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Effect of Biofertilizers on Growth and Establishment of Cashew Grafts under Nursery Condition

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A B S T R A C T

Evaluation of biofertilizers on growth and establishment of cashew grafts var. Chintamani-1 was studied under greenhouse condition. The grafts were planted in potting mixture, soil with farm yard manure and sand in the ratio of 2:1:1 (Soil: FYM; Sand) taken in polythene bags of size 12 cm x 18 cm, applied with biofertilizers such as *Azospirillum* spp., phosphorus solubilizing bacteria (PSB) and *Pseudomonas flourescens*, (single / dual / combinations) @ 10 g per graft plus vesicular arbuscular mycorrhiza (VAM), a soil based biofertilizer @10 g. The growth parameters *viz*. length, stem girth and number of leaves were found to be enhanced due to biofertilizer application. Also the biofertilizer inoculation had increased the percent success of grafts, total biomass and nitrogen and phosphorous uptake at the end of 150 days. The inoculation of consortia of three biofertilizers; *Azospirillum*, *Pseudomonas fluorescens* and VAM was efficient in getting maximum plant height (50.67 cm), stem girth (2.83 cm) and number of leaves (6.93 no.) and also found to be better than dual or single inoculation of these biofertilizers for growth and establishment as well as nutrient uptake in cashew grafts under nursery conditions.

Introduction

Cashew is a fast growing evergreen tropical tree growing to 12 m height and it is one of the largest spreading cash crops in Kolar District. The expansion of area under cultivation of this crop is increasing annually. The cultivation is increased by 53 per cent from 1993-94 to 2009-10 along with the yield (Kulkarni, *et al.*, 2012). In Kolar cashew is cultivated in an area of 2,210 ha (Maruthi Prasad *et al.*, 2015). It is highly suitable for the climatic conditions of Kolar district since it comes up very well in deep well drained sandy loams and prefers optimum monthly mean temperature of 25 degree centigrade and an annual rain fall of 1000 mm or more which can tolerate drought. The barren and unfertile soils can be put in to for cashew cultivation in large scale, besides restoration of mined soils (Singh *et al.*, 2003).

One of the limitations in cashew cultivation is the establishment of seedlings and grafted plants. The production of good quality grafted plants and their establishment in nursery is very much required to ensure survivability and further establishment in main fields. These limitations can be narrowed by use of biofertilizers such as *Azospirillum*, phosphorus solubilizing bacteria, *Pseudomonas flourescens* and Mycorrhizae. Biofertilizers such as *Azospirillum*, phosphorus solubilizing bacteria and Mycorrhizae are capable of improving the mineral nutrition of plants and enhance the soil fertility in low fertility soils. Microorganisms such as *Azospirillum* fix atmospheric nitrogen to be taken up by plants; *Pseudomonas fluorescens* control the soil borne pathogens, Mycorrhizae form symbiotic association with plant and supply unavailable phosphorus to plants respectively (Gurumurthy *et al.,* 2014). Phosphorus solubilizing bacteria are capable of solubilizing unavailable form of phosphorus into available form and make it available to plants (Veena *et al.*, 2009; Shankarappa *et al.*, 2012). Therefore, the effect of biofertilizers on the growth and establishment and nutrient uptake of grafted cashew plants was studied in a pot culture experiment.

Materials and Methods

The experiment on influence of biofertilizers on growth, establishment and nutrient uptake of grafted cashew plants, root stock: local variety and scion: chintamani-1 was conducted in poly house at Horticulture Research station, Hogalagere, Kolar.

The fifty days old rootstocks of cashew plants (local variety), of soft wood tissue were transferred to new polythene bags of size 12 cm x 18 cm that contained potting mixture, soil with farm yard manure and sand in the ratio of 2:1:1 (Soil: FYM; Sand). The potting mixture had pH - 6.95, Organic Carbon - 0.86 %, Available N- 228 kg ha^{-1}, Available P- 35 kg ha^{-1} and available Potassium 310 kg ha^{-1}. Applied with biofertilizers such as *Azospirillum lipoferum*, Phosphorus solubilizing bacteria (PSB- *Pseudomonas striata*), *Pseudomonas flourescence*, @ 10 g per graft to form single, dual, triple combinations (10g only) plus vesicular arbuscular mycorrhiza (VAM), soil based biofertilizer @10 g, where ever required. A total of fifteen treatments were formed. The moisture content of soil in poly bags was maintained at field capacity. On the next week the wedge grafting was carried out by using only the soft-wood tissues of the stock and scion (Nagabhushanam, 1985). The seedlings were decapitated at the soft-wood apical region to retain two pairs of leaves and wedge grafting was carried out with a 4-5 cm cleft on the rootstock and a small portion of the inner surface was removed to facilitate a perfect union of the wedge shaped scion. The scion (5 cm length) of variety, chintamani-1 was prepared by shaving a portion of the bark and tissue on either side, inserted in to wedge cut of stock and the union was secured by tying with a 30 cm polythene strip. The top of the scion was covered with a polythene cap to protect the apical portion of the scion from desiccation. The grafts were watered regularly once in a day to maintain moisture content of soil at field capacity. Polythene wrapping at the union was removed after 75 days after grafting to prevent girdling.

