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Response of ginger (*Zingiber officinale*) to *Azospirillum* inoculation at different levels of nitrogen application*

M GOVINDAN[1], K M SREEKUMAR[2] and MADHU SUBRAMANIAN[3]

Regional Agricultural Research Station, Kerala Agricultural University, Pilicode 671 353

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Ginger (*Zingiber officinale* Rosc.) prefers a rich soil and responds well to fertilizer nitrogen. However, the spiralling fertilizer prices as well as increased preference for organically grown spices necessitate exploitation of alternate methods of plant nutrition. The wide array of nitrogen fixing, growth-promoting microbes offers an exciting alternative that fits well with an ecofriendly nutrient management strategy. Azospirilla are nitrogen-fixing organisms whose association with plants have lead to enhanced growth and yield in a number of crops such as wheat, rice etc. (Chaitra and Patil 2007, Jain *et al*. 2007). These effects are not solely attributed to the N-fixing property of the bacteria, but also to its ability to secrete growth-promoting substances (Aguilar-Piedras *et al.* 2008) and enhancing mineral uptake by the roots (Okon and Itzigsohn 1995). The present study was aimed to determine the potential of plant growth-promoting rhizobacteria *Azospirillum* on growth and yield of ginger at different levels of fertilizer nitrogen.

The laboratory experiments and analysis were conducted at the Regional Agricultural Research Station, Pilicode, Kasaragod District, Kerala. The field trial was conducted at Pothankandam in Kannur District during 2004 and 2005. The soil was gravelly laterite with *p*H of 5.5, organic matter content of 1.05%, and low in available P_2O_5 and K_2O (6 kg/ha and 80 kg/ha, respectively). The experiment was laid out in a randomized block design with 3 replications. There were 6 treatment combinations, namely T_1:100% NPK (75:50:50 kg/ha), (as per the package of practices); T_2: 100% NPK and *Azospirillum*; T_3: 75% N + 100% P_2O_5 and K_2O; T_4: T_3 + *Azospirillum* T_5: 50% N + 100% P_2O_5 and K_2O and T_6: T_5 + *Azospirillum*

Ginger was planted in May on the receipt of pre-monsoon showers. Healthy and disease-free rhizomes were carefully selected and the rhizome bits of 15.0 g weight were used for planting. *Azospirillum brasilense* S10 from the culture collection of the laboratory was used for treating the rhizomes. The bacterium was grown on semisolid Malate medium for 72 hr and was mixed at the rate of 1400 ml of broth/8 kg carrier material- powdered sterilized lignite and incubated at room temperature (26–28°C) for a week. An aqueous slurry of the inoculum (10^9 cells/g) was prepared by adding 600 g of the inoculum to 1litre of water in which the rhizome bits were dipped for 20 min. and later used for sowing. The plants which were not inoculated served as control. The rhizomes were planted in beds (3 m^2) at a spacing of 20cm × 20cm and at a depth of 4–5 cm with at least one viable healthy bud facing upward.

The crop management and the nutrient schedule were followed as per the package of practices recommendations (Kerala Agricultural University 2003) unless otherwise stated. Fertilizers were given as urea, Rajphos and muriate of potash to meet the recommended quantities of NPK (75:50:50 kg/ha). Organic manures were applied @30 tonnes/ha. Full doses of P_2O_5 and 50% of K_2O were applied as basal dose. Half the quantity of N was applied 60 days after planting(DAP). The remaining quantity of N and K was applied 120 days after planting.

The rhizosphere soil samples were collected periodically and the total bacteria, nitrogen fixers and *Azospirillum* were assessed on 45, 90 and 120 days after planting following the serial dilution technique. The MPN (most probable number) technique was used for enumeration of *Azospirillum* (Hegazi *et al.* 1979). Total bacteria were estimated using the nutrient glucose agar medium and the total diazotrophs were estimated following the standard procedure. Growth and yield parameters like root length, plant height, number of tillers, number of roots/plant, primary and secondary finger characters, rhizome fresh weight etc. were recorded. The dry weights of shoots and soil were recorded after drying the samples in an oven at 80°C till constant weights were

*Short note

[1]Professor (e mail: mgpilicode @rediffmail.com); [2]Associate Professor (e mail: sreekumarkau @gmail.com), [3]Professor, Department of Agricultural Microbiology, College of Agriculture, Palakkad, Kerala 671 328

Table 1 Effect of inoculation of ginger with *Azospirillum* on the root length, rhizosphere microbial population and vegetative characters

Treatment	Root length (cm) *	Total bacteria × 10^7 *			Nitrogen fixers × 10^5*			Azospirillum × 10^5*			Tillers/ plant**	Plant height (cm) **	Leaves/ tiller**
T_1	18.40	113.4	164	183	96.2	134	121	51.1	86	74	17.21	54.22	12.45
T_2	22.60	127.5	173	214	110.3	142	120	67.2	90	85	19.12 (11.08)	58.48 (7.86)	15.45 (24.09)
T_3	17.30	106.1	145	162	94.0	114	102	47.3	77	62	15.84	52.38	13.32
T_4	21.50	121.0	162	191	107.2	138	117	64.1	82	74	18.16 (14.65)	57.10 (9.01)	14.25 (6.98)
T_5	15.10	102.3	110	126	85.8	91	85	42.1	63	56	12.11	41.51	14.21
T_6	18.90	115.8	124	133	99.4	117	108	58.2	79	62	15.23 (25.76)	46.91 (13.01)	15.35 (8.02)
CD (*P*=0.05)	3.61	11.8	7.01	9.31	12.1	10.3	6.82	10.6	6.12	9.1	1.20	3.61	2.12

* Recorded on 45th day after planting, ** Recorded on 160 th day after planting

Data in parentheses indicate the per cent increase in the parameter recorded due to inoculation of *Azospirillum* over the corresponding non-inoculated treatment

obtained. The protein content of the samples were determined as per AOAC (1990). The mean values of the data for two years were subjected to statistical analysis.

