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Assessing the Impact of Elite Indigenous *Azospirillum* Strains on Growth of Wheat (*Triticum aestivum* L.) Across Varied Nitrogen Regimes

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Abstract

The growth and productivity of wheat is mainly dependent on the availability of major essential plant nutrients. Of the major essential nutrients, nitrogen occupies a prime place and plays an important role in plant metabolism ultimately reflecting on crop production. In view of increasing cost and scarcity of nitrogenous fertilizers, exploring alternative approaches to supply crucial essential nutrients has become imperative. The use of plant growth-promoting rhizobacteria (PGPR), capable of performing biological nitorgen fixation and growth hormone production enhances growth and improves quality. Among PGPR members, genus *Azospirillum* are well known and commonly used bacteria in agriculture. A total of four native selected *Azospirillum* isolates *viz.,* UASDAZO-13, UASDAZO-21, UASDAZO-29 and UASDAZO-46 along with reference strain (ACD-15) were inoculated and evaluated on wheat growth at different nitrogen levels. Inoculation of efficient *Azospirillum* isolates along with a reference strain on wheat significantly increased seed germination, plant height, number of tillers and root parameters like root diameter, volume and surface area over uninoculated control (UIC). It was found that superior growth was observed in UASDAZO-29 inoculated with 75 per cent N application as per RDF.

Keywords : Wheat, *Azospirillum*, PGPR, Nitrogen levels

Wheat (*Triticum aestivum* L.) is one of the principal cereal crops grown worldwide and one of the important staple food of nearly 2.5 billion of world population. Among the major cereals grown in India, wheat stands second next to rice in area and production and stands first in productivity. In India, farmers annually apply considerable amount of nitrogen (N) fertilizers to ensure a higher crop yield. However, inorganic nitrogen fertilizer is a non-renewable source and its overuse is detrimental to the air, surface and groundwater environment and consequently, human health. Therefore, alternate methods are essential to reduce the need for inorganic nitrogen fertilizer while still meeting the nitrogen requirements of crops. The advancement of research involving microbiology in agriculture has intensified the use of plant growth-promoting bacteria (PGPB), capable of performing biological fixation of the atmospheric nitrogen (N), reducing the necessity of N fertilization in cereals and leguminous crops. Among PGPR members, genus *Azospirillum* are well known and commonly used bacteria in agriculture. *Azospirillum* is a diazotrophic bacteria that play a valuable role in the rhizosphere of many plants, fixes atmospheric nitrogen and converts it into plant-available form (Rehman *et al*., 2017). Nitrogen fixation, plant growth-promoting hormones production and consequently improving the water and nutrients uptake, increasing the insoluble-phosphates solubility, siderophores and vitamins production, synergistic relationship with other useful soil bacteria, nitrite production *etc.,* are some of the beneficial characteristics of this bacterium that ultimately enhances the efficiency and yield of crops (Zarea, 2017).

Inoculation of wheat seeds with *Azospirillum* spp. has been widely applied to boost crop growth and yields on different soils (Ahemad and Kibret, 2014). Also, with respect to method of application, both foliar and in-furrow bacterial applications were as effective as standard seed inoculation in providing wheat with nitrogen (Fukami *et al*., 2018). Thus, the use of *Azospirillum* spp. can help to bridge the gap between productivity and sustainability since inoculations based on this microorganism can improve plant growth and also reduce the use of nitrogen fertilizers which generate savings and greater profitability.

Material and Methods

A total of four native efficient *Azospirillum* isolates (UASDAZO-13, UASDAZO-21, UASDAZO-29 and UASDAZO-46) along with reference strain were inoculated and evaluated on wheat growth at different nitrogen levels. *Azospirillum* strain *i.e.,* ACD-15 (reference strain) collected from the Institute of Organic Farming, UAS, Dharwad, India. Efficient native *Azospirillum* isolates were isolated from different regions of Dharwad district following enrichment culture technique adopted by Dobereiner and Day (1976) in N-free malate semi-solid medium.

To study the effect of selected efficient native *Azospirillum* isolates on plant growth of wheat and its evaluation at different nitrogen levels, pot trials were conducted under greenhouse condition at the Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad.

For greenhouse studies, wheat (Variety: C-306) was cultivated in black soil using a Factorial Randomized Complete Block Design. The experiment involved 24 treatments with 3 replications. Seeds were bio-primed (Prasad *et al.,* 2020) and the nutrient application rate was set at 120 kg N, 60 kg P_2O_5 and 60 kg K_2O per hectare. The experiment consisted of inoculation of UASDAZO-13 (I_1), UASDAZO-21 (I_2), UASDAZO-29 (I_3), UASDAZO-46 (I_4) along with a reference strain, ACD-15 (I_5) and uninoculated control (I_6) treatments at four nitrogen levels *viz.,* 100% (N_1), 75% (N_2), 50% (N_3) and 0% (N_4). The observations related to this experiment like plant height, number of tillers per plant, root parameters and plant dry biomass were recorded at 60 DAS, respectively. Straw and grain yield of wheat was recorded at harvest. For root parameters, whea troots collected from pot with different treatments were washed with distilled water. After washing, roots were scanned under WinRHIZO scanner (Regents Instruments, Quebec, Canada) at 60 DAS. Nitrogen content of shoot and root was estimated by modified micro kjeldhal method as given by Jackson (1967).

