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Foliar application of nitrogen fixing bacteria increases growth and yield of canola grown under different nitrogen regimes

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ABSTRACT

A field study was conducted to evaluate the effects of foliar application of nitrogen fixing bacteria on canola growth and yield. *Azotobacter chroococcum* Strain 5 and *Azospirillum lipoferum* Strain 21 as well as mixture of them were used on aerial parts of two canola cultivars under different nitrogen regimes. The experiment was laid out in a randomized complete block design with factorial arrangement in three replications. The results indicated that foliar application of bacteria significantly promoted almost all agronomic traits. These positive effects amplified when both species of bacteria were applied simultaneously on the plants.

Use of biofertilizers containing beneficial microorganisms instead of synthetic chemical are known to improve plant growth through supply of plant nutrients and may help to sustain environmental health and soil productivity (O'Connell, 1992). So far considerable number of bacterial species mostly associated with the plant rhizosphere, have been tested and found to be beneficial for plant growth, yield and crop quality. They have been called 'plant growth promoting rhizobacteria (PGPR)' including the strains in the genera *Acinetobacter Alcaligenes*, *Arthrobacter*, *Azospirillium*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Rhizobium* and *Serratia* (Sturz and Nowak, 2000; Sudhakar et al., 2000). The improvement in plant growth and development in response to seed or root inoculation with various microbial inoculants through producing plant growth regulators has been reported by Zahir et al. (2004). However, little is known about promoting effects on yield and growth of floral and foliar inoculation with PGPR in crops.

In previous studies, foliar application of nitrogen-fixing bacteria could increase growth and yield in pea (Bahadur et al., 2007), mulberry (Sudhakar et al., 2000) and apricot (Esitken et al., 2002, 2003). In a study, Esitken et al. (2006) have shown that floral and foliar bacterial application in full bloom and shortly after flowering (cell division phase) of sweet cherry, stimulated fruit set and fruit development through the effect of IAA and *trans*-zeatin.

Since there is little information about PGPRs foliar application and their effects on agricultural crops productivity, comprehensive studies are needed. The objective of this study was to evaluate the effect of foliar application of bacteria and nitrogen fertilizer on winter canola production. Our hypothesis was that the application of *Azotobacter* and *Azospirillum* together and accompanied by N fertilizer would improve growth and canola seed yield.

To test the hypothesis, a field experiment was conducted in Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran during 2010 growing season. Two canola cultivars, Okapi and Zarfam and two different species of bacteria comprised of *Azotobacter chroococcum* (Strain 5) and *Azospirillum lipoferum* (Strain 21) as well as mixture of them were used under two different fertilizer regimes (application of 70 (N_1) and 100% (N_2) required nitrogen). The experiment was laid out in a randomized complete block design (RCBD) arranged in factorial with 16 treatments and three replications. The nitrogen fertilizer in the form of urea was applied according to results of soil analysis and certain treatments (84 and 120 kg N ha^{-1} in N_1 and N_2 treatments, respectively). Seeds were obtained from Seed and Plant Improvement Institute (SPII), Karaj, Iran and were sown by hand on 15th October with final plant density 830,000 plants per hectare. The plots were irrigated immediately after seed sowing. Weeds were controlled by hand

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Table 1
Main effects of cultivar, nitrogen regimes and rhizobacteria foliar application on agronomic traits of canola.

Factors	Levels	Plant height (cm)	Branch number	Seed number per silique	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Oil yield (kg ha^{-1})	Protein yield (kg ha^{-1})
Cultivars	Okapi	108 **b**	5 **a**	21 **a**	1871 **a**	39,718 **a**	790 **a**	462 **a**
	Zarfam	115 **a**	4 **b**	18 **b**	1309 **b**	22,546 **b**	545 **b**	332 **b**
Nitrogen regimes	100%	117 **a**	5 **a**	22 **a**	1824 **a**	38,048 **a**	775 **a**	486 **a**
	70%	106 **b**	4 **b**	16 **b**	1356 **b**	24,215 **b**	561 **b**	308 **b**
Bacteria	Control	105 **c**	3 **d**	16 **d**	1411 **c**	25,530 **c**	564 **c**	310 **d**
	Azotobacter	110 **bc**	4 **c**	19 **c**	1553 **b**	29,438 **b**	654 **b**	364 **c**
	Azospirillum	112 **b**	5 **b**	20 **b**	1600 **b**	30,930 **b**	680 **b**	397 **b**
	Azotobacter + Azospirillum	119 **a**	6 **a**	22 **a**	1796 **a**	38,628 **a**	773 **a**	516 **a**

