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SHORT COMMUNICATION

ENHANCED LEVELS OF SOIL NITROGEN AND ENDOGENOUS PHYTOHORMONES IN MAIZE (*ZEA MAYS* L.) INOCULATED WITH *AZOSPIRILLUM BRASILENSE*

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The para-nodulated maize plants grown in soil supplemented with *Azospirillum* thrice during the growth period performed better than the control plants. The para-nodules were formed along primary roots in the presence of 2,4-dichlorophenoxyacetic acid (2,4-D) and *Azospirillum*. The content of the endogenous hormones *viz.* indole acetic acid (IAA) and gibberellic acid (GA) was enhanced in para-nodulated maize plants cultivar Kiran. However, the level of abscisic acid (ABA) declined. The dry weight and leaf area of the para-nodulated plants increased as compared to the control plants. The ammonia concentration (56 % more) and nutrients were higher in the para-nodulated plants as compared to the control plants. The soil nitrate and ammonical N levels were higher in the pots with treated plants and inoculated with *Azospirillum* as compared to their levels in control pots. Bacterial phytohormone synthesis seems to be responsible for the *Azospirillum* mediated plant growth promotion.

Keywords: **Biofertilizers, gibberellic acid, indole-3-acetic acid, nitrogen, para-nodules, *Zea mays***

The rising cost of nitrogen fertilizers – driven by the rising cost of fossil fuels and need for improved sustainability make alternatives to nitrogen fertilizers more attractive. A number of field trials, carried out at different locations, have demonstrated that under certain environmental and soil conditions, inoculation with *Azospirillum* had beneficial effects on plant yields (Dobbelaere *et al.* 1999) because of their ability to convert atmospheric N_2 into ammonia that can be taken up by the plant (Steenhoudt and Vanderleyden 2000). Though contribution of the rhizobacteria through biological nitrogen fixation (BNF) is not considered very important, but our earlier studies have shown that para-nodules exhibited the nitrogenase activity and also have stimulatory effect on the growth and enzyme activities in the shoot (Saikia *et al.* 2007). In the present investigation, the performance of the maize crop in the presence of bio-fertilizers and in the absence of chemical fertilizers was studied to analyze the pattern of free ammonia and nutrient accumulation in plants as a result of para-nodulation, soil application of biofertilizer and changes in the endogenous levels of the hormones.

Seeds of *Zea mays* L. cv. Kiran were surface sterilized and germinated on sterile, acid-washed gravel culture at 25°C for 5-6 days. The para-nodules were induced using *Azospirillum brasilense* (strain Sp-29; 10^7 to 10^8 cells ml^{-1}) along with 1.0 ppm 2, 4-D (Saikia *et* al. 2007). The two weeks old seedlings after nodulation under laboratory conditions were transferred to pots (43 Kg soil with 2 Kg FYM) in the net house under natural conditions. Biofertilizer (20 ml pot^{-1}; 10^7 – 10^8 cells ml^{-1}) was applied before transplanting and 45 days after transplanting (DAT) in the pots with para-nodulated plants. No chemical fertilizers were applied to either treated or control plants.

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The dry weights and leaf area were recorded 30, 60 and 90 days after transplanting (DAT). The plant parts were dried at 70ºC till the constant dry weight was recorded. The leaf area was measured using Leaf Area Meter (LI- 3100, LI-COR). Ammonia present in unbound form was estimated by diffusing it as NH_3 from the tissue into boric acid traps (Martin *et al.* 1983) using Warburg's flasks. Ammonium borate formed was assayed (Novozamsky 1974). Nitrogen in the plant samples was estimated using auto analyzer (Gerhardt, autoanalyser). Phosphorus was estimated by following the method of Jackson (1973). Nitrate and ammonium in the soil samples were estimated by the method of Jackson (1973). Initial soil sample was taken two days after transplanting and second sampling was done at 50 DAT i.e after applying the biofertilizer in the soil (45 DAT). The plant hormones were extracted from the roots and leaves at 60 DAT by the modified procedure of kojima (1997). Indole acetic acid (IAA) and abscisic acid (ABA) were extracted as ether phase. The aqueous phase was used for the purification of gibberllins (GA). The reconstituted samples in methanol were analyzed by HPLC (P2000 liquid chromatograph), equipped with a variable wavelength UV-VIS detector.

