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Study of Effect of Plant Growth Promoting Rhizo Microorganisms on Success Rate of Cashew Grafts

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A B S T R A C T

Azatobacter chroococcum, *Bacillus megaterium*, *Pseudomonas fluorescens*, *Trichoderma viride* and *Glomus fasciculatum* were found to be efficient PGPR microorganisms. Hence, they were subjected to compatibility test by dual culture method. All the four PGPR microorganisms (*A. chroococcum, B. megaterium, P. fluorescens 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 organism alone or with other PGPR microorganism. Population density of inoculated organisms increased gradually up to grafting and there after slight decline was noticed.

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.

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 (Verma, 1993; SubbaRao, 1995). The beneficial effect of *Azotobacter* treatment has been reported in establishment of healthy and sturdy seedlings

(Sundara Rao *et al.*, 1963). 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 (Sperber, 1958 and Katznelson and Bose, 1959). Strobel and Nachmias (1985) 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.*

Materials 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.

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 Rhizomicro-organisms (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.

Date of sowing: 11/ 6/ 2008.

Variety: Root stock - Ullal – 1 and Scion – Chintamani -1

Design: CRD

Replications: 12

No of bags per treatment unit: 12

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: *Azotobacter chroococcum* + *Bacillus megaterium*

T_8: *Azotobacter chroococcum* + *Glomus fasciculatum*

T_9: *Azotobacter chroococcum* + *Pseudomonas fluorescens*

T_{10}: *Azotobacter chroococcum* + *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*

Date of grafting: 24/ 8/ 2008.

Observations recorded

Growth parameters

Germination percentage

Germination percentage was recorded on the 30^{th} 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 45^{th}, 75^{th} day after sowing and 45^{th} day after grafting.

Number of leaves

The number of fully opened leaves produced in a seedling was counted at 45^{th}, 75^{th} day after sowing and 45^{th} day after grafting.

Stem girth (cm)

The diameter of the stem below the first pair of leaves was measured with the help of Vernier – calipers at 45th, 75th day after sowing and 45th day after grafting. Finally the girth of the stem was calculated using the formula $C = 2\pi r$.

Recovery of graftable root stocks

Among the total number of germinated seeds in each treatment, the seedlings which have attained more than 25 cm in height and 1.6 cm in girth were counted and the rest were rejected and the recovery of good graftable seedlings was expressed in percentage.

Sprout initiation in grafts

The prepared graft was considered as sprouted, when rudimentary apical bud swollen and leaves were clearly visible.

Success of grafting (%)

The number of successful grafts was counted after the complete sprouting and commencement of new growth on scion at 45th day after grafting. Percentage of success was calculated based on the number of grafts prepared.

Root length (cm)

The root length was measured after depoting and washing the roots thoroughly from the end point of stem to the tip of the root.

Inoculation of PGPRM

Five grams of lignite based inoculum of plant growth promoting rhizomicroorganism was applied in the poly bag containing soil (2 – 3 cm below the soil surface), according to the treatment details.

Rising of root stock

The soaked seeds were sown in poly bags containing 2 kg of potting mixture with punch holes for drainage. The seeds were sown such that the stalk end facing upwards in polythene bags at about 2 – 3 cm below the soil surface. Germination of seeds started 15 days after sowing and continued till 30th day. Regular watering and weeding was attended.

Selection and preparation of seedling (root stock)

Healthy and vigorously grown seedlings having single main stem were selected for grafting. Seventy five days old seedling grown in the centre of polythene bag were used.

Preparation of scion

Three to four months old shoots of pencil thickness having terminal dormant buds were selected from the healthy trees and were precured by cutting the leaves keeping only the petiole attached. The scions were immediately wrapped in a wet cloth to avoid desiccation. Precuring was done seven days prior to the actual day of grafting.

Grafting method

Cashew seedlings (root stocks) grown for 75 days were selected for grafting. Grafting was done by soft wood method. The stock plant with new terminal growth was decapitated at a height varying from 10 - 18 cm from soil surface of poly bags and a vertical split of about 3 to 4 cm was made using a sharp knife and the care was also taken to retain 2 – 4 leaves well below the grafting point. A scion of 8 – 10 cm length was taken and the basal end was cut into a wedge form (3 – 4 cm long) and the stock in such a way that at least one side of the scion cambium makes

satisfactory contact with the cambium of root stock. The inserted portion was wrapped firmly with 1.5 cm wide and 30 cm long, 100 gauge white transparent polyethylene strip, so as to keep the stock and scion parts in firm contact and to prevent water entering into the grafted portion. A Pepsi poly tube was inserted over the scion to create higher humidity around the grafted portion (Plate 5a and 5b).