The *Azospirillum* inoculation efficiency (AIE) value was computed using the following formula :

$$\text{AIE (\%)} = \frac{\text{(Total biomass of inoculated plant) - (Total biomass of uninoculated plant)}}{\text{Total biomass of inoculated plant}} \times 100$$

Nitrogen uptake enhancement (NUEN) due to inoculation of *Azospirillum* was enumerated using the formula :

$$\text{NUEN (\%)} = \frac{\text{(Total N content of inoculated plant) - (Total N content of uninoculated plant)}}{\text{Total N content of inoculated plant}} \times 100$$

Statistical Analysis

The statistical analysis was done by using WASP :2.0 (Web Agri Stat Package 2) statistical tool and means were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

Effect of Efficient Indigenous *Azospirillum* Strains on Height and Tillers of Wheat under different Nitrogen Levels

The plant height and number of tillers per plant were recorded at 60 DAS and tabulated in Table 1. Among the different doses of N fertilization, inoculation of all the isolates and reference strain produced maximum height of plant at 75% N. Among the

TABLE 1
Influence of selected native *Azospirillum* isolates on plant height and number of tillers at different nitrogen levels in wheat

Treatments	Plant height (cm)					Number of tillers/plant				
	60 DAS					60 DAS				
Azospirillum isolates inoculation (I)	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I
UASDAZO-13 (I_1)	67.29 $^{c-f}$	69.88 $^{a-d}$	66.2 $^{e-g}$	62.5 ^{h}	66.47	4.11 ^{ab}	4.22 ^{ab}	3.88 $^{a-c}$	3.22 $^{b-d}$	3.86
UASDAZO-21 (I_2)	66.37 $^{e-g}$	71.49 ^{ab}	68.33 $^{b-e}$	61.71 ^{h}	66.97	4.22 ^{ab}	4.33 ^{ab}	4 $^{a-c}$	3.55 $^{a-d}$	4.03
UASDAZO-29 (I_3)	67.91 $^{c-e}$	72.82 ^{a}	71.29 ^{ab}	61.83 ^{h}	68.46	4.22 ^{ab}	4.44 ^{a}	4.11 ^{ab}	3.56 $^{a-d}$	4.08
UASDAZO-46 (I_4)	66.62 $^{d-f}$	69.14 $^{b-e}$	63.13 ^{gh}	61.54 ^{h}	65.11	4 $^{a-c}$	4.11 ^{ab}	3.78 $^{a-c}$	3.33 $^{a-d}$	3.80
ACD-15 (I_5)	70.49 $^{a-c}$	71.55 ^{ab}	68.5 $^{b-e}$	63.25 ^{gh}	68.21	4.11 ^{ab}	4.22 ^{ab}	4.11 ^{ab}	3.55 $^{a-d}$	4.00
UIC (I_6)	65.94 $^{e-g}$	64.41 $^{f-h}$	61.32 ^{h}	55.92 ^{i}	61.90	3.77 $^{a-c}$	3.44 $^{a-d}$	2.89 ^{cd}	2.44 ^{d}	3.14
Mean of N	67.44	69.88	66.46	61.13		4.07	4.13	3.79	3.28	

	S. Em (±)	C.D. (P = 0.01)	S. Em (±)	C.D. (P = 0.01)
Inoculation (I)	0.515	1.952	0.097	0.369
Nitrogen level (N)	0.420	1.594	0.079	0.301
Interaction (I × N)	1.029	3.905	0.195	0.738

Note : Means followed by same letters did not differ significantly. S. Em;Applicable to Duncan's Multiple Range Test
· Nitrogen level 1 (N_1) - 100% N as per RDF
· Nitrogen level 2 (N_2) - 75% N as per RDF
· Nitrogen level 3 (N_3) - 50% N as per RDF
· Nitrogen level 4 (N_4) - 0% N as per RDF

different treatments, inoculation of *Azospirillum* isolates at 75% N and 50% N *i.e.,* I_3N_2 (72.82cm), I_5N_2 (71.55 cm), I_2N_2 (71.49 cm) and I_3N_3 (71.29 cm) produced significantly higher plant height which was significantly more than height of plant at 100% N alone *i.e.,* I_6N_1 (65.94 cm). Similarly, inoculation of *Azospirillum* isolates at 75% N *i.e.,* I_3N_2 (4.44) produced more number of tillers which was significantly more than that of number of tillers per plant at 100% N alone (3.77). In the absence of N fertilization (0% N), all the inoculants significantly produced more number of tillers per plant which was more than that of uninoculated control (Plate 1 and Plate 2).

