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COMBINATION EFFECT OF *B. JAPONICUM* MUTATED AND NON MUTATED *B. JAPONICUM* STRAIN'S, *AZOTOBACTER* AND VAM FUNGI ON SOYBEAN

Kalpana Palghadmal

Department of Botany, Padmashri Vikhe Patil College of Arts, Science and Commerce Pravaranagar, Tal .Rahata, Dis. Ahmednagar, M. S.

Email: tapasekalpana8@gmail.com

ABSRACT:

Ten strains of *Bradyrhizobium japonicum* where isolated from ten localities from Pravara area and labeled as B_1, B_2, B_3, B_4, B_5 ..... B_{10} these isolates were tested for combination effect of *B. japonicum* (mutated and non mutated), *Azotobacter* and VAM fungi From the experimental finding it was interesting to note that the combination of B_1+B_2+B_4+VAM+*A.chroochoccum* showed significant increase in height and leaf area over mutated M_3 B_1+B_2+B_4

Keywords – Strain combination, *B. japonicum*, mutated, *Azotobacter* and VAM fungi

INTRODUCTION:

Soybean (*Glycine max* L. Merrill) is an important crop plant belonging to family Papiliniocaceae now called as golden bean contain 40% protein, 20% carbohydrates, 3.80% crude fibers, vit. A, vit. B and vit. C. In India it is cultivated during karif season in Maharashtra it is cultivated in different district. Nitrogen fixation in soybean takes place with nodule formation. Nodule development on soybean is a sort of communication between host root and bacteria i.e. release of isoflavonoids by host and reorganisation by expressing nod factor and invade root hair and multiply and form nodule

Nitrogen fixation process depends upon successive symbiosis between bacteria and host plant i.e. size, number of root nodules as well as biomass. Significant increase in number and size of nodule increases crop productivity of soybean, earlier flowering, and number of seed. (Asanuma 1980, Ibrahim and Mahmoud, 1989, Rahman, and Sonaria, 1990).

Besides nitrogen fixation, *B. japonicum* produce extracellular substances like vitamins, growth promoting substances which enhance the seed germination, quick initial growth of tap root and early plant development (Asanuma 1980 and Diatloff 1986).

Using strains combination and dual inoculation of *Rhizobium* and VAM fungi on nodulation, nutrient uptake and yield in legumes were effectively reported (Bagyaraj *et al.,* 1979, Subba Rao *et al.,* 1986).Das *et al.,* (1997) have studied the effect of VA-mycorrhiza and *Rhizobium* inoculation on nutrient uptake, growth attributes and yield of green gram (*Vigna radiata* L.) they have reported increase in shoot and root lengths, number of nodules, number of pods per plant, dry weight of pods and uptake of N and P with dual inoculation compared with the uninoculated control. Seed yield was significantly higher with *Rhizobium* + VAM compared with an inoculated. Bhattarai and Prasad (2003) have supported that dual inoculation of *B. japonicum* and *A. chroococcum* proved best in all the plant growth parameter of soybean. therefore efforts were

taken to investigate the combination effect of mutated and non mutated *B. japonicum* strain's, *Azotobacter* and VAM fungi on Soybean growth.

MATERIALS AND METHODS:

Uprooted soybean plants from ten localities at Pravaranagar (Dist. Ahmednagar) were brought to the laboratory, roots were washed, and pink nodules were collected in beaker and washed with distilled water. The nodules were surface sterilized in 0.1% $HgCl_2$ for 5 min, rinsed four times with sterilized distilled water, treated with 70% ethyl alcohol for 3 min. and repeatedly washed with sterilized distilled water. Nodules were crushed with 1 ml. water and suspensions of *B. japonicum* were made. Serial dilutions of nodule extract were prepared and 1ml of 10^{-6} dilution was spread on sterile Congo red Yeast Extract Mannitol Agar (CREYEMA) plates. The plates were incubated at $26^0C \pm 2^0C$ for 4 days. Large gummy colonies of *Rhizobia* appeared on CREYEMA plates within 3 days. Ten strains of *Rhizobia* were isolated as B_1, B_2,B_3,B_4,B_5 ..... B_{10} , strains were maintained on CREYEMA slants. The efficiency of isolates was studied against different strain combinations. The effect of *B.japonicum Azotobacter* and VAM was studied by the seed dressing technique. Variety DS 228 was selected for experimental study. Mutated B_1+B_2+B_4 strain (at 15 second UV exposure), non mutated B_1+B_2+B_4 strain, *Azotobacter chroochoccum* and *Glomus fassiculatum* were used. Observations regarding the growth of plant like height and leaf area were recorded at 15 days.

For mutation 2 days old cultures were poured in the sterilized plates in aseptic condition in front of laminar airflow. The plates were exposed to the UV radiation at a distance 58 cm from UV tube (15 wat/365 nm wavelength) till 20 seconds. After 5 second interval 1ml culture broth was taken out from the respective culture plates and was poured in freshly YEM liquid medium and incubated for 2 days. Mutants were identified on the pigment basis and mutation effect was studied.

RESULTS AND DISCUSSIONS:

The result summarized in the table 1 showed that isolates were rod shaped and showed size variation from 2.13 to 2.24 and 0.9 to 1.08 µm. These finding, are in confirmity with the finding of Mullen and Wollum (1989).

All the isolates under study were gram negative, non spore former and non capsulated. The *B. japonicum* isolates from different localities shows variation in their morphology but no change in staining reaction. These observations were also supported by Wright (1925), Jorhden (1984), Eaglesham *et al.*(1987) and Martha and Eaglesham (1989).

The results indicated in the table 2 supported that the mean height of the plant was significant and same in all the treatments over the control. Among these treatments B_1+B_2+B_4 + VAM + *A. chroococcum* was highly significant to improve the height and leaf area.

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Treatment	Height of plant (cm)			Mean height	Leaf area (cm)²			Mean leaf area
	P1	P2	P3	(cm)	P1	P2	P3	(cm)2
B1+B2+B4+VAM+*A. chroochoccum*	13.0	13.5	13.1	13.20	13.5	12.8	13.3	13.17
M 3 B1+B2+B4	12.4	12.2	12.0	12.20	13.5	12.8	13.3	13.17
B1+B2+B4+VAM	11.1	10.7	11.5	11.10	10.3	9.8	13.3	11.08
M3 +B2+B4+VAM+*A. chroochoccum*	11.9	12.3	11.7	11.97	13.3	12.8	12.5	12.83
B1+B2+B4 + *A. chroochoccum*	10.5	11.0	10.5	10.67	12.5	13.5	12.3	12.75
Control	7.5	8.2	8.0	7.90	12.5	11.8	12.3	12.17
	SE ±	0.1782			SE ±	0.5213		
	CD 5%	0.5179			CD 5%	0.5155		
	CD 1%	0.7001			CD 1%	2.0487		

ANOVA

Source of Variation	SS	df	MS	F value	P-value
Rows	50,3028	5,0000	10,0606	105,6634	2,53608
Columns	0,2011	2,0000	0,1006	1,0560	0,38363
Error	0,9522	10,0000	0,0952		
Total	51,4561	17,0000			

Source of Variation	SS	df	MS	F	P-value
Rows	9,528	5,000	1,906	2,335	0,118862
Columns	1,049	2,000	0,524	0,643	0,546350
Error	8,160	10,0000	0,816		
Total	18,736	17,000			