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Performance of nitrogen fixing bacteria with increasing nitrogen ratios on growth, maturity and biomass of winter wheat varieties

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Nitrogen fixing bacteria have the potential role in enhancing plant growth and production. To understand the role of microbes in wheat growth and in reduction of nitrogen doses, a field experiment was conducted at Agronomic research Farm, The University of Agriculture, Peshawar Pakistan. The experiment was designed in randomized complete block design with four replications. Synthetic nitrogen ratios (25%, 50%, 75% and 100%) and nitrogen fixing bacteria (*Azospirillum* and *Azotobacter*) were used on wheat varieties i.e. Shahkar-2013 and Pirsabak-2013. Nitrogen ratios significantly increased the days to maturity (187 days), plant height (97.9 cm), leaf area tiller^{-1} (142.1 cm^2), grains spike^{-1}(62) and biomass yield (10037 kg ha^{-1}) when wheat plots were treated with 100% nitrogen. Similarly, *Azospirillum* applied plots prolonged the maturity (181 days), increased the height of plants (94.3 cm), leaf area tiller^{-1} (130 cm^2), more grains spike^{-1} (53) and maximum biomass yield (9440 kg ha^{-1}). Pirsabak-2013 wheat variety showed maximum days to maturity (178), tallest plants (91.8 cm), higher leaf area tiller^{-1} (124.6 cm^2), more grains spike^{-1} (59.8), and maximum biomass yield (9085 kg ha^{-1}) as compared to Shahkar-2013. It was concluded that application of *Azospirillum* with 100% nitrogen improved the growth, maturity and biomass of Pirsabak-2013 wheat variety. Wheat variety Pirsabak-2013 along with *Azospirillum* and 100% nitrogen is recommended for the improved crop growth and biomass yield.

Keywords: Winter wheat; nitrogen fixing bacteria; *Azosprillium*; *Azotobacter*; nitrogen ratios.

INTRODUCTION

Wheat (*Triticum aestivum L.*) is cultivated worldwide and Pakistan is the 8th biggest wheat producer country. Wheat is also grown as fodder crop for animals feed because of some characteristics such as deliciousness, greater crude protein and digestibility as compared to other fodder crop (Krenzer, 2000). Its straw is used as animal feed and also for manufacturing papers (Iqtidar et al. 2006). Wheat was cultivated in Pakistan on an area of 9.1 million ha^{-1} with production of 25.2 million tones with average yield of 2.82 metric tons ha^{-1}, while in Khyber Pakhtunkhwa it was cultivated on an area of 0.77 million ha^{-1} producing 1.36 million tons with average yield of 1.72 metric tons ha^{-1} (MNFSR, 2014). The production of wheat in Pakistan was lower than major wheat cultivated countries. This decreased in yield is due to use of fertilizer improperly, imbalance fertilizer uses, at planting time less availability of quality seed and highly expenses of chemical fertilizer (Pathak et al. 2006).

Nitrogen play an important role in photosynthesis as it is a part of chlorophyll, protein, nucleic acid and all protoplasm. The leaf area for photosynthesis is roughly proportion to the amount of N supplied. The application of nitrogen at tillering stages resulted highest yield whereas late nitrogen application improved nitrogen recovery (Melaj et al. 2003). The application of nitrogen fertilizer had positive effect on number of tillers m^{-2}, number of grains $spike^{-1}$, thousand grain weight and grain yield of wheat crop (Kandil et al. 2011). Abedi et al. (2010) reported that the maximum wheat grain yield was achieved when the plants were treated with 160 kg N ha^{-1}. Farrer et al. (2006) investigated that application of nitrogen rates and timing had profound effect on quality of grain protein and forages.