Plate 1 : Influence of efficient *Azospirillum* isolate UASDAZO-29 (I_3) on growth of wheat under different nitrogen levels at 60 DAS

Plate 2 : Influence of *Azospirillum* isolate ACD-15 (reference; I_5) on growth of wheat under different nitrogen levels at 60 DAS

Early growth enhancement of *Azospirillum*-inoculated wheat was also observed in field-grown wheat (Karimi *et al.,* 2018). The number of tillers per m² in forages was positively influenced by N rates and by inoculation of *Azospirillum*. The increases obtained are attributed to the inclusion of additional nitrogen from both mineral source and biological fixation by bacteria through BNF, since the availability of N to the plant is fundamental for the survival and appearance of new tillers. (Benin *et al.*, 2012).

Effect of Efficient Indigenous *Azospirillum* Strains on Root Parameters of Wheat under different Nitrogen Levels

The root parameters *viz.,* average diameter, surface area of root and root volume was recorded by root analysis at 60 DAS and presented in Table 2. Among the different doses of N fertilization, inoculation of all the isolates and reference strain showed higher average root diameter, root surface area and root volume at 75% N except at uninoculated treatment where 100% N showed higher average root diameter. Among the different treatments, inoculation of *Azospirillum* isolates at 75% N *i.e.,* I_3N_2 (0.52 mm), I_5N_2 (0.52 mm), showed higher average root diameter which was more than average root diameter of all other treatments. Inoculation of *Azospirillum* isolates at 75% N *i.e.,* I_3N_2 (201.1 cm^2), I_2N_2 (198.9 cm^2), I_5N_2 (195.6 cm^2) and I_4N_2 (193.2 cm^2) showed higher root surface area which was more than root surface area of all other treatments. Similarly, inoculation of *Azospirillum* isolates at 75% N *i.e.,* I_3N_2 (2.10 cm^3), I_2N_2 (2.06 cm^3), I_5N_2 (2.02 cm^3) showed higher root volume which was more than root volume of all other treatments.

Plants being inoculated with *Azospirillum* are characterized by changes in root growth and morphology, such as enhancement of root elongation, root dry weight, promotion of root hair growth and root branching (El Sayed *et al.,* 2015), therefore occupying an enhanced soil volume. Dobbelaere *et al.* (2002) assessed the inoculation effect of *Azospirillum brasilense* on growth of spring wheat. They observed that inoculated plants resulted in better germination, early development and flowering and increased dry weight of both the root system and the upper plant parts as well as the N-uptake efficiency of plants.

Effect of Efficient Indigenous *Azospirillum* Strains on Plant Dry Biomass of Wheat under different Nitrogen Levels

The plant dry biomass *viz.,* shoot and root dry biomass was recorded at 60 DAS. The data pertaining to plant dry biomass are tabulated in Table 3.

Among the different doses of N fertilization, inoculation of all the isolates and reference strain showed higher plant dry biomass at 75% N except at uninoculated treatment where 100% N showed higher plant dry biomass. Among the different isolates and reference strain, inoculation of *Azospirillum* isolate

TABLE 2

Influence of selected native *Azospirillum* isolates on root diameter, root volume and root surface area of wheat at different nitrogen levels

