

Effect of application of biofertilizers to soybean (*Glycine max*) and nitrogen to tobacco (*Nicotiana tabacum*) in soybean – tobacco cropping system

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ABSTRACT

A field experiment was conducted during 2001-02 and 2002-03 at Jeelugumilli to find out the direct and residual effects of *Bradyrhizobium japonicum* and *Pseudomonas striata* inoculation on rainy season (*khari*) soybean [*Glycine max* (L.) Merr.] cv 'JS 335' and of directly applied N in winter (*rabi*) season FCV tobacco (*Nicotiana tabacum* L.) cv 'Kanchan' in irrigated Alfisols of Andhra Pradesh. Co-inoculation of soybean with *Bradyrhizobium* + *Pseudomonas* recorded significantly higher growth, yield attributes and grain yield than their individual inoculation and no inoculation. Green-manuring *in situ* with sunnhemp [*Crotalaria juncea* (L.) Rotar & Joy] followed by tobacco recorded significantly higher tobacco green-leaf and cured-leaf yields, and grade index compared with other treatments. Combined inoculation of *Bradyrhizobium* and *Pseudomonas* to soybean increased the tobacco green leaf yield, cured leaf yield and grade index by 10.5, 7.7 and 13.2% respectively compared with no inoculation. Application of 130 kg N/ha recorded significantly higher green-leaf and cured-leaf yields, and grade index compared with that of 110 kg N/ha. Higher total N, nicotine, lower reducing sugars and sugar: nicotine ratio in X and L leaf positions were observed in sunnhemp–tobacco system, followed by soybean (co-inoculation) – tobacco system. Higher total N, nicotine, lower reducing sugars and sugar : nicotine ratio were recorded with 130 kg N/ha than with 110 kg N/ha. Soybean (co-inoculation) – tobacco system gave additional net returns of Rs 1,710/ha over sunnhemp–tobacco system (Rs 40,670/ha). Higher B : C ratio of 1.65 was obtained with sunnhemp – tobacco system. It was concluded that combined inoculation of *Bradyrhizobium* and *Pseudomonas* to soybean improved the growth and yield of soybean and increased the succeeding tobacco yields, and application of 130 kg N/ha would be needed for tobacco succeeding soybean.

Key words: Biofertilizers, Direct effects, Economics, Leaf quality, Soybean, Sunnhemp green-manuring, Tobacco yield

Export-quality flue-cured Virginia (FCV) tobacco (*Nicotiana tabacum* L.) is cultivated mostly as a monocrop under irrigated Alfisols of East Godavari, West Godavari and Khammam districts of Andhra Pradesh in 24,838 ha, producing 48 million kg semi-flavour tobacco leaf annually (Tobacco Board, 2007). Crop rotation increases the yield and profit, and allows for sustained production. Soybean [*Glycine max* (L.) Merr.] has occupied a prominent niche in oilseed production in India. There is a scope in Andhra Pradesh also for soybean cultivation in larger areas. As soybean is not a native crop, effective strains of micro-organisms are lacking in those soils and response to *Bradyrhizobium japonicum* and *Pseudomonas striata* inoculation is expected (Subba Rao, 1986). Use of biofertilizers is one of the important components of integrated nutrient management. Phosphate-solubilizing bac-

teria are known to increase the availability of native and applied phosphorus. Some of the residual N (10-20 kg/ha) fixed by legume left in the soil is available to the succeeding tobacco crop (Collins and Hawks Jr., 1993). Tobacco-based cropping systems are advocated in traditional black soils and southern light soils of Andhra Pradesh (Singh and Srinivasulu, 1992). Earlier investigators reported reduction in yield and quality of tobacco succeeding soybean under rainfed vertisols (Harishu Kumar *et al.*, 1992). Studies on tobacco-based cropping system conducted earlier with 1 year cycle in irrigated Alfisols clearly showed the possibility of growing other crops preceding and succeeding tobacco (Kasturi Krishna *et al.*, 2004b). Previous experiments showed that FCV tobacco cv 'Kanchan' required 110-120 kg N/ha after sunnhemp [*Crotalaria juncea* (L.) Rotar & Joy] green manuring. Much work is not available on soybean – tobacco cropping system under irrigated conditions. Hence, a study on soybean - tobacco

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cropping sequence was undertaken to evaluate the direct and residual effects of *Bradyrhizobium japonicum* and *Pseudomonas striata* inoculation on performance of soybean and directly applied N in FCV tobacco in irrigated Alfisols of Andhra Pradesh.