The experiment was laid out in completely randomized design. All observations are the means of three replicates and data analysis was done using the standard statistical methods (Panse and Sukhatame 1967).

The dry weight of the plants increased (60 % at 30 DAT) in the para-nodulated plants as compared to the control plants (Fig. 1). The increase was 48 % at 90 DAT in the para-nodulated plants as compared to the control plants. In the para- nodulated plants the leaf area increased by 12-21 % as compared to the control plants (Fig. 1). The comparison of wheat plants inoculated with wild and mutant strain (less IAA synthesis) of *A. brasilense* clearly established the contribution of biofertilizer towards enhancing the biomass (Spaepen *et al*. 2008). The para- nodulated plants had higher level of free ammonia in both roots and leaves as compared to the control plants (Fig. 2). Higher levels of ammonia produced in the roots of the para-nodulated plants coincided with increased nitrogenase activity in roots of

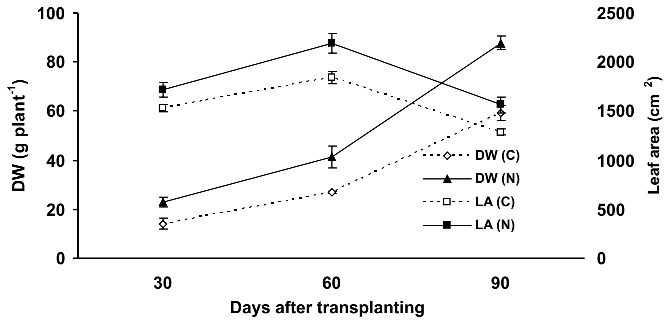

Fig. 1. Effect of inoculation with *A. brasilense* on dry weight (DW) and leaf area (LA) of para-nodulated and control maize plants at 30, 60 and 90 days after transplanting (DAT) in pots. (C=Control, N=Paranodulated plants ±=Standard error).

plants (Saikia *et al*. 2007). In our earlier studies, we have shown that the host plant was capable of supplying the necessary susbtrates for N_2 fixation (C_2H_2 reduction) of the bacteria residing mainly in the para-nodule (Saikia *et al*. 2006). Thus, plant bacteria relationship meets the essential prerequisites, which demonstrates that it is symbiotic.

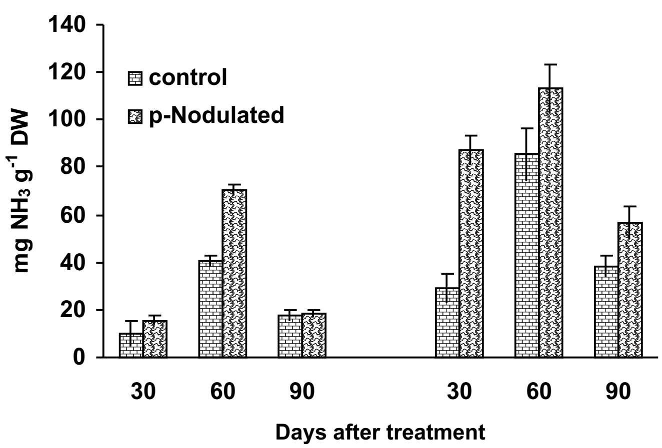

Fig. 2. Concentration of ammonia in the leaves and root tissues of para-nodulated and control maize plants at 30 and 60 DAT. Each bar represents ± SE of three replicates.

Nitrogen (N) and phosphorus analysis of the plants revealed that the content of these nutrients in the para-nodulated plants was significantly enhanced as compared to the control plants (Fig. 3). The N content increased up to 3 fold. Increase in N and P content in the cereals grown in the presence of biofertilizers has been reported earlier (Spaepen *et al.* 2008).

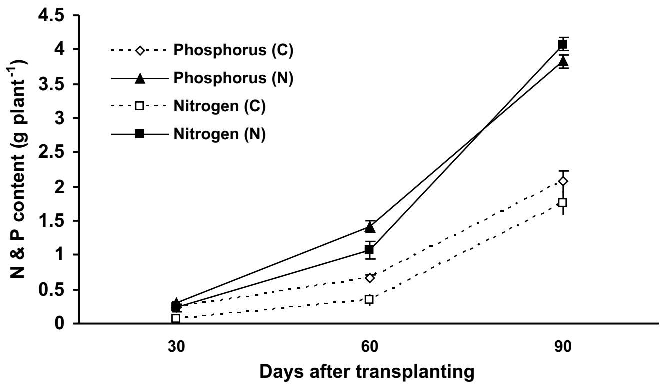

Fig. 3. Nitrogen (N) and phosphorus (P) content of the para-nodulated (N) and control (C) maize plants at 30, 60 and 90 DAT. Each line represents ± SE of three replicates.