Treatments	Root parameters at 60 DAS														
Azospirillum isolates inoculation (I)	Average Diameter (mm)					Surface Area (cm^2)					Root Volume (cm^3)				
	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I
UASDAZO -13 (I_1)	0.45 ^{fg}	0.47 $^{d-f}$	0.46 $^{e-g}$	0.41 ^{ij}	0.44	173.3 $^{h-k}$	187.2 $^{c-f}$	175.5 $^{g-j}$	158.6 ^{lm}	173.6	1.92 ^{cd}	1.96 $^{b-d}$	1.93 $^{b-d}$	1.68 ^{h}	1.87
UASDAZO -21 (I_2)	0.49 $^{b-d}$	0.51 ^{ab}	0.51 ^{ab}	0.46 $^{e-g}$	0.49	181.9 $^{e-h}$	198.9 ^{ab}	184 $^{d-g}$	160.4 ^{lm}	181.3	1.86 $^{d-f}$	2.06 ^{ab}	1.97 $^{b-d}$	1.74 $^{f-h}$	1.91
UASDAZO -29 (I_3)	0.5 $^{a-c}$	0.52 ^{a}	0.51 ^{ab}	0.46 $^{e-g}$	0.50	184.5 $^{d-g}$	201.1 ^{a}	190.2 $^{b-e}$	167.4 $^{j-l}$	185.8	1.93 $^{b-d}$	2.10 ^{a}	1.98 $^{a-d}$	1.75 $^{f-h}$	1.94
UASDAZO -46 (I_4)	0.48 $^{c-e}$	0.49 $^{b-d}$	0.47 $^{d-f}$	0.44 ^{gh}	0.47	178.9 $^{f-i}$	193.2 $^{a-d}$	184.7 $^{d-g}$	159.8 ^{lm}	179.2	1.85 $^{d-g}$	1.93 $^{b-d}$	1.88 ^{de}	1.67 ^{h}	1.83
ACD-15 (I_5)	0.50 $^{a-c}$	0.52 ^{a}	0.51 ^{ab}	0.45 ^{fg}	0.49	183.4 $^{d-g}$	195.6 $^{a-c}$	189.5 $^{b-e}$	164.1 ^{kl}	183.1	1.9 $^{c-e}$	2.02 $^{a-c}$	1.96 $^{b-d}$	1.73 ^{gh}	1.90
UIC (I_6)	0.45 ^{fg}	0.42 ^{hi}	0.39 ^{jk}	0.38 ^{k}	0.41	170.3 $^{i-k}$	152.7 ^{m}	127.2 ^{n}	114 ^{o}	141.1	1.78 $^{e-h}$	1.49 ^{i}	1.24 ^{j}	1.01 ^{k}	1.38
Mean of N	0.48	0.49	0.47	0.43		178.7	188.1	175.2	154.1		1.87	1.93	1.83	1.60	

	Average Diameter S. Em (±)**	Average Diameter C.D. (P = 0.01)	Surface Area S. Em (±)	Surface Area C.D. (P = 0.01)	Root Volume S. Em (±)	Root Volume C.D. (P = 0.01)
Inoculation (I)	0.004	0.014	1.584	6.007	0.020	0.075
Nitrogen level (N)	0.003	0.011	1.293	4.905	0.016	0.061
Interaction (I × N)	0.007	0.028	3.167	12.015	0.039	0.149

Note : Means followed by same letters did not differ significantly. S. Em; Applicable to Duncan's Multiple Range Test

- Nitrogen level 1 (N_1) - 100% N as per RDF
- Nitrogen level 2 (N_2) - 75% N as per RDF
- Nitrogen level 3 (N_3) - 50% N as per RDF
- Nitrogen level 4 (N_4) - 0% N as per RDF

TABLE 3

Total dry biomass of wheat as influenced by efficient native *Azospirillum* isolates at different nitrogen levels

Treatments	Plant dry biomass (g plant^{-1}) at 60 DAS														
	Shoot dry biomass					Root dry biomass					Total dry biomass				
Azospirillum isolates inoculation (I)	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I
UASDAZO -13 (I_1)	2.04 ^{i}	2.1 $^{g-i}$	2.05 ^{hi}	1.75 ^{j}	1.98	0.43 $^{h-k}$	0.44 $^{g-i}$	0.43 $^{h-j}$	0.39 ^{m}	0.42	2.47 ^{ij}	2.54 $^{g-i}$	2.47 ^{ij}	2.14 ^{k}	2.40
UASDAZO -21 (I_2)	2.08 ^{hi}	2.39 ^{e}	2.33 ^{e}	2.00 ^{i}	2.20	0.43 $^{h-k}$	0.45 ^{gh}	0.44 $^{g-i}$	0.41 $^{j-l}$	0.43	2.5 $^{h-j}$	2.84 ^{d}	2.77 ^{de}	2.42 ^{j}	2.63
UASDAZO -29 (I_3)	2.58 ^{cd}	2.85 ^{a}	2.67 ^{bc}	2.09 ^{hi}	2.55	0.52 ^{d}	0.60 ^{a}	0.57 ^{b}	0.46 ^{g}	0.54	3.10 ^{c}	3.45 ^{a}	3.24 ^{b}	2.55 $^{g-i}$	3.08
UASDAZO -46 (I_4)	2.15 $^{f-h}$	2.22 ^{f}	2.19 ^{fg}	2.02 ^{i}	2.14	0.44 $^{g-i}$	0.46 ^{g}	0.45 ^{gh}	0.43 $^{h-k}$	0.44	2.59 $^{f-h}$	2.68 ^{ef}	2.63 ^{fg}	2.44 ^{ij}	2.59
ACD-15 (I_5)	2.59 ^{cd}	2.74 ^{b}	2.54 ^{d}	2.05 ^{hi}	2.48	0.48 ^{f}	0.54 ^{c}	0.5 ^{e}	0.44 $^{g-i}$	0.49	3.07 ^{c}	3.29 ^{b}	3.05 ^{c}	2.49 $^{h-j}$	2.97
UIC (I_6)	2.03 ^{i}	1.78 ^{j}	1.69 ^{jk}	1.63 ^{k}	1.78	0.42 $^{i-l}$	0.37 ^{n}	0.36 ^{n}	0.32 ^{o}	0.37	2.45 ^{ij}	2.15 ^{k}	2.06 ^{kl}	1.95 ^{l}	2.15
Mean of N	2.24	2.35	2.24	1.92		0.45	0.48	0.46	0.41		2.70	2.82	2.70	2.33	