Enumeration of inoculated microbial population in the rhizosphere of cashew in nursery (both in root stocks and grafts)

For enumeration of PGPR microbial population, soil sample was collected carefully from the rhizosphere of cashew seedlings at different intervals and population density was determined by soil dilution plate method. Ten grams of the pooled soil sample of each treatment was collected from different replications. Each ten grams of soil was mixed in 90 ml sterilized water blank and mixed thoroughly to give 10^{-1} dilution. Subsequent dilutions up to 10^{-6} were made by transferring 1ml to 9 ml water blanks. For enumeration of *Azotobacter chroococcum*, *Bacillus megaterium*, *Pseudomonas fluorescens* and *Trichoderma viride*, different dilutions were selected. One ml of suspension from each dilution was transferred aseptically into sterile petriplates. Fifteen ml. of appropriate medium was poured into plates and gently rotated in clockwise and anticlock wise direction to let the suspension distribute uniformly in the medium. Three replications were maintained for each dilution. The plates were incubated at 27±1°C for 5 days and colonies were counted on a colony counter and population was estimated and expressed as CFU per gram dry weight of soil (Johnson and Curl, 1972).

The population of above said organisms was estimated at 30, 60 days after sowing and 30, 60 day after grafting (Note: During the estimation of *Bacillus* spp., the soil suspension was subjected to simmering for 10 minutes).

Procedure for enumeration of VAM spores

Fifty grams of rhizosphere soil was suspended in 500 ml of water and shaken thoroughly.

After the sedimentation of course sand, the suspension was decanted over a series of test sieves (a sieve set with 1mm pore size 350µm, 250 µm, 180 µm and 45µm arrange in the descending order). Then the content of 108µm and 45µm sieve were carefully washed into a conical flask it was shaken and allowed to settle for 30 sec and spores were trapped on a nylon mesh of 40 µm placed on a sieve. The nylon mesh was then transferred on to a glass petri plate the number of spores was then counted under stereomicroscope and expressed as spore number/ 50g dry soil.

After care of the grafts

The grafted plants were kept in poly house and watered regularly. The grafts sprouted in 11 - 18 days' time. The polythene cap was removed at this stage.

Results and Discussion

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 (Table 1 and 2).

Recovery of graftable seedlings

The data on recovery of graftable seedlings 75 DAS, number of days taken for sprout initiation in scion and success of grafts are presented in Table 2.

Table.1 Initial microbial population in the soil sample used for the study

Sl. No	Initial microbial population of soil	
1	Bacteria	23.00 x10^5
2	Fungi	10.50 x10^4
3	Actinomycetes	8.50 x10^3
4	*Azotobacter spp.*	10.50 x10^3
5	*Bacillus spp.*	13.50 x10^5
6	*Trichoderma spp.*	9.00 x10^2
7	*Pseudomonas spp.*	9.50 x10^3
8	VAM	18.00 spore/50g dry soil

Table.2 Effect of inoculation of PGPRM on graft characters of cashew seedlings under poly house condition

Treatments	Recovery of Graftable Seedlings (%) 75 DAS	Days taken for sprout Initiation after grafting	Success of Grafts (%) 45 DAG
T1 – Control	80.33^{g}	18.17^{a}	75.67^{g}
T2 - *Azotobacter chroococcum*	83.32^{ef}	17.07^{b}	87.06^{b}
T3 - *Bacillus megaterium*	82.23^{f}	17.01^{b}	82.26^{e}
T4- *Glomus fasciculatum*	85.63^{cd}	16.17^{bc}	83.20^{d}
T5 - *Pseudomonas fluorescens*	84.23^{de}	15.83^{c}	83.64^{d}
T6 - *Trichoderma viride*	86.11^{cd}	14.67^{d}	85.28^{c}
T7-*Azotobacter chroococcum* + *Bacillus megaterium*	85.63^{cd}	13.42^{ef}	87.12^{b}
T8-*Azotobacter chroococcum* + *Glomus fasciculatum*	85.32^{cd}	13.58^{e}	86.71^{b}
T9-*Azotobacter chroococcum* + *Pseudomonas fluorescens*	90.21^{a}	12.67^{efgh}	81.52^{f}
T10-*Azotobacter chroococcum* + *Trichoderma viride*	90.01^{a}	12.25^{ghi}	90.36^{a}
T11 - *Bacillus megaterium* + *Glomus fasciculatum*	89.22^{a}	11.67^{hi}	90.34^{a}
T12-*Bacillus megaterium* + *Pseudomonas fluorescens*	88.26^{ab}	11.75^{hi}	87.23^{b}
T13 - *Bacillus megaterium* + *Trichoderma viride*	86.78^{bc}	11.42^{i}	85.29^{c}
T14-*Pseudomonas fluorescens*+ *Trichoderma viride*	85.25^{cd}	13.25^{efg}	85.26^{c}
T15 - *Glomus fasciculatum*+ *Trichoderma viride*	86.72^{bc}	12.42^{fghi}	85.49^{c}
CD (P = 0.05)	**0.66**	**0.73**	**0.20**

Note: PGPRM = Plant Growth Promoting Rhizo microorganisms. DAS = days after sowing. DAG = Days After Grafting.

Maximum percentage recovery of graftable seedlings was recorded in the treatment receiving *Azotobacter chroococcum* with *Psuedomonas fluorescens* (90.21) followed by the treatment inoculated with *Azotobacter chroococcum* and *Trichoderma viride* (90.01) and the treatment receiving *Bacillus megaterium* with *Glomus fasciculatum* (89.22).