A total of fifteen treatments were tested in the experiment. The details of the treatments are; 1. Uninoculated control, 2. *Azospirillum lipoferum*, 3. Phosphorus solubilizing bacteria, 4. *Pseudomonas fluorescens* 5. Vesicular arbuscular mycorrhiza (VAM), 6. *Azospirillum* spp + phosphorus solubilizing bacteria, 7. *Azospirillum* spp + *Pseudomonas flourescence,* 8. *Azospirillum* spp + Vesicular Arbuscular Mycorrhiza, 9. Phosphorus solubilizing bacteria + *Pseudomonas fluorescens,* 10. Phosphorus solubilizing bacteria + VAM, 11. *Pseudomonas flourescence* + VAM, 12. *Azospirillum* spp +

phosphorus solubilizing bacteria + *Pseudomonas flourescence,* 13. *Azospirillum* spp + phosphorus solubilizing bacteria + VAM, 14. *Azospirillum* spp + *Pseudomonas fluorescens*+ VAM and 15. *Azospirillum* spp + phosphorus solubilizing bacteria + *Pseudomonas flourescence* + VAM. All the biofertilizers were procured from the Department of Agricultural Microbiology, University of Horticultural Sciences, Bangalore. The observations on parameters such as length and girth of the grafted plants and number of leaves at an interval of 30 days for 150 days was taken and total biomass, nitrogen and phosphorous content at the end of 150 days of grafting was estimated. The nitrogen and phosphorous of the oven dried plant samples were estimated by microkjeldahl and vanado-molybdate phosphoric acid methods respectively (Jackson, 1973) and the N and P uptake were worked out. Ten replications were maintained to test the per cent establishment and three replications for other parameters and were analyzed statistically using complete randomized design.

Results and Discussion

The evaluation of biofertilizers on growth and establishment of cashew grafts var. Chintamani-1 under greenhouse condition showed that the biofertilizers, nitrogen fixer, phosphate solubilizer, biocontrol agent and phosphorous mobilize in the form of *Azospirillum* spp., *Pseudomonas striata*, *Pseudomonas fluorescens* and VAM respectively had increased the growth and nutrient uptake. The growth, establishment and nutrient uptake was significantly superior over un-inoculated control.

All the biofertilizers showed positive influence on the height of the cashew grafts (Table 1). The inoculation of biofertilizers, consortia of *Azospirillum*, *Pseudomonas flourescence* and VAM showed maximum height (50.67 cm) at the end of 150 days after grafting and it was found to be on par with other treatments that received other combinations of three or four beneficial microorganisms. The increase in height was not prominent in any of the treatments in the initial stages, however, better growth was observed after 90 days after grafting. The un-inoculated control had a height of 38.33 cm i.e. only 10 cm growth from 28.33 cm in 150 days. The consortium of biofertilizer containing three or four beneficial microorganisms gave enhanced growth in terms of height (Akshitha *et al.*, 2014).

Similarly, the girth of grafted cashew showed positive response to biofertlilizer inoculation as presented in table 2. The consortia containing *Azospirillum*, *Pseudomonas fluorescens* and VAM produced maximum girth of 2.83 cm as against the control 2.03 cm observed at the end of 150 days after grafting. The biofertilizers consortia of three or four organisms were significantly superior over consortia of two organisms or biofertilizer of single organisms (Veena *et al.*, 2009). Although, a steady increase in girth was observed in all the treatments, it was more at the later part of the growth, after 90 days of grafting and the growth was superior in biofertilizer treated grafts.

The effect of biofertilizer inoculation on number of leaves produced in grafted cashew was significantly superior in the consortia of three or four organisms (Table 3). The treatment, *Azospirillum*, PSB, *Pseudomonas fluorescens* and VAM (7.66) showed maximum number of leaves which was on par with the treatment *Azospirillum*, *Pseudomonas flourescence* and VAM inoculation (6.93). The control treatment produced only 3.66 numbers of leaves at the end of 150 days after grafting. Here again the number of leaved had increased after 90 days of grafting and all the biofertilizers could increase the number.

