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Research Paper

Yield, nutrient availability and uptake in wheat (*Triticum aestivum*) as influenced by *Azotobacter* and nitrogen levels

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ABSTRACT

An experiment was carried out during the winter (*rabi*) season of 2014–15 and 2015–16 at Ludhiana, Punjab, with objectives of increasing nutrient availability, uptake and wheat (*Triticum aestivum* L.) yield with application of *Azotobacter* and nitrogen. Results showed that inoculation with *Azotobacter* and increased nitrogen levels significantly improved the yield attributes. Among the inoculation methods, seed inoculation with *Azotobacter* enhanced yield by 7.7% and 8.3% during 2014–15 and 2015–16, respectively, over un-inoculated control. The nutrient content, uptake and soil microbial count were also improved with the inoculation of *Azotobacter* and increase nitrogen levels. The seed and soil inoculations were statistically similar to each other for different parameters during both the years.

Key words : *Azotobacter*, Nitrogen, Nutrient uptake, Wheat, Yield

Environmentally sound and sustainable crop production is one of the major challenges faced by the agricultural scientist in twentyfirst century. It has become necessary to increase the production of food crops to provide food security to ever-increasing world population. However, the increased use of chemical fertilizers to harvest maximum yield potential adversely affects the soil health and biological system. Biofertilizers and other plant growth-promoting organisms are known to play an important role, as these are more eco-friendly than inorganic/ chemical fertilizers and simultaneously help in nutrient build up and improvement in soil health (Hayat *et al*., 2010).

Nitrogen is one of the most important inputs which affects growth and development of wheat to a great extent. Nitrogen constitutes about 78% of atmospheric gases, but this limitless reservoir of nitrogen is not directly available to the plants. However, some microbes like *Rhizobium*, *Azotobacter*, *Azospirillium* fix this inert nitrogen from the atmosphere and make this available to the plants through the enzyme nitrogenase (Bohlool *et al.,* 1992). *Azotobacter* represents the main group of free-living nitrogen-fixing bacteria. Apart from its nitrogen-fixing ability, *Azotobacter* synthesizes phytohormones like auxins, cytokinins and gibberellins (Mrkovacki and Milic, 2001). Hence, such biofertilizers play an important role in increasing the crop production. But the inoculation methods (whether the contact should be made through seed or soil) needs to be thoroughly established. Keeping this in view, an experiment was conducted to study the response of wheat to different inoculation methods of *Azotobacter* under various nitrogen levels.

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MATERIALS AND METHODS

The experiment was carried out at the Research Farm of Wheat Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, during the *rabi* season of 2014–15 and 2015–16. The soil was loamy sand, low in organic carbon and available nitrogen and medium in available phosphorus and potassium. The data on standard meteorological weeks (SMWs) during the 2 crop growing seasons were recorded at the meteorological observatory of the School of Climate Change and Agrometerology (SCCAM), PAU, Ludhiana. The weekly maximum temperature for 2 seasons ranged from 12.5ºC and 13.0ºC in December to 32.6 ºC and 35.1ºC in April, whereas the minimum temperature ranged from 5.2ºC and 4.9ºC in December to 18.1ºC and 20.1ºC in April during the winter season of 2014–15 and 2015–16 respectively. Weekly relative humidity ranged from 61 to 89% and 44 to 86% during the cropping seasons of 2014–15 and 2015–16 respectively. The total rainfall received during the cropping season of 2014–15 was 213 mm (in 26 days), whereas that received during the winter season 2015–16 was 71.4 mm (in 17 days). However, the normal average rainfall during the cropping season for the last 20 years was 105.4 mm.

The experiment was laid out in split-plot design with the methods of *Azotobacter* inoculation, viz. seed or soil inoculation and uninoculated control, in main plots and 4 levels of nitrogen (100%, 75% and 50% of recommended nitrogen and a control with no application of nitrogen) in subplots. The seed inoculation was done by moistening the seeds of variety 'WH 1105' and then treated with charcoal-based formulation of *Azotobacter*. For the soil-inoculation treatment, the biofertilizer was first mixed with soil @ 60 kg/ha and then applied along with the seed in manually opened furrow during the sowing. The sowing was done with *kera* (the furrow was open with manually operated plough and seed was placed in the furrow about 5–6 cm deep) method. The crop is raised as per standard recommended practices except for N which was applied according to the respective treatments.

