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Effect of Individual PGPRM on Percent Germination and Plant Height of Cashew Seedlings under Poly House Condition

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ABSTRACT

Azatobacter chroococcum, Bacillus megaterium, Pseudomonas florescens, Trichoderma viride and Glomus fasciculatum were found to be efficient PGPR microorganisms. Hence, they were subject ed to compatibility test by dual culture method. All the four PGPR microorganisms (A. chroococcum, B. megaterium, P. flourescens and T. viride) were found to be compatible under in vitro condition both on solid and in liquid media. Population density of inoculated PGPR microorganisms in the cashew rhizosphere at different intervals was found to be maximum in the treatments receiving that organisms alone or with other PGPR microorganism. Population density of inoculated organisms increased gradually up to grafting and there after slight decline was noticed.

Key words: *Azatobacter chroococcum, Bacillus megaterium, Pseudomonas florescens, Trichoderma viride*

INTRODUCTION

Cashew (*Anacardium occidentale* L.) a tropical plant of commercial importance, belongs to the family Anacardiaceae. It is one of the export oriented cash crops of our country. It is believed to be the native of tropical America, from where it was introduced to the Malabar Coast of India by early Portuguese settlers more than 500 years ago. It is a perennial, low spreading tree and can reach to the height of about 15 meters with number of primary and secondary branches. Earlier, it was grown mainly to check the soil erosion, but gradually it has gained commercial importance as a plantation crop and has assumed a prominent position in Indian economy, as an export oriented crop as it earns lot of foreign exchange.

Review of Literature

Application of biofertilizers is known to improve the soil fertility and crop productivity in several crops through atmospheric nitrogen fixation, solubilization of inorganic and organic phosphorus and other nutrients and synthesis of growth regulators. They also play an important role in improving germination, root proliferation and suppress plant diseases[6]. The beneficial effect of *Azotobacter* treatment has been reported in establishment of healthy and sturdy seedlings[5].

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Taking into consideration of the above all factors, it was thought appropriate to initiate an investigation on the use of *Azotobacter chroococcum, Bacillus megaterium, Trichoderma viride*, *Pseudomonas fluorescens* and the vesicular arbuscular mycorrhizal fungus *Glomus fasciculatum*, to study their role in nut germination along with other growth characters and also their influence on success rate of grafts which is a major problem in cashew, without chemical fertilizers in raising quality cashew root stocks.

Phosphate solubilizing microorganisms have been reported to solubilize inorganic forms of P by excreting organic acids that directly dissolve phosphate materials and chelate cationic forms of P ion[3,1]. Strobel and Nachmias[4], reported that application of *Agrobacterium* to bare root stocks of almond trees resulted in a striking increase in leaf number, stem diameter and shoot elongation during the first growing season no pathological reaction was seen with any of the plants used in their study. Similar positive growth effects were also recorded on initial growth of bare root stock of olive treated with *Agrobacterium.*

MATERIAL AND METHODS

The present investigation on the growth of cashew seedlings and success rate of grafts as influenced by different plant growth promoting rhizomicroorganisms was carried out during the year 2007- 08 in collaboration with All India Coordinated Research Project on cashew, nursery section at Agricultural Research Station (ARS), Chintamani, Kolar (Dist.), Karnataka. The details of the location of experimental site and methods followed are described in this chapter.

Location and climate

The Agricultural Research Station (ARS), Chintamani is situated on Kolar road about 7 km away from Chintamani town. It is located at 857 meter above the mean sea level with latitude of 13^0 24^1 N and longitude of 70^0 04^1 E.

The ARS is located in the eastern dry zone of Karnataka which generally receives an average annual rainfall of around 690 mm mainly distributed from May to November. During 2007 – 08 i.e. during the experimental period 197.5 mm of rainfall was received. The maximum daily temperature of 34.1^0C during April and the minimum of 25.3^0C during December were recorded. The highest evaporation of 9.8 mm was in March and the minimum of 3.1 mm was in December. The highest relative humidity of 81.8 percent was recorded during September and the lowest of 24.4 during March.

Plant Growth Promoting Rhizomicroorganisms (PGPRM)

PGPR microorganisms used in the study were *Azotobacter chroococcum* (free living nitrogen fixer),*Bacillus megaterium* ('P' solubilizing bacterium), *Trichoderma viride* and *Pseudomonas fluorescens* (PGPR microorganisms) and *Glomus fasciculatum* ('P'mobilizer).