Minimum recovery of graftable seedlings was recorded in control (80.33).

Days taken for sprout initiation

The data on number of days taken for sprout initiation in scion is presented in Table 2.

Minimum number of days taken for sprout initiation in the scion after grafting was observed in the treatment inoculated with *Bacillus megaterium* with *Trichoderma viride* (11.42) followed by the treatment which received *Bacillus megaterium* with *Glomus fasciculatum* (11.67) and *Bacillus megaterium* with *Psuedomonas fluorescens* (11.75).

Maximum number of days taken for sprout initiation was recorded in control (18.17).

Success of grafts (%)

The data on success of grafts, 45 DAG is presented in Table 2.

Maximum percentage of success of grafts was obtained in the treatment inoculated with *Azotobacter chroococcum* with *Trichoderma viride* (90.36) followed by *Bacillus megaterium* with *Glomus fasciculatum* (90.34) and *Bacillus megaterium* with *Psuedomonas fluorescens* (87.23). Minimum percentage of success of grafts was recorded in the treatment control (75.67).

The maximum stem girth of cashew root stocks at 45, 75 DAS and 45 DAG was obtained in the treatment receiving *Bacillus megaterium* with *Glomus fasciculatum*. At 45 DAS the next highest stem girth was observed in the treatments receiving *Pseudomonas fluorescens* with *Trichoderma viride* and *Glomus fasciculatum* with *Trichoderma viride*. Whereas at 75 DAS as well as at 45 DAG the next highest stem girth was obtained in the treatment receiving *Bacillus megaterium* with *Pseudomonas fluorescens* followed by the treatment which received *Azotobacter chroococcum* with *Glomus fasciculatum*.

The minimum stem girth of cashew seedlings was noticed in control. Similar reports of increase in growth parameters like plant height, stem diameter and shoot biomass by inoculation of *Gigaspora margarita* were reported by Balakrishna and Bagyaraj, (1994); Aneesha and Sathimoorthy (1997) in papaya, and Vinayaka and Bagyaraj (1990) in trifoliate orange and Lakshmipathy *et al.*, (2000) in cashew.

Maximum root length of cashew seedlings at 75 DAS was obtained in the treatments receiving *Azotobacter chroococcum* with *Bacillus megaterium* followed by *Bacillus megaterium* with *Glomus fasciculatum* and *Glomus fasciculatum* alone, lowest root length was recorded in control.

Shanmugam (1981) also observed similar effects of increased growth and root length in six months old *Citrus aurantifolia* seedlings when they were inoculated with *Glomus mosseae* or *Glomus etunicatum*. Dileep Kumar and Dube (1992) obtained similar results by VAM inoculation to tomato and egg plants.

Increase in height and better root development of soybean plants by inoculation of *Trichoderma harzianum* was also reported by Daiho and Upadhyay (1995).

Effect of PGPRM on shoot and root biomass of cashew seedling

Maximum shoot fresh and dry weight was observed in the treatment receiving *Pseudomonas fluorescens* with *Trichoderma viride* followed by *Glomus fasciculatum* alone.

Maximum root fresh and dry weight was obtained in the treatment receiving *Bacillus megaterium* with *Trichoderma viride* followed by the treatments which received *Pseudomonas fluorescens* with *Trichoderma viride* and *Bacillus megaterium* with *Pseudomonas fluorescens*. The minimum shoot and root fresh as well as dry weight was noticed in control. Similar results of increased fresh and dry weight of shoot and root was obtained by Lakshmipathy (2000) in cashew, Windham (1986) in tomato and tobacco, Chanway (1995) in *Tsugaheterophylla* and Meyer and Linderman (1986) in clover by inoculation with *Pseudomonas putida* and VAM.

Increase in growth parameters of cashew seedlings observed in the present study due to inoculation of PGPR microorganisms might be due to the mechanisms of production of growth regulating metabolites by the microbial agents (Mroz *et al.,* 1994; Glick, 1995), greater availability of nutrients by application of nitrogen fixers, P solubilizers, P mobilizers *etc*. (Rudresh *et al.,* 2005; Somani *et al.,* 1990; Pawar and Pawar 1998), synergistic interaction among the PGPR microorganisms which might have additive effect on growth and biomass of cashew seedlings (Raj *et al.,* 1981; Mayo *et al.,* 1986; Mosse, 1957), by increasing the deleterious effect on pathogens in presence of beneficial microorganisms and biocontrol agents. (Kumar *et al.,* 1998; Rakeshkumar *et al.,* 2004), by proliferation of beneficial PGPR microorganisms in the cashew nut rhizosphere (Oblisami *et al.,* 1985; Patil *et al.,* 1979; Lakshmipathy *et al.,* 2000 and Radhika *et al.,* 2005) and by biological control of plant pathogens (Krishna and Bagyaraj, 1993; Rakesh Kumar *et al.,* 2004 and Sunandadeene *et al.,* 2004).

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