The data for the effective tillers (ear-bearing tillers) were counted from 1 m row length from 5 locations in each plot before the harvesting of crop and then converted to number of effective tillers/m^2. Five ears were selected at random from each plot to measure ear length and their average value was expressed in cm and then threshed to record number of grains/ear. And 1,000-grain weight was recorded by taking a random sample of grain from each plot. The grain and straw samples from each plot were tested to estimate N, P and K content. For determination of N content, modified Kjeldahl's method was used. Phosphorus content was determined with the help of colorimeter by using Vanadomolybdate method. Uptake of N, P and K by grain and straw was then calculated by multiplying the per cent nutrient content in grain/ straw with their respective grain or straw yield.

The soil samples were taken from upper 0–15 cm layer of soil to analyze the microbial population. The isolation of bacteria from the samples was done by using the serial dilution and pour-plate method. The microbial count was calculated as:

$$\text{CFU/g soil} = \frac{\text{Count/plate}}{\text{Dilution used}}$$

RESULTS AND DISCUSSION

Yield attributes

The highest number of effective tillers, grains/ear and ear length were recorded with the seed inoculation of *Azotobacter,* which being statistically similar to soil inoculation was significantly better than no-inoculation (Table 1). Soil inoculation with *Azotobacter* too resulted in statistically higher number of effective tillers, grains/ear and ear length. Sarma *et al*. (2007) and Sepat *et al*. (2010) reported increase in density of effective tillers with the *Azotobacter* inoculation. The higher number of grains/ear with the *Azotobacter* inoculation was also reported by Gupta and Aggarwal (2008). The 1,000-grain weight was less influenced by the *Azotobacter* inoculation, as the grain weight recorded with the inoculation of *Azotobacter* was statistically similar to the un-inoculated control.

Application of full recommended N dose (125 kg/ha) significantly influenced the yield attributes, except 1000-grain weight. With an increase in N, the effective tillers increased significantly as compared to its lower nitrogen levels, the highest being recorded with the recommended dose of N. Rathi *et al*. (2014) also reported the significantly higher number of tillers with the increase in levels of nitrogen.

Nitrogen applied even at 50% of its recommended dose significantly influenced the number of grains/ear than no-nitrogen. The 75% recommended N registered grains/ ear at par with that recorded with 50% and 100% of recommended N. But the number of grains obtained with 100% recommended N were significantly better than 50% recommended N. Singh *et al*. (2016) also reported increase in number of grain/ear with the increase in the levels of N. Ear length too responded significantly to the increased levels of N during both the years. The recommended dose of N resulted in highest ear length which was significantly better than all the lower N levels. The increase in ear length and statistically similar 1,000-grain weight with inoculation of *Azotobacter* and increase in levels of N was also reported by Kachroo and Razdan (2006). The lower under suboptimal levels of available nitrogen (50% and 75% of recommended) resulted in poor plant growth and significantly lower yield attributes than the recommended dose of N. Moreover, 1,000-grain weight was least influenced by the inoculations and level of nitrogen.

Grain yield and straw yield

The seed or soil inoculation with *Azotobacter* resulted in significantly higher grain and straw yield than no inoculation. Seed inoculation with *Azotobacter* enhanced the grain yield by 8.1% over un-inoculated control. The soil inoculation with *Azotobacter* also enhanced grain yield by 6.5% over the control. Seed inoculation with *Azotobacter* obtained highest grain and straw yields but it was statistically similar to soil inoculation. The statistically similar grain yield with seed or soil inoculation with *Azotobacter* was also reported by Khandare *et al*. (2015). Gupta and Aggarwal (2008) reported increase in grain yields with

Azotobacter inoculation than the un-inoculated control. Zaidi and Khan (2005) and Rathi *et al*. (2014) also reported that inoculation resulted in higher straw yield with *Azotobacter* over than uninoculated control.

The nitrogen levels also significantly influenced the grain and straw yields of wheat. The highest grain (5.63 t/ha) and straw yields (7.37 t/ha) were obtained with recommended dose of N which was significantly better than all the lower N levels and the control. It was also observed that 75% recommended dose of N resulted in significantly better grain yield than 50% of recommended N, which in turn resulted in significantly better grain yield than the control (Table 1). The higher nutrient availability, improved growth and yield-attributing characters resulted in higher grain yield with recommended dose of N. These results confirm the findings of Negi *et al*. (2013).