Nitrogen fixing bacteria plays a very key role in enhancing soil fertility by fixing atmospheric nitrogen in association with plant roots and produces plant growth substances in the soil (Venkatashwarlu, 2008). The incorporation of bio-fertilizers in soil will reduce the individual use of chemical fertilizers and enhance soil microorganism (Subashini et al. 2007). Kandil et al. (2011) study the effects of nitrogen fixing bacteria (*Azotobacter sp.* and *Azospirillum sp.*) on wheat and founded that inoculated plants produced maximum plant height, grains $spike^{-1}$, 1000- grains weight, biomass yield and grain yield than non-inoculated pants. Application of organic manures particularly bio-fertilizers is the only option to improve the soil organic carbon for nourishment of soil quality and future agricultural productivity (Ramesh, 2008). Some researcher were observed that treatment of seeds with *Azospirillum sp.* and *Azotobacter sp.* in combination with organic fertilizers result in increasing growth and yield of wheat crop (Piccinin et al. 2013). The seed treatment with biofertilizer can improve the number of harvested grains by 6.1% and grain yield 8.0% by 260 kg ha^{-1} in wheat (Zorita and Canigia, 2009). *Azotobacter*, not only provides nitrogen, but also synthesizes growth promoting hormones such as IAA and GA, *Azospirillum* also helps in plant growth and increases the yield of crops by improving root development , mineral uptake etc.

In bio-fertilizers the beneficial microorganisms protect plants from pests and diseases and enhanced the plant growth (El-yazeid et al. 2007). Many researchers reported that nitrogen fixing bacteria significantly improved yield and yield component of wheat crop. The experimental results showed that biofertilizers not only increased yield but also reduced the consumption of chemical fertilizers (Yousefi and Barzegar, 2014). Environmental pollution caused by chemical fertilizers is one of the main problem now a days. Usage of bio-fertilizers, can reduce chemical fertilizers used. Biofertilizer is not important only for the reduction of quality of chemical fertilizers but also for receiving better yield in sustainable agriculture. It is environmental friendly and has been proved to be economical as compared to chemical fertilizers (Hafeez et al. 2002).

Therefore, an experiment was designed to study the effects of nitrogen ratio and nitrogen fixing bacteria on growth, maturity and biomass of wheat.

MATERIALS AND METHODS

A field experiment entitled “performance of nitrogen fixing bacteria with decreasing nitrogen ratios on growth, maturity and biomass of winter wheat varieties” was conducted at Agronomy Research Farm, The University of Agriculture Peshawar during Rabi season 2014-15. The experiment was laid out in randomized block design with four replications. The net plot size of 2.1 x $4m^2$ having 7 rows, 5m long and 30cm apart was used. Different strain of Inoculants of nitrogen fixing bacteria was *Azotobacter* and *Azospirillum*. Different treatments i.e Control (T1), Recommended N (T2), *Azotobacter*-Alone (T3), *Azospirillum*-Alone (T4), *Azotobacter* + N25% (T5), *Azospirillum*+ N25% (T6), *Azotobacter* + N50% (T7), *Azospirillum* + N50% (T8), *Azotobacter* + N75% (T9), *Azospirillum* + N75% (T10), *Azotobacter* + N100% (T11), *Azospirillum* + N100% (T12) were applied for two wheat varieties (Shahkar-2013 and Pirsabak-2013). The quantity of each nitrogen fixing microbes culture was used @ 500g ha^{-1} and was treated with seed before sowing. Recommended dose of NPK @ 120:90:60 kg ha^{-1}. Half of N was applied at the time of sowing whereas the remaining half was applied at tillering stage. A composite soil sample was taken to determine the basic physio-chemical properties before starting experiment (Table. 1). Urea and single super phosphate (SSP) was applied as source of N and P respectively. Plots was irrigated and weeded as and when required. Other agronomic and cultural practice such as irrigation weeding and hoeing etc was done