Preparation of inocula:

Standard cultures of *Bacillus megaterium, Azotobacter chroococcum, Pseudomonas fluorescens* and *Trichoderma viride* were grown as liquid cultures in Nutrient broth, Waksman No. 77 broth, King's B broth and Potato dextrose broth respectively, at 27± 1^0c on a rotary shaker at 150 rpm for 5 days to get the maximum population. When cell density was 10^9/ml of the broth culture, it was mixed with the carrier materials such as lignite and talc separately. The carrier based inocula were used for further studies

Polyhouse evaluation

The experiment on the evaluation of the microbial inoculants was conducted under poly house condition during Kharif season (Plate 4a and 4b). The experimental details are as follows.

1) Date of sowing: 11/ 6/ 2008.
2) Variety: Root stock - Ullal – 1 and Scion – Chintamani -1
3) Design: CRD
4) Replications: 12
5) No of bags per treatment unit: 12
6) Treatment details:

T_1: control
T_2: *Azotobacter chroococcum*
T_3: *Bacillus megaterium*

T_4: *Glomus fasciculatum*
T_5: *Pseudomonas fluorescens*
T_6: *Trichoderma viride*
T_7: *Azotobacterchroococcum* + *Bacillus megaterium*
T_8: *Azotobacter chroococcum* + *Glomus fasciculatum*
T_9: *Azotobacter chroococcum* + *Pseudomonas fluorescens*
T_{10}: *Azotobacterchroococcum* + *Trichoderma viride*
T_{11}: *Bacillus megaterium* + *Glomus fasciculatum*
T_{12}: *Bacillus megaterium* + *Pseudomonas fluorescens*
T_{13}: *Bacillus megaterium* + *Trichoderma viride*
T_{14}: *Pseudomonas fluorescens* + *Trichoderma viride*
T_{15}: *Glomus fasciculatum*+ *Trichoderma viride*

7) Date of grafting: 24/ 8/ 2008.

Observations recorded

Growth parameters

Germination percentage

Germination percentage was recorded on the 30th day after sowing.

Seedling height (cm)

The height of seedlings (cm) was measured from the soil surface in poly bags to the growing point end of the leaves. This was measured at 45th, 75th day after sowing and 45th day after grafting.

EXPERIMENTAL RESULTS

The results of the experiment conducted on cashew to study the effect of inoculation of plant growth promoting rhizomicroorganisms (PGPRM) on cashew rootstock growth and success of grafting, are presented in this chapter.

Screening of plant growth promoting rhizomicroorganisms (PGPRM)

The data on germination percentage and plant height of cashew seedlings as influenced by individual PGPRM is presented in table 1.

Ten PGPR microorganisms were used to screen their efficiency on cashew nut germination and seedling height. Maximum germination percentage was obtained in the treatment which received *Pseudomonas fluorescens* (91%), followed by the treatments inoculated with *Bacillus megaterium* (88.56%), *Trichoderma viride* (87.56%), *Glomus fasciculatum* (84%) and *Azotobacter chroococcum* (85.33%). Seedling height recorded at 2 MAS was also, found to be maximum (29.51 cm) in *Trichoderma viride* inoculated treatment, followed by the treatments which received *Pseudomonas fluorescens, Glomus fasciculatum* (27 cm), *Bacillus megaterium* (26.83 cm) and *Azotobacter chroococcum* (26.33 cm).

Compatibility of PGPRM

Compatibility of PGPRM on solid and liquid medium by dual culture test is presented in Table 2**.**

Growth on solid medium (mm)

Maximum growth of *Azotobacter chroococcum* (12.66), *Bacillus megaterium* (7.33), *Pseudomonas fluorescens* (8.00) and *Trichoderma viride* (75.66) was obtained on solid medium when these organisms were grown individually as control. When *Azotobacter chroococcum* was grown with *Pseudomonas fluorescens* the growth of *Azotobacter chroococcum* (11.66) was slightly inhibited. When *Bacillus megaterium* was grown with *Trichoderma viride* the growth of *Bacillus megaterium* (6.66) was slightly inhibited. Similarly the growth of *Pseudomonas fluorescens* with *Azotobacter chroococcum* and growth of *Trichoderma viride* with *Pseudomonas fluorescens* was slightly inhibited. However the inhibition of PGPR microorganisms was not statistically significant.

Growth in liquid medium (CFU x 10^8/ml)

Maximum population density of *Bacillus megaterium* (29.33), *Pseudomonas fluorescens* (31.00) and *Trichoderma viride* (24.00) was obtained in liquid medium when these organisms were grown individually as control. The population of *Azotobacter chroococcum* was found highest when it was dual inoculated with *Pseudomonas fluorescens* and slightly inhibited with *Bacillus megaterium*. The population density of *Bacillus megaterium* was

slightly inhibited with *Trichoderma viride*, *Azotobacter chroococcum* and with *Pseudomonas fluorescens*. The population of *Pseudomonas fluorescens* was also slightly inhibited, when it was coinoculated with *Azotobacter chroococcum* or *Trichoderma viride*. The population density of *Trichoderma viride* was also slightly inhibited when it was grown with *Azotobacter chroococcum* or *Pseudomonas fluorescens* or *Bacillus megaterium* in liquid medium.