	S. Em (±)**	C.D. (P = 0.01)	S. Em (±)	C.D. (P = 0.01)	S. Em (±)	C.D. (P = 0.01)
Inoculation (I)	0.016	0.059	0.003	0.012	0.018	0.068
Nitrogen level (N)	0.013	0.049	0.003	0.010	0.015	0.055
Interaction (I x N)	0.031	0.119	0.006	0.024	0.036	0.135

Note : Means followed by same letters did not differ significantly. S. Em; Applicable to Duncan's Multiple Range Test

· Nitrogen level 1 (N_1) - 100% N as per RDF
· Nitrogen level 2 (N_2) - 75% N as per RDF
· Nitrogen level 3 (N_3) - 50% N as per RDF
. Nitrogen level 4 (N_4) - 0% N as per RDF

at 75% N *i.e.,* I_3N_2 (2.85g) showed significantly higher shoot dry biomass which was more than shoot dry biomass of all other treatments. Similarly, inoculation of *Azospirillum* isolate at 75% N *i.e.,* I_3N_2 (0.60g) showed significantly higher root dry biomass which was more than root dry biomass of all other treatments. In the absence of N fertilization, all the inoculated isolates *i.e.,* I_3N_4 (2.55g), I_5N_4 (2.49g), I_4N_4 (2.44g), I_2N_4 (2.42g) and I_1N_4 (2.14g) recorded significantly higher total dry biomass which was more than uninoculated control (1.95g) and also recorded higher total dry biomass than 50% N alone.

Inoculation with *Azospirillum brasilense* enhanced fresh weight and dry weigh by 60 and 54 per cent respectively, in inoculated seedlings of wheat after 90h treatment. The use of nitrogen fertilizers in combination with *Azospirillum* produced significantly higher green and dry matter yields than those from inoculated or fertilized alone (Dayamani *et al.,* 2011).

Increased root, shoot and total dry matter due to the inoculation of *Azospirillum* isolates could be attributed to biological nitrogen fixation and production of plant growth promoting substances (Mohamed *et al.,* 2018).

Effect of Efficient Indigenous *Azospirillum* Strains on Nitrogen Concentration of Wheat under different Nitrogen Levels

Nitrogen (N) concentration *viz.,* shoot N concentration and root N concentration was recorded at 60 DAS. The data related to nitrogen concentration are tabulated in Table 4.

Among the different doses of N fertilization, inoculation of all the isolates and reference strain showed higher shoot and root N concentration at 75% N except at uninoculated treatment where 100% N showed higher shoot and root N concentration. Inoculation of *Azospirillum* isolates I_3N_1 (1.71%),

TABLE 4

Effect of inoculation of efficient native *Azospirillum* isolates on shoot and root N concentration in wheat

Treatments	N concentration (%) at 60 DAS									
Azospirillum isolates inoculation (I)	Shoot N %					Root N %				
	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I
UASDAZO-13 (I_1)	1.3 $^{f-i}$	1.34 $^{e-g}$	1.27 $^{f-i}$	1.16 ^{j}	1.27	0.5 ^{hi}	0.52 ^{gh}	0.49 ^{i}	0.46 ^{j}	0.49
UASDAZO-21 (I_2)	1.37 ^{e}	1.67 ^{b}	1.58 ^{c}	1.33 $^{e-h}$	1.49	0.58 ^{ef}	0.64 ^{c}	0.6 ^{de}	0.51 $^{g-i}$	0.58
UASDAZO-29 (I_3)	1.71 ^{ab}	1.75 ^{a}	1.67 ^{b}	1.39 ^{de}	1.63	0.67 ^{b}	0.71 ^{a}	0.69 ^{ab}	0.62 ^{cd}	0.67
UASDAZO-46 (I_4)	1.34 ^{ef}	1.44 ^{d}	1.39 ^{de}	1.27 $^{f-i}$	1.36	0.53 ^{g}	0.57 ^{f}	0.58 ^{ef}	0.5 ^{hi}	0.55
ACD-15 (I_5)	1.54 ^{c}	1.71 ^{ab}	1.68 ^{b}	1.34 $^{e-g}$	1.57	0.59 ^{ef}	0.64 ^{c}	0.63 ^{c}	0.57 ^{f}	0.61
UIC (I_6)	1.25 ^{i}	1.09 ^{k}	1.06 ^{kl}	0.99 ^{l}	1.10	0.46 ^{j}	0.42 ^{k}	0.41 ^{kl}	0.39 ^{l}	0.42
Mean of N	1.42	1.50	1.44	1.25		0.56	0.58	0.57	0.51	