MATERIALS AND METHODS

The field experiment was conducted during the rainy (*kharif*) and winter (*rabi*) seasons from 2001-02 to 2002-03 at Central Tobacco Research Institute Research Station, Jeelugumilli (17° 11' 30" N and 81° 07' 50" E at 150 m above mean sea-level), West Godavari district, Andhra Pradesh under semi-arid tropical climate on fixed permanent plots. The soil was sandy loam (0-22.5 cm) and deeper layers were (22.5-45 cm) sandy clay, classified Typic Haplustalfs with pH (1:2.5) 6.0, electrical conductivity (1:2.5) 0.18 dS/m and organic C (0.20%). The available soil N, P and K were 135, 18 and 86 kg/ha, respectively. Soil chlorides were 21 mg/kg. The treatments consisted of 6 main plots in rainy season and 2 subplots in winter season. During rainy season (June-September), soybean was grown with four biofertilizer treatments, viz. no inoculation, inoculated with either *Bradyrhizobium* or *Pseudomonas* or both along with sunnhemp green-manuring and fallow plots, in a randomized block design. During winter season, each main plot was divided into two sub-plots, and N levels (110 and 130 kg/ha) were allotted to tobacco in split-plot design, replicated three times.

Sunnhemp was sown @ 50 kg/ha in the first week of June and was incorporated *in situ* before flowering in the first week of August. A uniform dose of 30+26.2+25 kg/ha of N, P and K was applied through urea, single super-phosphate and potassium sulphate before sowing soybean. Soybean seed after inoculation with required biofertilizer cultures was sown at 30 x 10 cm spacing on the same day of sunnhemp sowing in rainy season and harvested at physiological maturity. Tobacco seedlings (60 days old) were planted at 100 x 60 cm spacing. Nitrogen (as per treatment) and 99.9 kg K/ha were applied in three splits at 7-10, 25-30 and 40-45 days after planting (DAP) in 1:2:1 proportion. The entire dose of P (26.2 kg/ha) was applied basal along with N and K at 7-10 DAP. The N, P and K were applied through calcium ammonium nitrate, diammonium phosphate and potassium sulphate, respectively. Soybean cv 'JS 335' and tobacco cv 'Kanchan' were used in this study. The crops were raised with recommended package of practices. During rainy season different growth parameters, yield attributes and yield of soybean were recorded, and harvest index and seed-production efficiency were calculated. During winter season, the data on tobacco green-leaf and cured-leaf yields were recorded and grade index was calculated. The cured leaf

samples collected from X and L positions were analysed for total N, nicotine, sugars and chlorides as per the standard procedures. The tobacco leaf-equivalent yield was computed by converting the soybean yield to tobacco yield based on their market prices. Cost of cultivation of tobacco included the sunnhemp cost of cultivation : sale price of soybean and tobacco cured leaf was Rs 10 and 42/kg, respectively.

RESULTS AND DISCUSSION

Biomass and N accumulation of sunnhemp

Sunnhemp produced 33.6 and 35.4 t/ha fresh biomass in 55-60 days, i.e. 3.36 and 3.54 t/ha dry matter with a N content of 3.22% on dry-weight basis. This added 108 and 114 kg N/ha during 2001-02 and 2002-03, respectively. These findings support the results of Kasturi Krishna *et al.* (2004b) who reported addition of 50 t/ha fresh matter of *Crotalaria juncea* with *in situ* green-manuring.