Table 1: Effect of individual PGPRM on percent germination and plant height of cashew seedlings under poly house condition

Treatments	Germination (%)	Plant height (cm) 2 MAS
Azotobacter chroococcum	85.33 [d]	26.33 [b]
Beijerinckia spp,	74.23 [h]	20.33 [d]
Bacillus megaterium	88.56 [b]	26.83 [b]
Aspergillus awamori	79.00 [f]	20.51 [d]
Trichoderma viride	87.56 [c]	29.51 [a]
Trichoderma harzianum	75.68 [g]	20.54 [d]
Pseudomonas fluorescens	91.00 [a]	27.00 [b]
Pseudomonas striata	74.32 [h]	22.98 [c]
Glomus fasciculatum	84.00 [e]	27.00 [b]
Glomus mossae	78.56 [f]	21.00 [cd]
CD (P = 0.05)	**0.25**	**0.95**

Note: PGPRM = Plant Growth Promoting Rhizo microorganisms.

Table 2: Growth of plant growth promoting rhizomicroorganisms (PGPRM) in dual culture with other PGPRM on solid and in liquid medium

	Growth on solid medium (mm)				Growth in liquid medium (CFU x 10^8/ml)			
Treatments	***A.c***	***B.m***	***P.f***	***T.v***	***A.c***	***B.m***	***P.f***	***T.v***
Control	12.66	7.33	8.00	75.66	24.00	29.33	31.00	24.00
T.v	12.33	6.66	8.00	-	24.00	26.66	29.66	-
P.f	11.66	7.00	-	74.33	24.00	28.00	-	22.66
B.m	12.00	-	7.66	75.00	23.33	-	30.00	23.00
A.c	-	7.33	7.33	74.66	-	27.66	29.33	22.00

Note:
Ac - Azotobacter chroococcum, Bm - Bacillus megaterium,
Gf - Glomus fasciculatum, Pf - Pseudomonas fluorescens,
Tv - Trichoderma viride
PGPRM = Plant Growth Promoting Rhizo microorganisms.

DISCUSSION

Cashew is commercially propagated by soft wood grafting using root stock raised with seed of a known cashew variety. At present, due to various reasons the cashew grafting success rate is very less. The success of soft wood grafting is mainly dependent on vigourness of stock in attaining the desired height and girth at grafting position. It has been demonstrated by many scientists that the enhanced growth could be achieved by the

inoculation of biofertilizers to the root stock raising medium[6,2].

Therefore, an attempt was made to assess the influence of PGPR microorganisms on growth of cashew root stocks and success rate of grafting.

***In vivo* and *in vitro* screening of plant growth promoting rhizomicroorganisms (PGPRM)**

Standard cultures of ten PGPR microorganisms collected from the Dept. of Agri microbiology were inoculated individually to the polybags containing potting mixture and on which cashew seedlings were grown for a period of 2 months. Based on the germination and growth characters of cashew seedling as influenced by the efficient PGPR microorganisms, *Azotobacter chroococcum*, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Trichoderma viride* and *Glomus fasciculatum* were selected for further studies.

Compatibility of PGPRM

Compatibility studies under *In vitro* by dual culture test showed that the growth of *Azotobacter chroococcum*, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Trichoderma viride* individually with other PGPR microorganisms on solid media was not affected significantly. However, a slight inhibition of *Azotobacter chroococcum* was observed when it was grown on solid media with *Pseudomonas fluorescens*. Similarly, the growth of *Bacillus megaterium* with *Trichoderma viride*, growth of *Pseudomonas fluorescens* with *Azotobacter chroococcum* and growth of *Trichoderma viride* with *Pseudomonas fluorescens* was slightly inhibited.

Growth of *Azotobacter chroococcum*, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Trichoderma viride* individually with other PGPR microorganism in liquid medium was also not affected significantly. However, a slight inhibition in the growth of *Azotobacter chroococcum* with *Bacillus megaterium*, the growth of *Bacillus megaterium* with *Trichoderma viride* or with *Azotobacter chroococcum*, the growth of *Pseudomonas fluorescens* with *Azotobacter chroococcum* or with *Trichoderma viride* and the growth of *Trichoderma viride* with *Azotobacter chroococcum* or with *Pseudomonas fluorescens* was observed.

A slight inhibition of individual PGPR microorganism when they were with another PGPR microorganisms on solid medium as well as in liquid broth might be due to competition and antibiosis. However, the literature pertaining to compatibility studies between Plant Growth Promoting Rhizomicroorganisms are limited.

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