	Shoot S. Em (±)	Shoot C.D. (P = 0.01)	Root S. Em (±)	Root C.D. (P = 0.01)
Inoculation (I)	0.011	0.041	0.004	0.014
Nitrogen level (N)	0.009	0.034	0.003	0.012
Interaction (I x N)	0.022	0.082	0.008	0.028

Note : Means followed by same letters did not differ significantly. S. Em; Applicable to Duncan's Multiple Range Test

- Nitrogen level 1 (N_1) - 100% N as per RDF
- Nitrogen level 2 (N_2) - 75% N as per RDF
- Nitrogen level 3 (N_3) - 50% N as per RDF
- Nitrogen level 4 (N_4) - 0% N as per RDF

I_3N_2 (1.75%), I_5N_2 (1.71%) showed significantly higher shoot N concentration and inoculation of *Azospirillum* isolate at 75% N *i.e.,* I_3N_2 (0.71%) and at 50% N *i.e.,* I_3N_3 (0.69%) showed significantly higher root N concentration which was more than shoot and root N concentration of all other treatments. In the absence of N fertilization, all the inoculated isolates *i.e.,* I_3N_4 (0.62%), I_5N_4 (0.57%), I_2N_4 (0.51%), I_4N_4 (0.5%) and I_1N_4 (0.46%) recorded significantly higher root N concentration which was more than uninoculated control (0.39%) and also recorded higher root N concentration than 75% N alone *i.e.,* I_6N_2 (0.42%).

The increased per cent N content in wheat in the present investigation due to inoculation of *Azospirillum* may be attributed to increased population of free living N_2-fixer, which has then probably increased the uptake of nutrients by means of altering the root surface characters involved in nutrient uptake these findings are in line with the results of Subba rao (1993) who observed increased percent 'N' in crops due to inoculation with *Azospirillum*.

Effect of Efficient Indigenous *Azospirillum* Strains on inoculation Efficiency and Nitrogen Uptake Enhancement of Wheat under different Nitrogen Levels

The efficiency of *Azospirillum* inoculation and enhancement in nitrogen uptake due to inoculation of isolates at different doses of N fertilization was examined and results are tabulated in Table 5.

Among the different doses of N fertilization, inoculation of isolates and reference strain showed higher *Azospirillum* inoculation efficiency at 75% N except in UASDAZO-13 inoculation where 50% N with UASDAZO-13 inoculation showed higher *Azospirillum* inoculation efficiency. Among the different isolates and reference strain, inoculation of UASDAZO-29 at 75% N *i.e.,* I_3N_2 (37.67%) and at 50% N *i.e.,* I_3N_3 (36.46%) showed significantly higher *Azospirillum* inoculation efficiency compared to other treatments.

Also, inoculation of isolates and reference strain showed higher nitrogen uptake enhancementat 75%

Table 5

***Azospirillum* inoculation efficiency and Nitrogen uptake enhancement by efficient *Azospirillum* isolates at different nitrogen levels**

Treatments	*Azospirillum* inoculation efficiency (%)					Nitrogen uptake enhancement (%)				
Azospirillum isolates inoculation (I)	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I
UASDAZO-13 (I_1)	0.6 m	15.45 i	16.76 i	8.76 j	10.37	5.43 r	18.8 m	16.67 n	14.66 o	13.89
UASDAZO-21 (I_2)	2.14 l	24.19 e	25.69 d	19.19 h	17.91	12.35 p	34.55 e	32.69 f	24.81 j	26.10
UASDAZO-29 (I_3)	20.84 fg	37.67 a	36.46 a	23.52 e	29.60	28.24 h	38.51 a	37.69 b	30.81 g	33.81
UASDAZO-46 (I_4)	5.29 k	19.89 gh	21.91 f	20.02 gh	16.44	8.96 q	24.85 j	25.1 j	22.05 k	20.24
ACD-15 (I_5)	20.14 gh	34.59 b	32.43 c	21.63 f	27.09	19.74 l	35.48 d	36.45 c	27.42 i	29.77
UIC (I_6)	0 m	0 m	0 m	0 m	0.00	0 s	0 s	0 s	0 s	0.00
Mean of N	8.15	21.89	22.29	15.28		12.45	25.36	24.77	19.96	

	S. Em (±)	C.D. (P = 0.01)	S. Em (±)	C.D. (P = 0.01)
Inoculation (I)	0.233	0.884	0.088	0.335
Nitrogen level (N)	0.190	0.721	0.072	0.273
Interaction (I X N)	0.466	1.767	0.176	0.669

Note : Means followed by same letters did not differ significantly. S. Em;Applicable to Duncan's Multiple Range Test
· Nitrogen level 1 (N_1) - 100% N as per RDF
· Nitrogen level 2 (N_2) - 75% N as per RDF
· Nitrogen level 3 (N_3) - 50% N as per RDF
· Nitrogen level 4 (N_4) - 0% N as per RDF

N except in UASDAZO-46 inoculation treatment, where 50% N (I_4N_3) showed higher nitrogen uptake enhancement compared to 75% N level.