The level of the growth promoting hormones significantly increased in both roots and leaves of the para-nodulated plants as compared to the control plants. The concentration of both IAA and GA was higher in the roots as well as in the leaves of the para-nodulated plants (Fig. 4). IAA increased by more than 2 fold and GA was 44 % more in the leaves of the para-nodulated plants as compared to the control plants. The levels of ABA in both roots as well as leaves of the para-nodulated plants declined by 15 and 26 %, respectively, as compared to the control plants. The increase in IAA and GA might be due the production of phytohormones by the rhizobacteria (Spaepen and Vanderleyden 2007, Spaepen *et al*. 2008). Studies have indicated that endophytic bacteria viz. *Azospirillum* have beneficial effects on cereals as these microorganisms are capable of producing GA (MacMillan 2002). In bacteria, probably there is no role for GAs except that they might play a role as signaling factor towards host plant. The production of phytohormones by plant growth promoting rhizobacteria and the enhancement of endogenous levels in plants is important. The soil nitrate and ammonium levels were higher in the pots sown with inoculated plants as compared to the pots with the control plants (Fig. 5). It is possible that the degenerating bacteria improved N content of the soil. It is well known that part of the N is available to the plants after the death and lyses of the bacteria (Shantaram and Mattoo 1997).

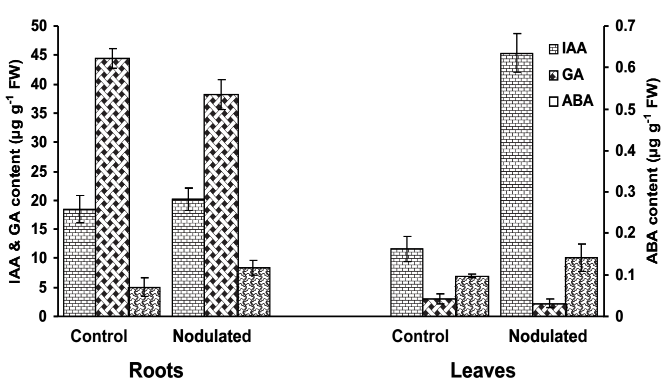

Fig. 4. Endogenous content of phytohormones (IAA, GA and ABA) in the leaves and roots of the para-nodulated and control maize plants at 60 DAT. Each bar represents ± SE of three replicates.

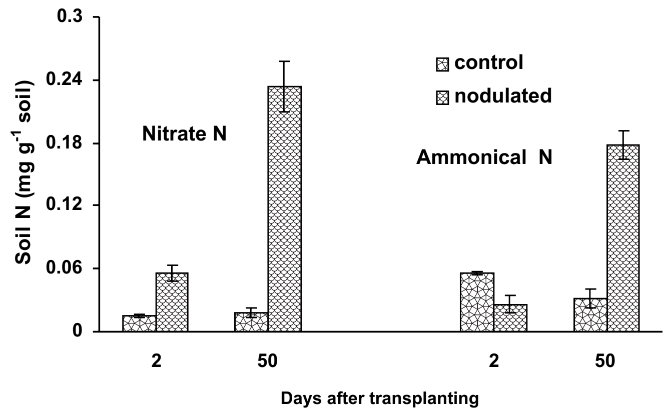

Fig. 5. Total soil nitrate nitrogen and ammonical nitrogen in the soil from pots inoculated with *A. brasilense* and control pots. Each bar represents ± SE of three replicates.

Based on our results, it appears that the process of enhanced growth triggered by *Azospirillum* inoculation is not solely due to N_2 fixation, but may be a consequence of different mechanisms including phytohormone synthesis and their release to the plants. Mechanisms by which plant growth promoting rhizobacteria promote increases in nutrients and yields are not fully elucidated, the synthesis of phytohormones including gibberellins (Bottini *et al*. 2004) and absorption of these by the crop plants might be the key factors.

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