Means within each column followed by the same letter are not statistically different at $\alpha = 0.05$ by DMRT test.

during growing season. At flowering stage, foliar application of bacteria was performed. Bacteria suspension, *Azotobacter chroococcum* Strain 5 and *Azospirillum lipoferum* Strain 21 (Obtained from Soil and Water Research Institute, Tehran, Iran) were sprayed on the plants using backpack spryer according to the methods of Okon and Itzigsohn (1995) and Zahir et al. (2004). Inoculant was containing 10^8 bacteria per millilitre. 17 g l^{-1} cohesive substance and one drop of Tween 20 were added into the solution to better contact between bacteria and leaves. Foliar application was performed in the evening after sunset. At physiological maturity stage one square meter of each plot was harvested randomly and canola agronomic traits were registered. In addition, oil and protein percentage were measured by Inframatic 8620 Percor. Oil and protein yield were calculated by multiplying seed yield by oil or protein percentage, respectively. All data were first analyzed by ANOVA to determine significant ($P \leq 0.05$) treatment effects. Significant differences between means were determined using Duncan's multiple range test (DMRT).

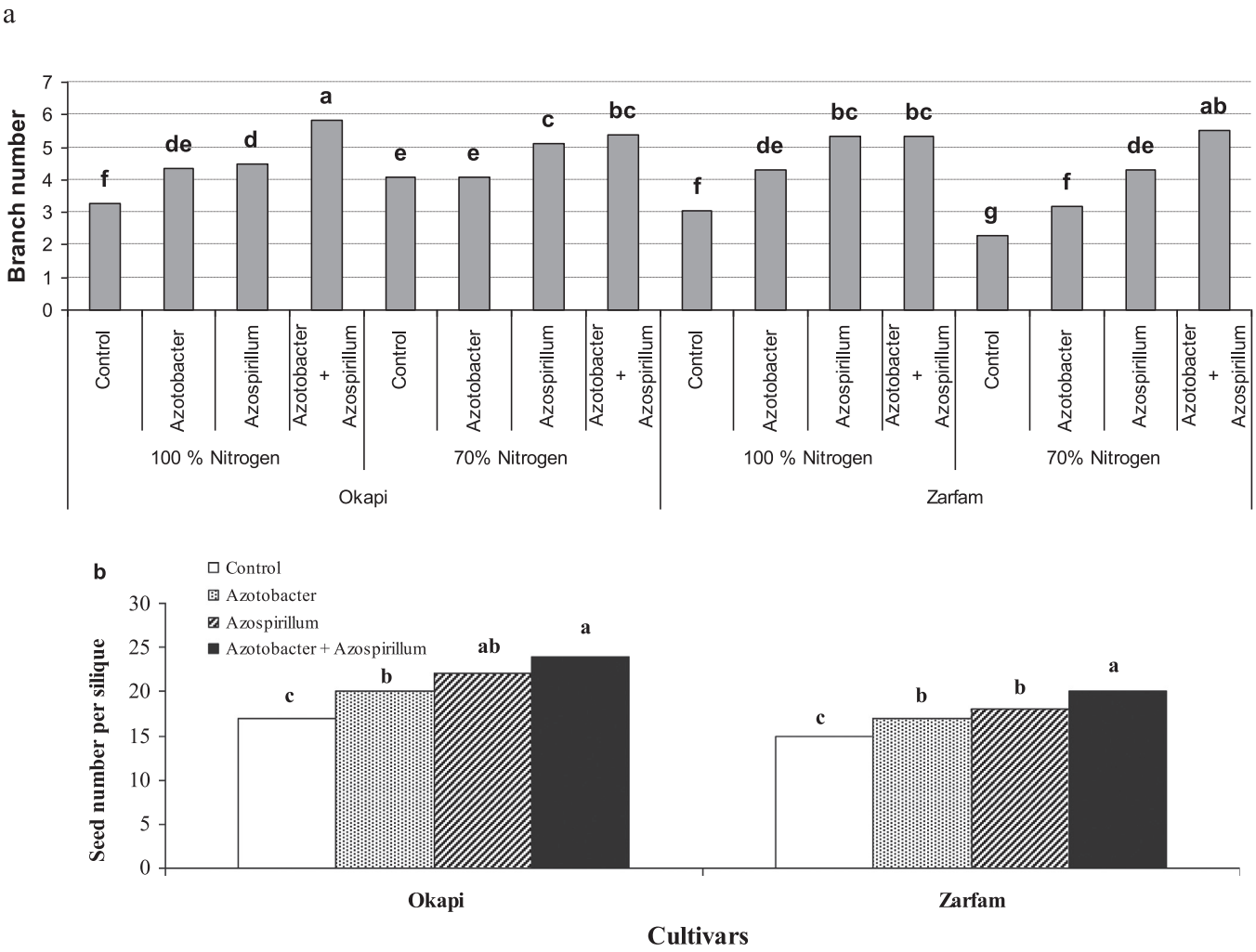

Fig. 1. Interaction among cultivar, nitrogen regimes and rhizobacteria on branch number (a) and interaction between cultivar and rhizobacteria on seed number per silique (b). Means followed by the same letter are not statistically different at $\alpha = 0.05$ by DMRT test.

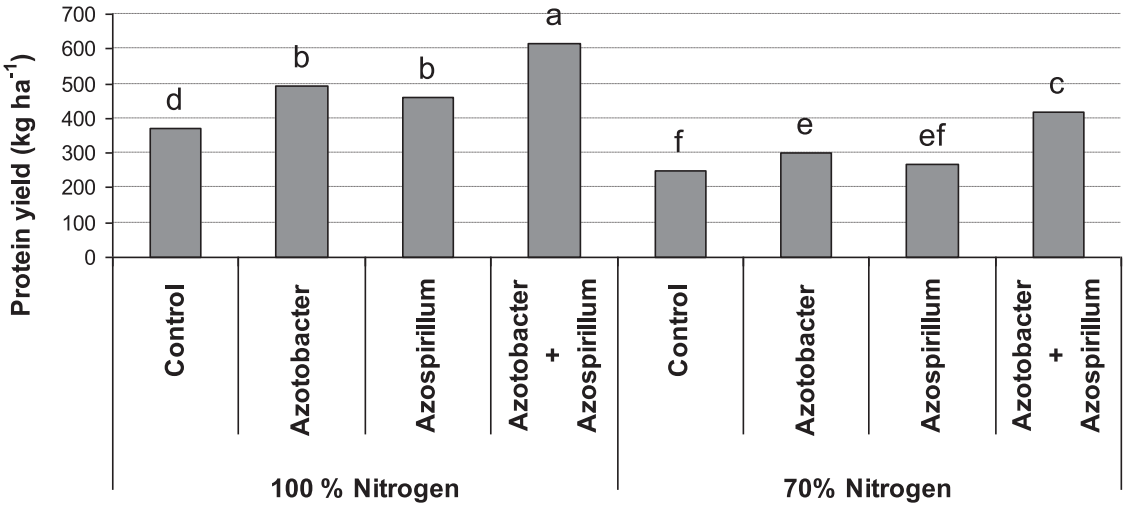

Fig. 2. Interaction between regimes and rhizobacteria on protein yield. Means followed by the same letter are not statistically different at $\alpha = 0.05$ by DMRT test.