The increased efficiency and nitrogen uptake enhancement provided by the inoculation with *Azospirillum* positively influenced wheat growth, reducing the N doses applied in top-dressing and favoring higher yields. Similar results were obtained by Nunes *et al.* (2015), where wheat yields were 7.6 per cent higher with inoculation of A. *brasilense* when compared to control, even in an area with high N availability.

Effect of Efficient Indigenous *Azospirillum* Strains on Wheat Yield under different Nitrogen Levels

The grain and straw yield were recorded at harvest and tabulated in Table 6. Among the different doses of N fertilization, inoculation of all the isolates and reference strain produced maximum grain and straw yield at 75% N. Among the different treatments, inoculation of *Azospirillum* isolates at 75% *i.e.,* I_3N_2 (8.36g plant^{-1}) produced significantly higher grain yield which was significantly more than grain yield at 100% N alone *i.e.,* I_6N_1 (6.06g plant^{-1}). Similarly, inoculation of *Azospirillum* isolates at 75% N *i.e.,* I_3N_2 (20.30 g plant^{-1}) produced more straw yield which was significantly more than that of straw yield at 100% N alone (17.68g plant^{-1}). In the absence of N fertilization (0% N), all the inoculants significantly produced more grain and straw yield which was more than that of uninoculated control.

Inoculation of *Azospirillum* with plants usually results in increase in plant's dry weight, flowering and grain production. Improvement of yield caused by these PGPR could often be attributed to an increase in root development. Developed root system allows plant to uptake water and minerals in a better way. Similar experiments also showed that inoculation of wheat with *Azospirillum* increases the productivity of wheat (Ayyaz *et al.,* 2016).

This investigation clearly showed that the selected efficient native *Azospirillum* isolates were effective

TABLE 6

Influence of selected native *Azospirillum* isolates on wheat yield at different nitrogen levels

Treatments *Azospirillum* isolates inoculation (I)	Grain yield (g plant^{-1})					Straw yield (g plant^{-1})				
	N_1	N_2	N_3	N_4	Mean of I	N_1	N_2	N_3	N_4	Mean of I
UASDAZO-13 (I_1)	6.15 ^{jk}	6.28 ^{ij}	6.09 ^{kl}	5.41 ^{m}	5.98	17.88 $^{h-j}$	17.08 $^{f-h}$	16.89 $^{h-j}$	15.33 ^{k}	16.80
UASDAZO-21 (I_2)	6.45 ^{gh}	6.89 ^{e}	6.63 ^{f}	5.33 ^{jk}	6.33	18.19 ^{ef}	19.38 ^{c}	19.06 ^{d}	15.95 $^{g-i}$	18.15
UASDAZO-29 (I_3)	7.49 ^{c}	8.36 ^{a}	7.96 ^{b}	5.64 ^{e}	7.36	19.75 ^{b}	20.30 ^{a}	19.81 ^{b}	16.14 ^{fg}	19.00
UASDAZO-46 (I_4)	6.32 ^{hi}	6.62 ^{f}	6.58 ^{fg}	5.35 ^{jk}	6.22	18.05 $^{f-h}$	18.37 ^{e}	18.27 ^{ef}	16.76 ^{ij}	17.86
ACD-15 (I_5)	6.83 ^{e}	7.45 ^{c}	7.19 ^{d}	5.51 ^{fg}	6.75	19.26 ^{cd}	19.92 ^{b}	19.72 ^{b}	15.92 $^{g-i}$	18.71
UIC (I_6)	6.06$^{l\ m}$	5.54 ^{n}	5.48 ^{n}	4.78 ^{o}	5.47	17.68 ^{j}	17.14 ^{kl}	17.00 ^{lm}	15.10 ^{m}	16.73
Mean of N	6.55	6.86	6.66	5.34		18.47	18.70	18.46	15.87	

	S. Em (±)	C.D. (P = 0.01)	S. Em (±)	C.D. (P = 0.01)
Inoculation (I)	0.026	0.100	0.126	0.441
Nitrogen level (N)	0.022	0.082	0.099	0.346
Interaction (I × N)	0.053	0.200	0.282	0.987