Nitrogen, phosphorus and potassium content and uptake

Nitrogen content in plant at 60 and 90 days after sowing (DAS) was significantly improved when seed inoculation was done with *Azotobacter* over no inoculation (Table 2). Soil inoculation with *Azotobacter* too improved the N content over un-inoculated control at 60 and 90 DAS. Increasing the level of N from 0% to 100% recommended dose increased the N content significantly at each level during all the growth stages (60 and 90 DAS) in a 2-year study (Table 2).

Table 1. Yield-attributing characters and yield of wheat as influenced by inoculation with *Azotobacter* and varied levels of nitrogen (pooled data of 2 years)

Treatment	Effective tillers (m^2)	1,000-grain weight (g)	Grains/ear	Ear length (cm)	Grain yield (t/ha)	Straw yield (t/ha)
Inoculations						
No inoculation	277.4	40.3	38.4	11.1	4.44	5.88
Seed inoculation with *Azotobacter*	293.2	40.5	40.7	12.3	4.80	6.35
Soil inoculation with *Azotobacter*	286.9	40.4	40.1	12.1	4.73	6.23
SEm±	2.2	0.6	0.3	0.1	0.05	0.08
CD (P=0.05)	6.9	NS	1.0	0.5	0.16	0.21
Levels of N						
No N (control)	233.6	40.1	36.0	10.8	3.39	4.83
50% Rec. N	279.9	40.2	39.3	11.5	4.46	5.78
75% Rec. N	303.2	40.6	41.3	12.1	5.16	6.63
Rec. N (125 kg/ha)	326.8	40.8	42.4	12.8	5.63	7.37
SEm±	4.5	0.5	0.5	0.2	0.07	0.08
CD (P=0.05)	13.0	NS	1.5	0.6	0.20	0.23

Rec., Recommended

Table 2. Nitrogen content and its uptake by wheat as influenced by inoculation with *Azotobacter* and nitrogen levels (pooled data of 2 years)

Treatment	N content (%)		N uptake (kg/ha)	
	60 DAS	90 DAS	60 DAS	90 DAS
Inoculations				
No inoculation	2.74	1.26	71.1	87.8
Seed inoculation with *Azotobacter*	2.95	1.37	85.5	101.2
Soil inoculation with *Azotobacter*	2.90	1.34	81.8	97.3
SEm±	0.03	0.01	1.4	1.4
CD (P=0.05)	0.09	0.04	4.4	4.5
Levels of N				
No N (control)	2.46	1.08	54.2	67.4
50% Rec. N	2.74	1.24	67.8	85.3
75% Rec. N	2.99	1.42	88.9	104.1
rec. N (125 kg/ha)	3.26	1.55	107.0	124.8
SEm±	0.03	0.02	2.0	1.6
CD (P=0.05)	0.09	0.05	5.6	4.6

Rec., Recommended

The N uptake by wheat at 60 and 90 DAS was also influenced significantly with the *Azotobacter* inoculation and N levels. The seed or soil inoculation with *Azotobacter* being statistically similar to each other, showed significantly higher N uptake by wheat at 60 and 90 DAS than un-inoculated control during both the years (Table 2). Seed and soil inoculation with *Azotobacter* significantly improved the N and K uptake by wheat grain and straw over the un-inoculated control (Table 3). The seed inoculation with *Azotobacter* significantly improved the P uptake in wheat grain. However, the different inoculation methods resulted in statistically similar P uptake by wheat straw. An increase in N level from 0 to 100% recommended dose significantly enhanced the N uptake by wheat at 60 and 90 DAS as well as in grain and straw. Similar trend was observed for the P and K uptake by wheat grain and straw where 100% recommended N resulted in significantly higher P and K uptake than the lower levels of N and the control.

The improved growth and higher dry-matter production with recommended N resulted in higher N, P and K uptake by wheat grain and straw. Khandare *et al*. (2015) also reported increased nutrient uptake with *Azotobacter* inoculation and increased N levels.

Soil analysis

Available N, P and K contents after the wheat harvest remained unaffected with inoculation methods of *Azotobacter* (Fig. 1). Inoculation with *Azotobacter* resulted in higher but statistically similar N, P and K availability than the no-inoculation.

Application of full recommended nitrogen recorded significantly higher available soil N than 50% of recommended dose and the control, but it was statistically similar to 75% of recommended N. The full recommended N optimized the availability of N to plant throughout the growing season and it also balanced the N status of soil after the harvesting of crop during both the years. The available P and K contents in the soil were less influenced by the application of different N doses. Kachroo and Razdan (2006) also reported the similar results.