Soybean growth and yield

Growth parameters, viz. dry matter/plant at 60 days and harvest, plant height, and the number and dry weight of nodules were more in co-inoculation of soybean with *Bradyrhizobium* + *Pseudomonas* followed by their individual inoculation, and lower values were recorded where no inoculation was done (Tables 1, 2). Yield attributes, viz. total pods/plant, pods/plant and weight of pods/plant, were significantly higher with dual inoculation of *Bradyrhizobium* + *Pseudomonas* to soybean than their individual inoculation and no inoculation. Seeds/pod, seeds/plant, 100-seed weight, seed yield, biological yield, harvest index and seed-production efficiency were also higher with combined inoculation of *Bradyrhizobium* + *Pseudomonas* to soybean than their individual inoculation and no inoculation. Since grain yield is a cumulative effect of yield attributes, slight improvement in all yield attributes resulted in higher yield. Thus, dual inoculation of soybean with *Bradyrhizobium* + *Pseudomonas* recorded significantly higher yields than their individual inoculation and no inoculation. Individual inoculation with *Bradyrhizobium* or *Pseudomonas* also significantly increased the grain yield of soybean compared with no inoculation. These results corroborate with the findings of Poonam and Pant (2002).

Residual soil fertility and nutrient uptake by soybean

The change in organic C over a period of two years in soybean-tobacco system was marginal. However, it was higher with *in situ* incorporation of sunnhemp - tobacco system compared to soybean - tobacco and fallow - tobacco systems (Table 5). Soil available N also increased due to sunnhemp incorporation - tobacco, followed by

soybean (co-inoculation) - tobacco treatment. Increase in organic C content and soil available N due to incorporation of green manure was reported by Kasturi Krishna *et al.* (2004b). Contribution of organic C content and available N to soil through left over biomass (leaf fall and nodulated roots) of soybean was reported by Prakash *et al.* (2002). Nutrient uptake by soybean was higher in soybean co-inoculated with *Bradyrhizobium* + *Pseudomonas* compared with their individual inoculations or no inoculation. These findings corroborate with the findings of Praharaj and Dhingra (2001).

Residual effect on tobacco

Sunnhemp (*in situ* green manuring) - tobacco recorded significantly higher green-leaf and cured-leaf yields, and grade index compared with other treatments (Table 3). Dual inoculation of *Bradyrhizobium* and *Pseudomonas* to soybean being on a par with individual inoculation of *Bradyrhizobium* or *Pseudomonas* to soybean, recorded significantly higher green-leaf and cured-leaf yields, and grade index of succeeding tobacco compared with soybean without inoculation. Dual inoculation of *Bradyrhizobium* and *Pseudomonas* to soybean increased the green-leaf yield of tobacco by 10.5%, cured-leaf yield by 7.7% and grade index by 13.2% compared with soybean without inoculation. Individual inoculation of soybean with *Bradyrhizobium* and *Pseudomonas* increased the yield of succeeding tobacco green-leaf by 6.46 and

7.26%, cured-leaf yield by 4.12 and 5.15%, and grade index by 10.9 and 11.62%, respectively compared with soybean without inoculation. In soybean only the fallen leaf material, left-over stubbles and residual biologically fixed N₂ was available to tobacco. Higher yields with sunnhemp green-manuring might be owing to ploughing in of the green matter into the soil in addition to symbiotic biological N₂ fixed through its roots. As the soils are sandy loam, addition of organic matter through green-manuring might have also improved soil physical properties, especially water-holding capacity, and thereby nutrient-use efficiency (Krishna Reddy *et al.*, 2006).

The lower yield of tobacco in soybean (no inoculation) - tobacco than fallow - tobacco might be due to lack of effective strains of native *Bradyrhizobium* in the soil. Soybean owing to its high protein and oil content might have depleted most of the symbiotically fixed N₂ and to some extent other nutrients. Przednowek *et al.* (2004) also reported that soybean crop of 3.06 t/ha grain yield removed 223, 30.6 and 107 kg N, P and K /ha and left only 5 kg/ha biologically fixed N₂ in the soil.

