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## Genotype-dependent response to *Azospirillum* treatment in yield and nitrogenase activity in *Brassica juncea* L.

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Twelve advanced lines of mustard (*Brassica juncea* L.) were evaluated for response to treatment with *Azospirillum brasilense* in yield and a few components. Two lines gave yield increases with concomitant increases in nitrogenase activity and *Azospirillum* population. In general, there were desirable and significant correlations between plot yield, nitrogenase activity and  $\log_e$  (*Azospirillum* population). The results point to the possibility of utilizing genotype-dependent response to *Azospirillum* in breeding programmes and also for exploring new avenues of *Azospirillum*-based cultural practices in sustaining improved productivity.

In the context of imperative need for increasing and sustaining productivity in oleiferous Brassicae, the utility of bio-fertilizing agents assumes crucial importance. *Azospirillum* has been reported to be a good microaerophilic nitrogen fixer in several plants<sup>1,2</sup>. Its potential to increase grain yields has been recorded in crops like sorghum, pearl millet, ragi and barley, though the magnitude of improvement varied with the crop and genotypes. The utility of such biofertilization in Brassica did not appear

to have been examined or reported so far. This paper summarizes the results of a pilot study on the response of *Brassica juncea* to seed treatment with *Azospirillum brasilense*.

Ten advanced lines of *B. juncea* developed in the Oilseeds Unit of the Indian Agricultural Research Institute, and the two checks, Pusa Bold and Pusa Barani formed the material. Seeds of the lines were coated with *A. brasilense* following the procedure outlined by Subba Rao *et al.*<sup>3</sup> Thus in each line there were two treatments—control, and treated with *Azospirillum*. The material was grown in a split-plot design with three replications, with the lines allotted to main plots and the two treatments to sub-plots. The plot size was five rows of 5 m length, with inter-row space of 60 cm and inter-plant distance of 10 to 15 cm. The crop was raised under normal agronomic and cultural practices. The crop was free of diseases; but aphids were controlled by timely application of the insecticide, Rogor.

Observations were recorded on plot yield (g) and on the following characters on samples of five plants, with mean values being used for statistical analysis: plant height (cm), number of primary branches, number of secondary branches and biomass (g). The presence of *Azospirillum* in the rhizosphere soil was detected by the most probable number (MPN) method<sup>4</sup>. The bacterial population in the endorhizosphere was estimated by macerating one gram of fresh roots after surface-sterilizing the roots with 0.1% chloramine-T for 30 min and making to 10-fold serial dilutions in sterile distilled water. The MPN counts were taken according to Alexander<sup>5</sup> and the population expressed as per g dry soil or per g fresh weight of roots. The cell count values were transformed to log scale to remove non-normality for further analysis. The acetylene reduction assay suggested by Hardy *et al.*<sup>6</sup> was used to assess the nitrogenase activity in excised roots.

The variation among the lines was significant for plot yield and the three characters— $\log_e$  (*Azospirillum* population in rhizosphere, RHS),  $\log_e$  (*Azospirillum* population in endorhizosphere, ERS) and nitrogenase activity (NGA). Treatment variation and the line  $\times$  treatment interaction were significant for biomass, ERS and NGA; they were not significant for plot yield.

The range of variation among the lines was high in the control lines for all characters except primary and secondary branches (Table 1). *Azospirillum* treatment enhanced the mean and the range of variation still further. Overall, plot yield showed a little but non-significant depression under treatment. In general, the coefficient of variation (CV) for the treated lines was much higher than the corresponding values for control, though for *Azospirillum* cell count and nitrogenase activity there were either no differences or a nominal decrease in the CV of treated lines.

However, a few cultures responded to the *Azospirillum* treatment and could show significant improvement over control for primary branches, biomass and plot yield (Table 2). The improvement was relatively higher for other