Soil bacterium, *Azospirillum* has been found to be very much associated with the rhizosphere of ginger (Table 1). Both the inoculated as well as uninoculated treatments recorded *Azospirillum* in the rhizosphere. Their population varied between 42×10^5 and 67.2×10^5 on 45th day and 63×10^5 and 90×10^5 on 90th day. It can be seen that the total bacterial population as well as that of nitrogen fixers and *Azospirillum* were significantly higher in the root zone of inoculated plants by 45th day itself. Inoculation with *Azospirillum* also appear to have stimulated root growth as is evident from the fact that the mean root length for inoculated plants was 21.0 cm while the corresponding figure for non-inoculated plants was 16.9 cm. The fact that first dose of N was applied only 60 day after planting and also the consistent superiority of inoculated plants over non-inoculated for same level of fertilizer nitrogen clearly brings out the positive effect of *Azospirillum* on initial root growth. *Azospirillum* is reported to have the ability to modify plant root architecture. Nitric oxide has been described to mediate indole-3-acetic acid (IAA)-signaling pathways in plants, leading to both lateral and adventitious root formation. Creus *et al.* (2005) and Molina-Favero *et al.* (2008) reported the participation of nitric oxide in the *Azospirillum*-promoted lateral root formation. In all the combinations of fertilizer nitrogen, *Azospirillum* application enhanced root length of ginger. Maximum root length was observed in T_2-1 inoculating *Azospirillum* along with application of 100% fertilizer nitrogen. Inoculation favored build-up of *Azospirillum* in the root zone. The total bacteria and nitrogen fixers were also higher in all the treatments which received *Azospirillum* inoculation.

The production of tillers/plant was also influenced by *Azospirillum* inoculation. There was an increase of 11.08, 14.65 and 25.76% in tiller production over the corresponding treatments which received only fertilizer nitrogen at 100, 75 and 50% respectively. The number, girth and height of the tillers are considered to be attributing to yield in ginger. (Ravindran and Nirmal Babu 2004). Azospirilla are known to produce plant growth-promoting substances. The higher root production and tiller production lead to better nutrient uptake, growth and yield. This was reflected in the plant height and number of leaves/tiller. Even at 100% fertilizer nitrogen level there was a positive effect of inoculation in plant height and number of leaves produced.

Yield parameters, like number of fingers, length and girth of fingers were recorded on 160th day and also at the time of harvest. The number of fingers produced was highest at 100% fertilizer nitrogen + *Azospirillum* which was 22.48% higher than its corresponding control. At 75% fertilizer nitrogen also there was good response to inoculation (21.9%). At 50% N level, the production of fingers was low. The fresh weight of rhizome was recorded on 160th day and at harvest Maximum yield was obtained in the treatment T_2 (100% N fertilizer + *Azospirillum*), followed by T_4 (75% fertilizer nitrogen + *Azospirillum*). It can be seen that inoculation at 75% N resulted in 190.5 g which is higher than the yield obtained by applying 100% Nitrogen alone. It is clearly evident from the data that *Azospirillum* is effective under field conditions in enhancing yield. Rames (2008) also had observed that *Azospirillum brasilense* Sp7 significantly increased rhizome weight of ginger. Inoculation resulted in significant difference in the protein content of rhizome at all the levels of fertilizer nitrogen. The increase over the corresponding non-inoculated treatments were 12.42%, 15.12% and 17.03% at 100%, 75% and 50% fertilizer nitrogen levels. At harvest, the highest protein content was observed in T_2 (100% fertilizer nitrogen + inoculation).

Ginger is considered as a soil nutrient exhausting crop. It requires sufficient amounts of nutrients for proper growth and yield. The dominant approach so far to meet this requirement has been to depend heavily on chemical fertilizers, which cannot be sustainable from economic or ecological point of view. A strong case exists for integrating farmer and nature-friendly technologies in nutrient management strategies. The present study provides strong evidence that nitrogen-fixing and growth-promoting microbes can be a vital component of such a strategy by proving that in the case of ginger *Azospirillum* can help effectively replace at least 25% of chemical nitrogen fertilizer.

SUMMARY

Field trials were carried out during 2004 and 2005 to study the efficacy of *Azospirillum*, a nitrogen-fixing rhizobacterium as a biofertilizer for ginger at different levels of fertilizer nitrogen. The treatment of *Azospirillum* with 100% fertilizer nitrogen was found to be superior to all other treatments in terms of maximum root length, number of fingers, yield and starch content at harvest. Inoculation also resulted in increased protein content of rhizome at all the levels of fertilizer nitrogen.

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