As can be seen from Table 1, plant height increased due to mixture of *Azotobacter* and *Azospirillum*. However single application of each bacterium increased plant height compared with control treatment. It has been reported that inoculation of plants with *Azospirillum* could result in significant changes in various growth parameters, such as increase in plant biomass, nutrient uptake, tissue nitrogen content, plant height, leaf size and root length (Bashan et al., 2004). Positive interactions between free living, nitrogen-fixing rhizosphere bacteria belonging to the genera *Azotobacter* and *Azospirillum* and various field-grown crops have been recorded in a number of studies (De Freitas, 2000). Basha et al. (2006) found that the foliar application of PGPR provided a superior efficiency in the management of fungal diseases on chickpea, and Esitken et al. (2006) reported a positive effect of a PGPR foliar spray on sweet cherry yield. Vijayan et al. (2007) founded that foliar application of *Azotobacter chroococcum* could alleviate inhibiting effects of salinity in mulberry plants. In addition, Grichko and Glick (2001) found that inoculated tomato plants with PGPR have higher tolerance to waterlogging stress. Three-way interaction was significant on branch number. The most branch number was observed when both *Azotobacter* and *Azospirillum* were applied. In general, foliar application of bacteria, in particular mixture of them, increased branch number in canola cultivars (Fig. 1a). This increase might be due to increase in vegetative growth and simulation of lateral buds to grow because of nitrogen fixation and secreted plant hormones such as auxins, gibberellins and cytokinins (Saxena and Tilak, 1994; De Silva et al., 2000) by bacteria. Zahir et al., (2004) have reported that these bacteria increase flower buds number and postpone senescence and flower abscission via plant hormones secretion. Several studies have shown that beneficial microbes, such as *Azotobacter* and *Azospirillum*, are not only effective in terms of nitrogen fixation but also exhibit other favourable properties, including production of growth hormones (Remus et al., 2000; Okon and Itzigsohn, 1995). On the other hand, the highest seed number per silique was produced when both species of bacteria were applied on Okapi cultivar. Control treatments, whether Okapi or Zarfam, had the lowest seed number per silique (Fig. 1b). The stimulating effects of these microorganisms are attributed to their efficiency in supplying better grow conditions with dissolved immobilized nutrients and producing phytohormones, which could stimulate nutrient and water absorption as well as photosynthesis, leading to increased plant growth and yield (Remus et al., 2000).

It is worth mentioning that seed yield in Okapi cultivar was more than Zarfam cultivar (Table 1). There was no significant difference between two nitrogen rates in Okapi cultivar while in Zarfam seed yield increased with increasing in nitrogen availability (through N fertilizer and especially bacteria application). In case of biological yield, more nitrogen application produced more vegetative parts. Furthermore, application of bacteria improved biological yield especially when both species of bacteria were applied (Table 1). The biofertilizers foliar application has many advantages: (i) the nitrogen is being fixed close to its place of assimilation (De Freitas, 2000), (ii) the nitrogen-fixing bacteria on the phylloplane can act as antagonists to many plant pathogens if the right strains are used (Liu et al., 2016; Bahadur et al., 2007), (iii) there is enough food material for bacteria on the phylloplane in the form of leaf leachates and degrading cuticle which is better suited to symbiotic nitrogen-fixing bacteria (Sen, 1988), and (iv) the bacteria face less competition by other microflora on the phylloplane than in soil (Kozdroja et al., 2004). The enhancing effect of rhizobacteria on seed and biological yield was reported by many researchers (Zahir et al., 2004; Sudhakar et al., 2000). Such an improvement might be attributed to N_2-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances (Salantur et al., 2006).

From Table 1, oil and protein yield increased with increasing nitrogen rate. In addition, bacteria application improved oil and protein yield. There was no significant difference between the two species of bacteria on oil yield production while *Azospirillum* was more effective than *Azotobacter* in increasing protein yield. The highest protein yield was observed when both bacteria were applied at the same time along with application of 100% required nitrogen (Fig. 2). Xia et al. (1990) found that the oil concentration of rapeseed increased in response to rhizobacteria inoculation.

In summary, the results of this study showed the combined application of *Azotobacter* and *Azospirillum* with nitrogen fertilizer improved canola productivity compared to their individual application. The results showed that the efficiency of nitrogen fertilizer increased in the presence of rhizobacteria. Therefore, the combined application of nitrogen fertilizer with rhizobacteria could result in decreasing the amount of nitrogen requirement.

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