Table.1 Plant height of cashew grafts as influenced by biofertilizers

Treatments	Plant height (cm) at different days					
	0	30	60	90	120	150
Uninoculated control	28.33	31.00	31.67	32.33	31.33	38.33
Azospirillum spp. (AZO)	32.33	32.33	34.67	37.00	40.00	42.33
Phosphorus solubilizing bacteria (PSB)	30.67	31.00	32.67	32.33	39.00	42.00
Pseudomonas flourescens (PF)	29.67	31.00	34.67	35.33	39.00	42.67
Vesicular Arbuscular Mycorrhiza (VAM)	26.33	31.67	33.00	36.67	43.33	44.33
AZO + PSB	31.33	33.67	38.67	37.67	43.00	46.33
AZO + *PF*	29.33	34.33	37.67	40.00	42.00	45.00
AZO + VAM	28.67	33.67	37.00	39.00	42.00	43.33
PSB + *PF*	27.33	32.00	33.00	35.00	40.67	42.67
PSB + VAM	31.00	32.00	35.33	40.33	45.67	45.33
PF + VAM	34.00	36.33	37.00	39.67	39.00	47.67
AZO + PSB + *PF*	29.00	34.00	36.00	38.67	42.67	47.67
AZO + PSB + VAM	33.00	36.33	39.33	42.00	44.67	46.00
AZO + *PF*+ VAM	32.00	34.00	39.00	40.00	46.00	50.67
AZO + PSB + *PF* + VAM	29.33	33.67	37.33	43.67	51.00	48.00
S Em ±	0.854	0.750	0.964	1.092	0.939	0.854
CD @ 5%	2.400	2.095	2.681	3.036	2.573	2.350

Table.2 Stem girth of cashew grafts as influenced by biofertilizers

Treatments	Plant girth (cm) at different days					
	0	30	60	90	120	150
Uninoculated control	1.13	1.30	1.33	1.47	1.68	2.03
Azospirillum spp. (AZO)	1.37	1.53	1.63	1.80	1.94	2.40
Phosphorus solubilizing bacteria (PSB)	1.37	1.47	1.53	1.57	2.27	2.00
Pseudomonas flourescens (PF)	1.27	1.33	1.53	1.60	1.73	2.33
Vesicular Arbuscular Mycorrhiza (VAM)	1.43	1.57	1.67	1.93	2.03	2.20
AZO + PSB	1.33	1.47	1.67	1.60	1.70	1.97
AZO + *PF*	1.20	1.47	1.63	1.83	1.90	2.63
AZO + VAM	1.33	1.50	1.67	1.80	1.73	2.67
PSB + *PF*	1.37	1.47	1.50	1.63	2.00	2.40
PSB + VAM	1.43	1.50	1.67	1.83	1.90	2.23
PF + VAM	1.20	1.53	1.73	2.00	1.73	2.53
AZO + PSB + *PF*	1.20	1.40	1.43	1.83	1.93	2.23
AZO + PSB + VAM	1.43	1.70	1.90	2.10	2.27	2.63
AZO + *PF*+ VAM	1.33	1.40	1.87	1.93	2.33	2.83
AZO + PSB + *PF* + VAM	1.40	1.60	1.80	2.20	2.63	2.73
S Em ±	0.028	0.340	0.467	0.0521	0.060	0.060
CD @ 5%	0.079	0.094	0.129	0.144	0.166	0.168

Table.3 Number of leaves of cashew grafts as influenced by biofertilizers

Treatments	No of leaves at different days				
	30	60	90	120	150
Uninoculated control	1.00	2.00	3.00	3.33	3.66
Azospirillum spp. (AZO)	1.00	2.00	3.00	4.00	4.10
Phosphorus solubilizing bacteria (PSB)	1.00	2.00	3.00	4.33	4.66
Pseudomonas flourescens (PF)	1.00	2.00	3.00	4.00	4.33
Vesicular Arbuscular Mycorrhiza (VAM)	1.00	2.00	3.00	4.00	4.33
AZO + PSB	1.00	2.00	3.00	4.33	4.66
AZO + *PF*	1.00	2.00	3.00	4.00	4.10
AZO + VAM	1.00	2.67	3.67	5.33	5.87
PSB + *PF*	1.00	2.00	3.33	5.00	5.13
PSB + VAM	1.00	2.33	3.67	5.33	5.66
PF + VAM	1.00	2.33	4.00	4.33	4.97
AZO + PSB + *PF*	1.00	3.00	4.67	6.00	6.30
AZO + PSB + VAM	1.67	3.00	4.33	6.00	6.63
AZO + *PF*+ VAM	1.67	3.67	5.00	7.00	6.93
AZO + PSB + *PF* + VAM	1.67	3.33	5.33	7.67	7.66
S Em ±	0.079	0.102	0.129	0.171	0.081
CD @ 5%	0.218	0.274	0.360	0.477	0.224