Note : Means followed by same letters did not differ significantly. S. Em; Applicable to Duncan's Multiple Range Test
· Nitrogen level 1 (N_1) - 100% N as per RDF
· Nitrogen level 2 (N_2) - 75% N as per RDF
· Nitrogen level 3 (N_3) - 50% N as per RDF
· Nitrogen level 4 (N_4) - 0% N as per RDF

in increasing growth and N content in wheat. Among the selected efficient native *Azospirillum* isolates, UASDAZO-29 was found to be efficient for the growth and productivity of wheat at different nitrogen levels. It was found that superior growth and yield was observed in UASDAZO-29 inoculated with 75% N application as per RDF which implies that 25% of N application can be reduced by replacing it with *Azospirillum* inoculation. Therefore, the best alternative is use of *Azospirillum* for inoculation as it could be used to reduce the nitrogen requirement and also growth promotion in wheat.

References

Ahemad, M. and Kibret, M., 2014, Mechanisms and applications of plant growth promoting rhizo bacteria : current perspective. *J. King Saud. Univ. Sci.,* **26** (1) : 1 - 20.

Benin, G., Bornhofen, E., Beche, E., Pagliosa, E. S., Silva, C. L. D. and Pinnow, C., 2012, Agronomic performance of wheat cultivars in response to nitrogen fertilization levels. *Acta Sci. Agron.*, **34** (3) : 275 - 283.

Dayamani, K. J., Savalgi, V. P., Krishnaraj, P. U. and Harinikumar, K. M., 2011, Performance of azide resistant mutants of *Azospirillum* spp. on growth and re-uptake of maize (*Zea mays* L.). *Mysore J. Agric, Sci.,* **45** (2) : 279 - 283.

Dobbelaere, S., Croonenborghs, A., Thys, A., Ptacek, D., Okon, Y. and Vanderleyden, J., 2002, Effect of inoculation with wild type *Azospirillum brasilense* and *A. irakense* strains on development and nitrogen uptake of spring wheat and grain maize. *Biol. Fertil. Soil.,* **36** (4) : 284 - 297.

Dobereiner, J. and Day, J. M., 1976, First international symposium on nitrogen fixation. In: *Proceedings of International Symposium on Nitrogen Fixation*, Ed. Newton, W. E. and Nyman, C. J., Washington State University Press, Pullman, W.A., pp. : 518 - 538.

El Sayed, H., Althubiani, A. and Kabli, S., 2015, Enhancement of plant growth by soil inoculation with *Azospirillum brasilense* HM1 isolated from soil of Saudi Arabia. *Int. J. Curr. Microbiol. Appl. Sci.,* **4** : 238 - 248.

Fukami, J., Cerezini, P. and Hungria, M., 2018, *Azospirillum*: benefits that go far beyond biological nitrogen fixation. *AMB Express*, **8** (1) : 1 - 12.

Jackson, M. L., 1967, Soil chemical analysis, prentice hall of India Private Limited, New Delhi, pp. : 111 - 203.

Karimi, N., Zarea, M. J. and Mehnaz, S., 2018, Endophytic *Azospirillum* for enhancement of growth and yield of wheat. *Environ. Sustainabil.*, **1** (2) : 149 - 158.

Mohamed, M. G., Mohamed, H. M., Gameh, M. A. and El-Rewainy, H. M., 2018, Isolation and characterization of *Azospirillum* isolates from soil and their effect on growth and yield of wheat (*Triticum aestivum* L.) under different levels of nitrogen fertilizer. *Assiut J. Agric. Sci.,* **49** (3) : 107 - 116.

Nunes, P. H. M. P., Aquino, L. A., Santos, L. P. D. D., Xavier, F. O., Dezordi, L. R. and Assunēćo, N. S., 2015, Yield of the irrigated wheat crop subjected to nitrogen application and to inoculation with *Azospirillum brasilense*. *Rev. Bras. Cienc. Solo.*, **39** (1) : 174 - 182.

Prasad, S. R., Rani, K. and Rajatha, K. D., 2020, Seed bio-priming: Plant growth promoting micro organisms in enhancing crop productivity and stress tolerance-A Review. *Mysore J. Agric. Sci.*, **54** (3) : 1 - 18.

Rehman, A. U., Khan, A. Z., Muhammad, A. and Jalal, A., 2017, Performance of nitrogen fixing bacteria with increasing nitrogen ratios on growth, maturity and biomass of winter wheat varieties. *Biosci. Res.,* **14** (2) : 114 - 121.

Subba Rao, N. S., 1993, Nitrogen fixing bacteria associated with plantation and orchard plants. *Can. J. Microbiol.,* **29** : 863 - 866.

Zarea, M. J., 2017. *Azospirillum* and wheat production. *Probiot. Agroecosyst.*, pp. : 329 - 348.