used in further experiments. These pure cultures are always maintained in active stage by sowing seeds at regular time intervals.

***Treatment of tissue cultured seedlings of RAH-9750 with AMF*:** RAH-9750 cotton seedlings along with the seven different pure cultures of AMF were sown in polythene bags (20x15cm) containing sterilized soil. The bags were given sterilized water regularly. After 90 days the seedlings were carefully extricated and the different parameters were recorded. Phillips and Hayman (1970) procedure were employed for clearing and staining the roots. Percentage of infection was calculated by the formula of Giovannetti and Mosse (1980). Best AMF pure culture may be selected for further studies.

***Preparation of standard inoculum of bioinoculants*:** The standard inoculums 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, *Azospirillum* PPK-27 and *Bacillus* sp PU-1 were isolated from Kothagudem, Khammam and Achampet soils, respectively and maintained as pure cultures in laboratory of Microbiology, Palamuru University.

***Treatment of tissue cultured seedlings of RAH-9750 with bioinoculants and R1-R2 pure culture of AMF*:** *Rhizobium* sp. RHPU-7 (Hafeez *et al*., 2004), *Azospirillum* sp. PPK-27, *Bacillus* sp. PU-1 are novel species which were indentified based on the 16S rRNA genomic sequence & maintained as pure cultures in the laboratory. One µl of each bioinoculant was added at the time of transferring the seedlings to the pots along with the R1-R2 pure culture of AMF. The following treatments were used in Single, Dual, Triple & Multi combinations.

Single combination : R+R1-R2, R+ *Rhizobium*, R+ *Bacillus*, R+ *Azospirillum*.

Dual combination: R+R1-R2+ *Rhizobium*, R+R1-R2+ *Bacillus*, R+R1-R2+ *Azospirillum*.

Triple combination : R+R1-R2+ *Rhizobium* + *Azospirillum*, R+R1-R2+ *Azospirillum* + *Bacillus*, R+R1-R2+ *Rhizobium* + *Bacillus*.

Control : For every combination one control was maintained.

The seedlings were transferred to the pots containing sterilized soil. The pots were given sterilized water regularly. After 90 days the seedlings were carefully extricated and 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).

***Measurement of growth parameters of regenerated seedlings of RAH-9750*:** The observations on different growth parameters were recorded after 90 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 & shoot samples were measured physically on the top loading balance & resulting weight were recorded as shoot & 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.

Statistical analysis: Data of Table 2 and Table 3 was subjected to Pearson correlation coefficient and p-value is also provided.

RESULTS

The AMF cultures were single spore cultures and were identified based on their morphology and cellular characters and compared with original descriptions of Schenck and Perez and Among all seven pure cultures, R1-R2 pure culture has shown the best growth in all parameters, which was identified as *Glomus mossae* and hence it was taken for further investigations. The effect of these above AMF pure culture on seeds and plant parameters were studied.

***Shoot induction and multiple shoot induction*:** Among three different cultivars RAH-9750 was found to be the best cultivar for shoot initiation and multiple shoot induction (Table 2). All treated cultivars showed the bud proliferation and initiation in between 5 to 7 days. The media with different combination of hormones was effective in the induction of sprouting of existing buds for production of shoots of each explant (Hazra *et al*., 2000). It seems that all the treatments assayed were able to cause shoot induction with 79% efficiency. Seedling cotyledonary nodal explants were more responsive to BA compared to the other explants (Table 1). There was no progress on the treatments during the first week of observations. Shoot regeneration occurred via adventitious proliferation from the axillary portion. Meristem based regeneration methods have been used successfully for transformation in cotton (McCabe and Martinell, 1993) Rapid shoot growth and multiple shoot induction performed after 4 weeks old culture. MS media supplemented with BA (1.5mg/l) with combination of auxin like NAA (0.1mg/l) in RAH-9750. When compared to BA, the Kn showed slow growth of shoot and some cases stunted growth. There was no much difference in micro shoot height with different concentration of Kn. Low level concentrations of Kn with NAA resulted formation of callus at the cut end of the explants (Table 1). When MS media was supplemented in higher concentration of Kn with NAA, the cotyledonary nodal explants were similar in all the cultivars and shoot initiation has taken place within 3-5 days**.** They did not induce any multiple shoot formation at different level of growth of explants. Viable micro shoots were grown vigorously and were transferred for the development of multiple shoot formation. When elongated shoots were maintained on MS media supplemented with hormonal concentration of BA (1.5mg/l) with NAA (0.1mg/l), the first and second subcultures produced six to eight multiple shoots per explant (Table 1).

Table 1. Effect of the different phytohormones on percentage of shoot initiation and shoot length of coteledonary nodal explants of the Indian cotton cultivars after 30 days of culture.