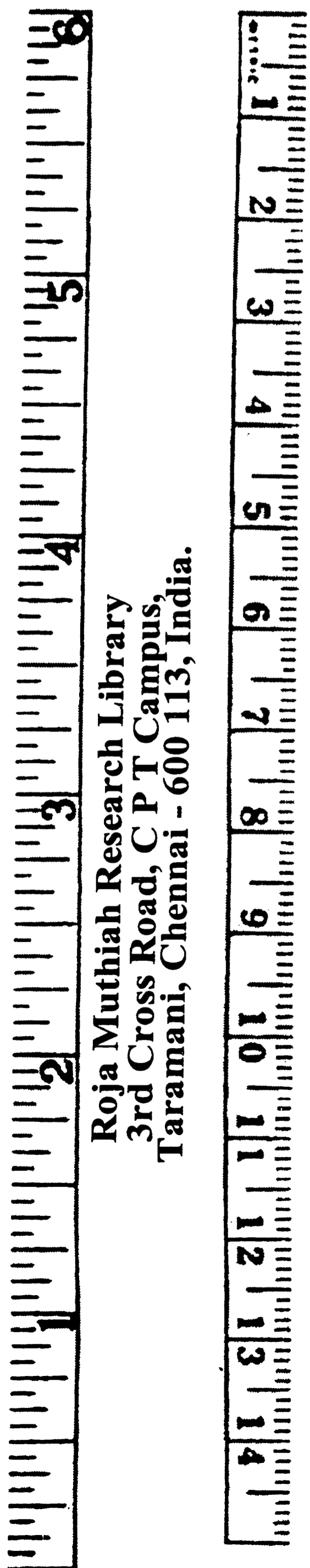


Table 1. Mean and range of variation for various traits in *B. juncea*

Character	Control			Treated			Genetic variance among lines
	Range	Mean	CV	Range	Mean	CV	
Plant height (cm)	148-204	173	9	151-208	174	10	228.27
Primary branches	5-7	6	8	6-7	6	7	0.0012
Secondary branches	10-17	13	15	9-18	13	20	0.55
Biomass (g)	68-127	106	18	78-176	110	27	64.96
Plot yield (g)	1220-2987	2391	22	1267-3060	2291	25	0.27
$\log_e$ ( <i>Azospirillum</i> population)							
Rhizosphere (per g soil)	9-14	12	15	15-23	18	12	2.95
Endorhizosphere (per g fresh root weight)	7-10	8	11	11-16	12	12	0.43
Nitrogenase activity ( $\mu\text{mol C}_2\text{H}_4/\text{h/g}$ fresh root weight)	4-13	6	48	6-22	11	39	12.10

Table 2. Significant improvement (%) in some characters with application of *A. brasilense* in *B. juncea*.

Character	Treated > Control		Treated < Control	
	Cultivar	Improvement	Cultivar	Improvement
Primary branches	DIRA 326	30	—	—
	DIRA 329	20	—	—
	DIRA 333	22	—	—
Secondary branches	DIRA 326	55	DIRA 335	24
	DIRA 329	23	Pusa Bold	19
	DIRA 333	43	Pusa Barani	29
	DIRA 337	24		
Biomass	DIRA 326	160	DIRA 335	31
			Pusa Bold	30
Plot yield	DIRA 313	11	DIRA 18	26
	DIRA 335	8	DIRA 344	12

characters than for plot yield. The yield increase was not accompanied by simultaneous improvement in other characters. Some lines showed significant decrease in the values of secondary branches, biomass and plot yield under *Azospirillum* treatment. The results are thus suggestive of a genotype-dependent response to *Azospirillum* treatment.

The observed improvements could be associated with significant and desirable correlation between yield (YLD) and some of the component characters defining increased nitrogenase activity. In particular, RHS, NGA and ERS were worth mentioning ( $r$ : YLD-RHS = 0.546\*; YLD-NGA = 0.457\*; RHS-ERS = 0.896\*). Further, the regression of plot yield on nitrogenase activity was significant (at  $P = 0.05$ ) under *Azospirillum* treatment ( $b = 1.638^*$ ) and not significant under control ( $b = 2.027$ , not significant). In turn, the regression of the increase in nitrogenase activity in lines on the increase in RHS was significant ( $b = 5.428^*$ ). Thus the results would suggest that *Azospirillum* count in rhizosphere could have accentuated nitrogenase activity resulting in better nitrogen remobilization and thereby in increased yields. Such a view is also supported by the

desirable and positive increases in the values of RHS, ERS and NGA in cultures that recorded increases in plot yield (Table 3). But such increases were not observed in the relatively high-yielding checks, Pusa Bold and Pusa Barani, which recorded high increases in nitrogenase activity. It is possible that the *Azospirillum* population had already reached the required threshold in the check varieties; as a consequence, further increases in cell count and hence nitrogenase activity could not result in proportionate increases in yield. By the same logic, lines like DIRA 313, DIRA 335 which did not possibly reach such thresholds in *Azospirillum* population and nitrogenase activity could record appreciable yield increases (Table 3). Thus the observation that there is genotype-dependent response for *Azospirillum* treatment in *B. juncea* gains further ground. Earlier work has also shown differential response to *Azospirillum* in varying growth environments and various crops<sup>7,8</sup>. In particular, *Azospirillum* treatment combined with low doses of nitrogenous fertilizer gave responses equivalent to those obtainable under high doses of N application, entailing considerable saving in fossil fuel

**Table 3.** Increase in mean values of some characters in *Azospirillum* treatment over control in specific lines of *B. juncea*

Line	Biomass	Plot yield	Log <sub>10</sub> ( <i>Azospirillum</i> population)		Nitrogenase activity
			Rhizosphere (per g soil)	Endorhizosphere (per g fresh root weight)	
DIRA 313	21.8	0.21	5.92	4.94	4.75
DIRA 335	- 34.7	0.23	6.36	3.30	7.50
Pusa Bold	- 32.9	- 0.21	6.61	6.32	6.59
Pusa Barani	- 11.0	- 0.14	9.05	8.60	9.17
Isd	43.78	0.38	2.44	2.05	2.87

depleting chemical fertilizers. With such possibilities, breeding for higher productivity in *B. juncea* begets an expanded horizon in which (i) increases due to combined application (of low doses) of N with *Azospirillum*, and (ii) exploitation of genotype environment-dependent *Azospirillum* response becomes feasible propositions to reckon with.

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