Table.4 Total dry weight, N content and P uptake of cashew grafts at 150 days

Treatments	Establish ment (%)	Total dry wt. (g^{-1}plant)	N uptake (mg^{-1}plant)	P uptake (mg^{-1}Plant)
Uninoculated control	80	11.70	463.33	61.00
Azospirillum spp. (AZO)	100	12.27	521.00	63.67
Phosphorus solubilizing bacteria (PSB)	100	11.47	502.33	70.33
Pseudomonas flourescens (PF)	100	11.80	507.33	69.33
Vesicular Arbuscular Mycorrhiza (VAM)	100	12.02	549.67	65.00
AZO + PSB	100	14.40	547.67	87.33
AZO + *PF*	100	14.63	504.33	79.00
AZO + VAM	100	14.63	501.00	88.33
PSB + *PF*	100	14.53	514.00	87.33
PSB + VAM	100	14.50	521.67	88.00
PF + VAM	100	14.47	573.67	91.00
AZO + PSB + *PF*	100	15.73	577.33	90.67
AZO + PSB + VAM	100	15.63	587.33	93.00
AZO + *PF*+ VAM	100	15.90	601.33	97.00
AZO + PSB + *PF* + VAM	100	15.90	610.67	98.67
S Em ±		0.3981	12.2935	2.5327
CD @ 5%		0.7804	24.0952	4.9642

Establishment of grafted plants, biomass output and nutrient uptake was observed to be high in biofertilizers treated cashew grafts (Table 4). The success of grafted plants to establish was 100 percent in biofertilizer treated grafts as against 80 percent observed in control. All the biofertilizers irrespective of their application as single, dual, triple or four organisms in a consortium produced better establishment. The establishment of grafts, increased graft height, girth and number of leaves due to biofertilizer inoculation had reflected on biomass output (Shankarappa *et al.,* 2012; Vijendrakumar *et al.,* 2014). The total dry matter content was high in biofertilizer treated grafts. Highest biomass of 15.90 g was observed in the treatments *Azospirillum*, PSB, *Pseudomonas fluorescens* and VAM and *Azospirillum*, *Pseudomonas flourescence* and VAM inoculation (15.90).

These treatments were on par with other treatments of triple organisms inoculations. Similarly, the nitrogen uptake (610.67 mg) and phosphorous uptake (98.67 mg) per plant was found to be highest in the plants treated with consortia of biofertilizers that contained all the four organisms and it was on par with consortia that contained combinations of three organisms. The enhanced height, girth and number of leaves of cashew grafts and better nutrient uptake and biomass growth, observed due to inoculation of single or multiple biofertilizers is attributed to the production of various growths promoting hormones by the beneficial microorganisms such as IAA, Gibberellic acid, and cytokinins (Veena *et al.*, 2009; Shankarappa *et al.*, 2012) as well as the availability of fixed nitrogen by *Azospirillum*, phosphate solubilization by *Pseudomonas striata* (Akshitha *et al.,* 2014; Vijendrakumar *et al.,* 2014), phosphate mobilization by the mycorrhizal fungi (Gurumurthy *et al.,* 2014), biocontrol activity of *Pseudomonas fluorescens* (Akshitha *et al.,* 2014; Vijendrakumar *et al.*, 2014) and the synergistic growth promotion by the consortia of biofertilizers used.

In conclusion, the experiment on effect of biofertilizers establishment and nutrient uptake of cashew grafted plants showed that the inoculation of consortium of three biofertilizers; *Azospirillum*, *Pseudomonas fluorescens* and VAM was efficient in getting maximum plant height (50.67 cm), stem girth (2.83 cm) and number of leaves (6.93 no.). Also the inoculation of consortia of these three biofertilizers showed better nutrient uptake and growth at the end of 150 days in nursery. The inoculation of consortium of three biofertilizers was found to be on par with inoculation of consortium containing four biofertilizers but found to be superior over dual inoculation, single inoculation and over control.

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