Total N, nicotine, reducing sugars, sugars : nicotine ratio and chlorides in leaf are important quality parameters, which are influenced by the preceding crops grown (Kasturi Krishna *et al.*, 2004b). In general, the quality characters were within the acceptable limits of good quality leaf. Total N and nicotine concentration increased and reducing sugars concentration and sugars : nicotine ratio

Table 1. Growth and yield attributes of soybean as affected by biofertilizer inoculation (pooled mean of 2 seasons)

Treatment	Dry matter (g/plant)		Plant height at harvest (cm)	Branches/plant at harvest	Nodules/plant		Pods/plant		Filled seeds/pod	Seeds/plant	100-seed weight (g)
	60 DAS	Harvest			Number	Dry weight (mg)	Number	Weight (g)			
No inoculation	11.59	15.08	50.8	4.62	27.2	100.8	19.35	6.54	2.22	42.49	11.05
<i>Bradyrhizobium japonicum</i>	12.61	15.96	54.7	4.72	37.2	130.2	20.51	7.45	2.24	45.48	11.60
<i>Pseudomonas striata</i>	12.65	16.13	55.0	4.74	39.5	131.1	20.43	7.48	2.24	45.30	11.61
<i>B. japonicum</i> + <i>P. striata</i>	13.10	16.33	57.7	4.83	46.5	153.0	20.96	8.03	2.25	46.66	12.15
SEm±	0.16	0.28	1.0	0.07	0.6	1.3	0.09	0.12	0.02	0.44	0.17
CD (P=0.05)	0.47	0.81	2.8	NS	1.6	3.6	0.25	0.34	NS	1.28	0.48

Table 2. Yield, harvest index and seed-production efficiency of soybean as affected by biofertilizer inoculation (pooled mean of 2 seasons)

Treatment	Seed yield (t/ha)	Biological yield (t/ha)	Stover yield (t/ha)	Harvest index (%)	Seed - production efficiency (kg/ha/day)
No inoculation	1.57	5.03	3.46	31.2	16.06
<i>Bradyrhizobium japonicum</i>	1.76	5.32	3.56	33.0	18.06
<i>Pseudomonas striata</i>	1.77	5.38	3.61	32.9	18.12
<i>B. japonicum</i> + <i>P. striata</i>	1.89	5.44	3.55	34.7	19.39
SEm±	0.03	0.09	0.07	0.32	0.28
CD (P=0.05)	0.08	0.03	NS	0.91	0.81

Table 3. Yield and quality of FCV tobacco as affected by biofertiliser inoculation to soybean and directly applied N to tobacco (Pooled mean of two seasons)