Cultivar	Phytohormones (mg/l)			Explants response (%)	Length of Shoot (cm) (Mean±SD)
	BA	Kn	NAA		
RAH-9750	1.5	0.1	0.1	94	2.25±0.71
GTHV-302	1.5	0.1	0.1	72	2.81±0.40
LH-1802	1.5	0.1	0.1	78	3.02±0.58
F-2036	1.5	0.1	0.1	62	1.68±0.71
Y-668	1.5	0.1	0.1	60	2.66±0.90
ARB-8901	1.5	0.1	0.1	80	2.05±0.29
N-32	1.5	0.1	0.1	82	2.81±0.32
AK-32	1.5	0.1	0.1	70	1.83±0.40
AR-20	1.5	0.1	0.1	65	2.58±0.30

SD – standard deviation

Table 2. Effect of AMF on 90 days old RAH-9750 regenerated cotton seedlings

Treatment of AMF Pure ultures	Mycorrhizal colonization (%)	Height of the plant (cm)		Plant fresh weight (g)		Plant dry weight (g)	
		Shoot	Root	Shoot	Root	Shoot	Root
R[a]-Control	-	15.8	10.2	10.7	0.84	0.73	0.52
R+V1[b]	74.3	20.5	16.8	3.68	1.25	0.97	0.82
R+V2[c]	80.3	24.8	16.5	2.25	1.32	0.99	0.89
R+V3 [d]	47.8	13.7	8.92	1.34	0.88	0.72	0.46
R+S1[e]	62.0	14.3	10.0	2.03	0.96	0.84	0.59
R+S2[f]	61.1	15.2	11.1	1.35	0.99	0.90	0.60
R+P1-P2[g]	72.2	16.7	13.9	1.54	1.51	0.95	0.82
R+R1-R2[h]	87.5	38.4	17.7	4.49	2.79	2.45	1.59
r		0.551	0.702	0.709	0.609	0.526	0.642
p-value		p<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

Where [a]-RAH-9750 Cotton cultivar, [b, c, d, e, f, g, h] are AMF pure cultures; *r* = Pearson orrelation coefficient

The effect of inoculations of AMF pure culture on plant height and root length of RAH-9750 cotton was studied (Table 2). The data evidently indicated that the plant which was getting R1-R2 AMF pure culture showed the maximum mycorrhizal colonization i.e. 87.5%. Its shoot length was 38.4 cm, root length was 17.7 cm, fresh shoot weight was 4.49 g, fresh root weight was 2.79g, dry shoot weight was 2.45 g and dry root weight was 1.59 g. (Table 2).

The effect of inoculations of AMF pure culture along with bioinoculants on plant height and root length of RAH-9750 was studied (Table 3). The data clearly indicated that the plant which got the multicombination of bioinoculants along with AMF pure culture, i.e. R1-R2 pure culture *Rhizobium sp.* RHPU-7, *Azospirillum sp.* PPK-27, *and Bacillus sp.* PU-1 showed the best growth. Its mycorrhizal colonization was 88%. Plant shoot length was reported to be maximum, i.e. 19.9 cm, root length was 14.5 cm, shoot fresh weight was 1.49 g , root fresh weight was 0.97 g, shoot dry weight was 0.98g, root dry weight was 0.61g. In Triple combination with R+R1-R2+*Rhizobium+Azospirillum*, the best growth in all parameters such as height, fresh weight, dry weight was recorded. Also it's mycorrhizal colonization was found to be greater than R+R1-R2+*Azospirillum+Bacillus* while the least colonization was recorded in R+R1-R2+*Rhizobium+Bacillus*. In Dual combination, the best growth was shown by R+R1-R2+*Rhizobium*. It's height, fresh weight, dry weight & mycorrhizal colonization were found to be superior to R+R1-R2+*Bacillus* where as the least colonization was found in R+R1-R2+*Azospirillum.* In Single combination, best growth was shown by R+R1-R2. It's height, fresh weight, dry weight & mycorrhizal colonization were found to be superior to R+Rhi, R+*Bacillus* while least colonization was found in R+*Azospirillum*.

In the recent decades, there has been a focus on the development of regeneration systems through shoot meristamatic tissues. (Agarwal *et al*., 1997; Hemphill *et al*., 1998; Sathyavathi *et al*., 2002). Regeneration from the pre existing meristamatic tissues as explants like seedling cotyledonary nodal explants, shoot tips, nodal explants, shoot axillary buds explants and shoot meristems was direct and simple. Theoretically, each excised meristamatic tissue should develop into a rooted plant which is already a programmed tissue for further growth. However, the yield of shoots *in vitro* from isolated meristamatic tissues depends on the incidence of contamination and rooting efficiency (Gould *et al*., 1991). The induction of multiple shoots in explants varied with hormonal concentration and the age of explants. MS basal medium without BAP or Kn did not support the induction of multiple shoots. In the shoot proliferation and multiple shoot induction, BA is very important cytokinin in single or combination with auxins was reported by many scientists (Agarwal *et al*., 1997, Sathyavathi *et al*., 2002).