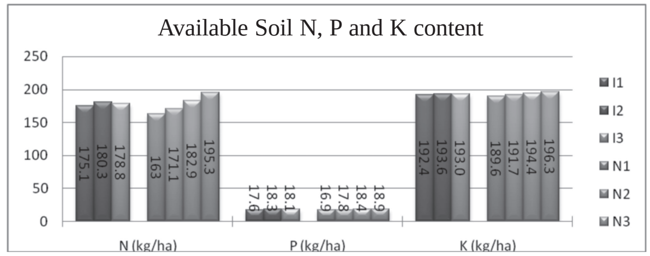

Fig. 1. Effect of *Azotobacter* inoculation and nitrogen levels on available soil N, P and K content after the crop harvesting

Soil microbial count

The *Azotobacter* count (CFU/g soil) at different growth stages of wheat was significantly improved with the seed and soil inoculation of *Azotobacter* as compared to un-inoculated control in both the years (Fig. 2). The seed inoculation with *Azotobacter* recorded the highest CFU throughout the crop-growth period than soil inoculation and no-inoculation treatment.

The levels of nitrogen also influenced the CFU of *Azotobacter* (Fig. 3). The *Azotobacter* count increased with the increase in nitrogen dose. An increase in N dose from zero to 100% recommended significantly increased the CFU of *Azotobacter*. The highest CFU was always recorded with recommended nitrogen at all the growth stages of wheat in both the years. Zaidi and Khan (2005)

Table 3. Effect of *Azotobacter* inoculation and nitrogen levels on nitrogen, phosphorus and potassium uptake (kg/ha) by wheat grain and straw (pooled data of 2 years)

Treatment	Grain			Straw		
	N	P	K	N	P	K
Inoculations						
No inoculation	62.6	9.5	14.2	27.5	1.8	51.5
Seed inoculation with *Azotobacter*	72.4	11.2	16.3	32.7	2.0	58.3
Soil inoculation with *Azotobacter*	69.9	10.5	16.0	31.0	1.9	56.6
SEm±	1.1	0.3	0.2	0.5	0.1	0.7
CD (P=0.05)	3.6	1.1	0.9	1.6	NS	2.6
Levels of N						
No N (control)	46.5	6.6	9.9	21.2	1.2	40.5
50% Rec. N	63.4	9.7	14.5	27.6	1.7	50.0
75% Rec. N	77.1	11.9	17.3	33.4	2.1	61.0
Rec. N (125 kg/ha)	86.2	13.5	20.4	39.4	2.6	70.4
SEm±	1.4	0.4	0.3	0.7	0.1	1.1
CD (P=0.05)	3.9	0.9	1.0	2.1	0.2	3.0

Rec., Recommended

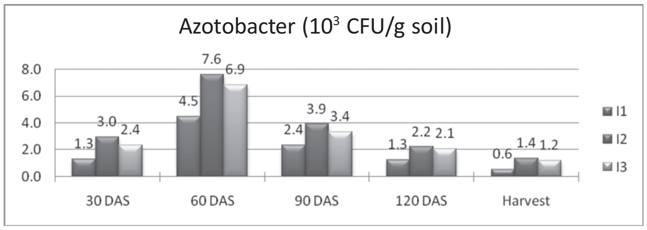

Fig. 2. Soil microbial count as influenced by *Azotobacter* inoculation (I1, No inoculation; I2, seed inoculation with *Azotobacter;* I3, soil inoculation with *Azotobacter*)

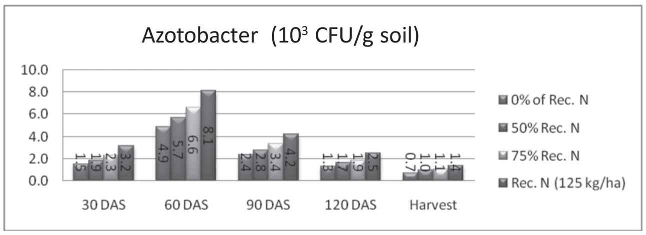

Fig. 3. Soil microbial count as influenced by nitrogen levels

also reported improved soil microbial count with the *Azotobacter* inoculation.

It was concluded that the seed inoculation with *Azotobacter* and recommended N application resulted in higher yield and nutrient uptake by wheat than un-inoculated control. The *Azotobacter* inoculation (either seed or soil) also significantly improved soil microbial count than no inoculation; however, the available N, P and K content after crop harvesting was not significantly influenced with the inoculation of *Azotobacter*.

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