Treatment	Tobacco yield (t/ha)		Tobacco-equivalent yield (t/ha)	Total N (%)		Nicotine (%)		Reducing sugars (%)		Sugars: nicotine		Chlorides (%)		
	Green-leaf	Cured-leaf		X	L	X	L	X	L	X	L	X	L	
<i>Biofertilizer inoculated to soybean</i>														
No inoculation	15.16	1.94	1.29	2.31	2.41	3.20	2.10	3.00	18.73	16.44	9.18	5.27	0.66	0.76
<i>Bradyrhizobium japonicum</i>	16.14	2.02	1.43	2.44	2.76	3.62	2.46	3.46	16.53	14.88	6.94	4.39	0.68	0.80
<i>Pseudomonas striata</i>	16.26	2.04	1.44	2.46	2.66	3.53	2.37	3.31	17.92	15.23	7.78	4.74	0.70	0.78
<i>B. japonicum</i> + <i>P. striata</i>	16.94	2.09	1.46	2.54	2.87	3.65	2.57	3.56	13.89	11.99	5.53	3.43	0.65	0.76
Sunn hemp green-manuring	20.14	2.41	1.72	2.41	2.86	3.77	2.64	3.69	12.14	9.94	4.70	2.75	0.62	0.74
Fallow	15.42	2.01	1.38	2.01	2.29	3.02	2.01	2.88	18.80	16.74	9.67	5.97	0.66	0.75
SEM±	0.38	0.05	0.04	0.06	0.07	0.06	0.07	0.08	0.31	0.35	0.36	0.18	0.03	0.03
CD (P=0.05)	1.13	0.14	0.10	0.16	0.19	0.19	0.20	0.23	0.91	1.03	1.07	0.54	NS	0.08
<i>N level to tobacco (kg/ha)</i>														
110	16.09	2.03	1.42	2.31	2.52	3.36	2.24	3.16	16.88	14.82	7.95	4.76	0.69	0.66
130	17.26	2.13	1.48	2.41	2.76	3.57	2.48	3.47	15.79	13.58	6.65	4.09	0.64	0.87
SEM±	0.15	0.02	0.01	0.02	0.02	0.02	0.01	0.02	0.11	0.13	0.06	0.10	0.02	0.02
CD (P=0.05)	0.42	0.05	0.03	0.05	0.06	0.06	0.03	0.06	0.32	0.38	0.18	0.03	NS	0.06

decreased from X to L positions. Higher values of total N and nicotine in leaf were recorded in the sunnhemp - tobacco system, followed by soybean (dual inoculation) - tobacco system. This trend could be attributed to higher organic matter and residual N in these crop sequences. Lowest concentration of total N and nicotine was found in fallow - tobacco system due to lower organic C and residual N in this system. The concentration of reducing sugar in the leaf was lowest in sunnhemp - tobacco system, followed by soybean (dual inoculation) - tobacco system. Higher levels of reducing sugars and sugar : nicotine ratio in tobacco leaf in X and L positions were recorded in fallow - tobacco system. Lower levels of sugars are associated with higher levels of nicotine (Gopalachari, 1984).

Direct effect on tobacco

Application of 130 kg N/ha recorded significantly higher green-leaf, and cured-leaf yields, and grade index compared with that of 110 kg N/ha (Table 3). The significant increase in green-leaf yield, cured-leaf yield and grade index might be due to the utilization of most of the symbiotically fixed N and to some extent other nutrients by soybean crop for protein synthesis and lipid metabolism. Przednowek *et al.* (2004) also reported slight benefit of N through soybean to the following cereals.

There was a significant increase in total N and nicotine concentration with 130 kg N/ha. Reducing sugars and sugar : nicotine ratio were significantly higher with 110 kg N/ha than with 130 kg N/ha. Nitrogen is a component of the nicotine molecule and is important in the synthesis of this constituent of tobacco. The concentration of N in leaves is positively correlated with nicotine and negatively correlated with starch and sugar concentrations. Thus in the present study, an increase in the rate of N fertilizer increased the concentration of total N and nicotine and decreased the sugar and sugar : nicotine ratio. Kasturi Krishna *et al.* (2004a) also reported that increase in N rate resulted in higher total alkaloids and lower sugar concentrations. The chloride content ranged from 0.62 to 0.87%, which was much less than the acceptable limit of 1.5% in leaf in all the treatments in both the plant positions.

Tobacco leaf equivalent yield

The tobacco leaf-equivalent yields of different systems (Table 3) showed that the maximum yield (2.54 t/ha) was obtained from soybean (dual inoculation) - tobacco system, followed by soybean (*Pseudomonas*) - tobacco (2.46 t/ha) and soybean (*Bradyrhizobium*) - tobacco (2.44 t/ha), because of higher yield of rainy season soybean in addition to tobacco yield in this system. The addition of biofertilizers to soybean also increased the productivity of

Table 4. Economics of soybean – tobacco cropping system under irrigated conditions (mean data of 2 seasons)