The lower level of BA concentration will promote the high rate proliferation of explants and induce the multiple shoot induction (Saeed Rauf *et al*., 2005) whereas higher concentrations of BA will result in the low level proliferation (Sulekha *et al*., 2000) and single shoot production. The seven days old seedling cotyledonary nodal explants yield the maximum multiple shoot induction (6-8 shoots/explants) on MS (BA 1.5mg/l + NAA0.1mg/l). The lower and higher level concentrations of Kn promote the single shoot proliferation but sometimes the higher

Table 3. Effect of AMF and other bioinoculants on 90days old RAH-9750 regenerated cotton seedlings:

Type of Combination	Treatment of AMF & other bioinoculant	Mycorrhizal Colonization	Height of the plant (cm)		Plant fresh weight		Plant dry weight(gm)	
			Shoot	Root	Shoot	Root	Shoot	Root
Single combination	R-Control	-	17.0	10.1	0.99	0.72	0.54	0.24
	R^a+R1-$R2^e$	88	20.5	14.0	1.40	0.89	0.81	0.45
	R+Rhi^b	77	18.2	13.1	1.13	0.75	0.67	0.39
	R+Bac^c	73	18.1	12.3	1.58	0.76	0.73	0.42
	R+Azo^d	70	17.8	10.5	1.52	0.55	0.69	0.28
Dual combination	R+R1-$R2^e$+Rhi^b	85	18.9	13.8	1.33	0.85	0.76	0.52
	R+R1-$R2^e$+Bac^c	80	18.3	13.3	1.35	0.90	0.80	0.50
	R+R1-$R2^e$+Azo^d	71	18.0	10.6	1.07	0.77	0.71	0.40
Triple combination	R+R1-$R2^e$+Rhi^b+Azo^d	83	18.9	13.8	1.25	0.92	0.78	0.49
	R+R1-$R2^e$+Azo^d+Bac^c	73	18.2	12.3	1.20	0.78	0.76	0.46
	R+R1-$R2^e$+Rhi^b+Bac^c	78	18.4	12.6	1.14	0.82	0.75	0.49
Multiple combination	R+R1-$R2^h$+Rhi^b+Azo^d+Bac^c	88	19.9	14.5	1.49	0.97	0.98	0.61
r			0.896	0.925	0.173	0.822	0.724	0.753
p-value			P<0.05	p<0.05	p<0.05	p<0.05	p<0.05	p<0.05

Where [a]-RAH-9750 Cotton cultivar, [b]-Rhizobium, [c]-Bacillus.[d]-Azospirillum, [e]-AMF pure culture, *r* = Pearson orrelation coefficient

concentration of Kn promotes to proliferate the callus induction (Stelly *et al*., 1989; Garshasbi *et al*., 2012) at the cut ends of the explants, which results in the stunted growth and the leaf turns yellow in color. This may be due to the endogenous hormonal regulations. To overcome this problem, finally we adopted *in vitro* technology to micro shoots. Grafting is a very useful technique and is commonly used in horticultural crops. Unrooted micro shoots, were grafted with normal in vitro grown seedlings as previously described method of Luo and Gould (1999), Jin and others (2006). In this procedure it was observed that the plant has good rooting efficiency and recovery with maximum of 90% survival rate.

Efforts have been made to couple this regeneration procedure with Agrobacterium- mediated transformation for rapid introduction of agronomical value-added traits like disease - resistance, herbicidal - resistance and drought - resistance directly into high-fiber-yielding cotton germplasm.

The effect of inoculations of AMF pure culture i.e. R1-R2 on plant height & root length of RAH-9750 was studied. Data of Table-2 clearly indicates that the plant which was provided with R1-R2 pure culture showed the best growth when comparing with other inoculations of AMF. Its mycorrhizal colonization is 87.5% shoot length is 38.4, root length is 17.7, fresh shoot weight is 4.49 g, fresh root weight is 2.79g, dry shoot weight is 2.45 g & dry root weight is 1.59 g was recorded.

The effect of combined inoculation of AMF pure culture i.e. R1-R2 & bioinoculants on plant height & root length of RAH-9750 cotton was studied. The data of Table-3clearly indicates 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. Its mycorrhizal colonization was found to be superior to Single, Dual & Triple combinations i.e. 88. Plant shoot length is maximum, i.e. 19.9, root length is 14.5, shoot fresh weight is 1.49 g , root fresh weight is 0.97 g, shoot dry weight is 0.98g, root dry weight is 0.61g, was recorded.

Tissue culture regenerated RAH-9750 cotton seedlings along with the AMF and other bioinoculant combinations have shown increased capacity to tolerate the harsh conditions of environment. They not only tolerate well to the adverse effects of environment but also give the best yield of cotton. The Multi combination of bioinoculants along with AMF pure culture resulted in the significant increase in shoot & root length of plant. So Multi combination was proved to be superior because this combination was tested with different soil types of Mahabubnagar District and it has showed the similar results in all soil types.

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