Treatment	Cost of cultivation (× 10 ³ Rs/ha)			Gross returns (× 10 ³ Rs/ha)			Net returns (× 10 ³ Rs/ha)	B : C ratio
	Rainy season	Winter season	Total	Rainy season	Winter season	Total		
Soybean - no inoculation								
– 110 kg N/ha to tobacco	8.06	57.63	65.69	15.65	79.09	94.74	29.05	1.44
– 130 kg N/ha to tobacco	8.06	58.31	66.37	15.65	83.50	99.15	32.78	1.49
Soybean - <i>Bradyrhizobium japonicum</i>								
– 110 kg N/ha to tobacco	8.11	57.63	65.74	17.58	82.91	100.49	34.75	1.53
– 130 kg N/ha to tobacco	8.11	58.31	66.42	17.58	87.07	104.65	38.23	1.58
Soybean - <i>Pseudomonas striata</i>								
– 110 kg N/ha to tobacco	8.11	57.63	65.74	17.66	83.33	100.99	35.25	1.54
– 130 kg N/ha to tobacco	8.11	58.31	66.42	17.66	87.74	105.40	38.98	1.59
Soybean - <i>B. japonicum</i> + <i>P. striata</i>								
– 110 kg N/ha to tobacco	8.14	57.63	65.77	18.90	85.64	104.54	38.77	1.59
– 130 kg N/ha to tobacco	8.14	58.31	66.45	18.90	89.92	108.82	42.38	1.64
Sunnhemp green manuring								
– 110 kg N/ha to tobacco		61.63	61.63		99.37	99.37	37.74	1.61
– 130 kg N/ha to tobacco		62.31	62.31		102.98	102.98	40.67	1.65
Fallow								
– 110 kg N/ha to tobacco		57.63	57.63		82.15	82.15	24.52	1.42
– 130 kg N/ha to tobacco		58.31	58.31		86.48	86.48	28.17	1.48

Table 5. Status of soil-organic C, available N after soybean, and N and P uptake by soybean

Treatment in rainy season	Soil organic C (%)		Available soil N after soybean (kg/ha)		N uptake by soybean (kg/ha)	
	2001-02	2002-03	2001-02	2002-03	N	P
Soybean - no inoculation	0.22	0.23	132	135	130	11.9
Soybean - <i>Bradyrhizobium japonicum</i>	0.23	0.25	139	143	143	13.6
Soybean - <i>Pseudomonas striata</i>	0.23	0.25	139	143	144	14.4
Soybean - <i>B. japonicum</i> + <i>P. striata</i>	0.25	0.27	141	146	151	15.0
Sunnhemp green-manuring	0.29	0.31	149	153		
Fallow	0.20	0.20	135	138		

the soybean - tobacco system compared with that of fallow - tobacco. The higher tobacco-equivalents in sunnhemp - tobacco system compared with those in fallow - tobacco may be because of improvement in organic C content (0.30%) and soil-available N due to addition of sunnhemp biomass and available nutrient status in the soil.

Economics

Perceptible differences were noticed among the treatments for net returns and benefit : cost ratio (Table 4). The highest net returns were obtained from soybean (co-inoculation) – tobacco system, followed by sunnhemp (*in situ* green manuring) – tobacco system. These were followed by soybean (*Bradyrhizobium* or *Pseudomonas*) – tobacco system. Soybean (co-inoculation) – tobacco system (Rs 42,380/ha) gave additional net returns of Rs 1,710/ha than sunnhemp - tobacco system (Rs 40,670/ha). Higher B : C ratio of 1.65 was obtained with sunnhemp - tobacco sys-

tem. The highest net returns with soybean (co-inoculation)–tobacco system were due to additional returns from soybean.

It was concluded that combined inoculation of *Bradyrhizobium* and *Pseudomonas* to soybean improved the growth and yield of soybean and also increased the succeeding tobacco yields compared with their individual inoculation or no inoculation. Nitrogen dose of 130 kg N/ha would be needed for tobacco succeeding soybean.

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