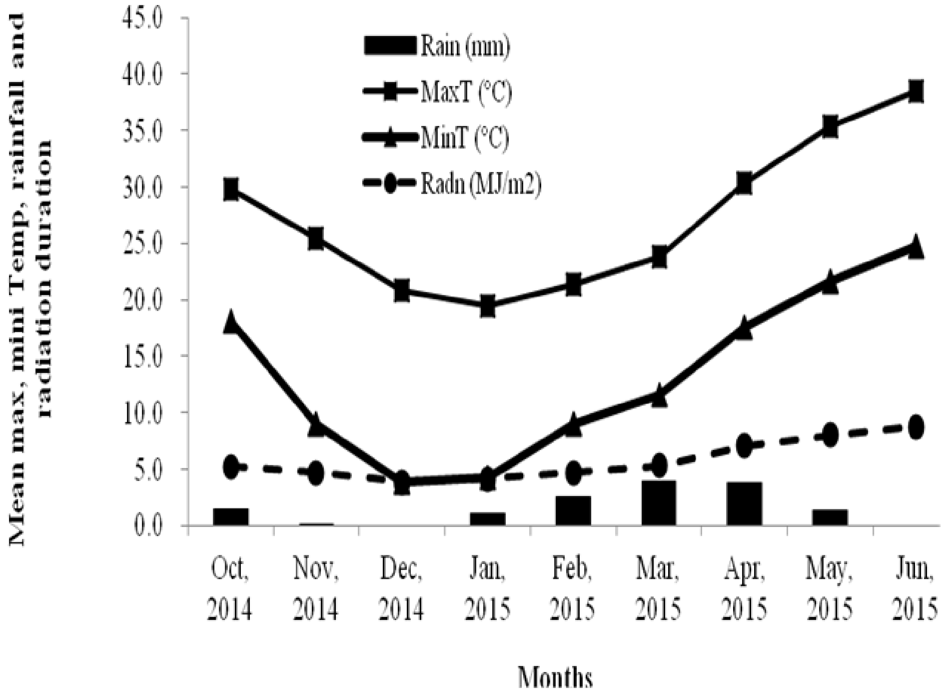

Figure:1 Monthly maximum, minimum temperature (^oC), rain fall (mm) and radiation hours during growing season 2014 and 2015.

Table: 1. Before sowing soil physical and chemical properties.

Parameters	Value
pH	7.54
Sand	11%
Clay	29%
Silt	60%
Soil texture	Silt Clay Loam
EC	0.31dsm^{-1}
Organic Matter	0.45%
Caco$_3$	15.1%
Total N before sowing	0.21 mg kg^{-1}

uniformly. Mean monthly maximum, minimum temperature (^oC), rain fall (mm) and radiation hours during growing season 2014-2015 is presented in figure 1.

Parameters studied

Days to maturity:

Days to physiological maturity was calculated by counting the number of days from planting to 80% physiological maturity.

Plant height (cm):

Plant height data was recorded by measuring the height of randomly selected 10 plants in each subplot at physiological maturity and averaged.

Leaf area tiller^{-1} (cm^2):

Data leaf area tiller^{-1} was recorded by measuring three leaf tiller^{-1} of five randomly selected plants with the help of meter rod from each sub plot and then average height was calculated.

Grains Spike^{-1}:

Data on grain spike^{-1} was taken by counting wheat grains in randomly selected five spikes in each plot and was average accordingly.

Biomass yield (kg ha^{-1}):

For biological yield data five central rows was harvested in each plot and was sun dried, weighed and then was converted to kg ha^{-1}.

Statistical analysis:

The data was analyzed statistically using analysis of variance techniques appropriate for randomized complete block deign. Significant differences among treatment were determined using least significant difference (LSD) (Jan et al. 2009).

RESULTS AND DISCUSSION

Days to maturity:

Data concerning days to maturity indicated in (Table 2). Analysis of the data reported that nitrogen ratios and nitrogen fixing bacteria significantly affected days to physiological maturity. All the interactions were found non-significant. Among the planned mean comparison control vs rest, Nitrogen fixing bacteria (NBF) (alone) vs nitrogen and NBF (alone) vs Mix were found significant while only nitrogen vs rest was found non-significant. Among the nitrogen ratios plots with 100% nitrogen was applied, delayed maturity (187 days) while early maturity (173 days) were attained when nitrogen was applied at 25%. Plots treated with *Azospirillum* delayed maturity (181), whereas *Azotobacter* decreased number of days for maturity (180). Maturity was less in control plots (165 days) than rest of the treated plots (180 days). Statistical analysis of data showed that the effect of nitrogen ratios, nitrogen fixing bacteria and control vs rest have significantly influenced days to maturity. Both interactions were found non-significant. Maturity was delayed in urea treated with nitrogen fixing bacteria while physiological maturity was earlier in control plots. it’s because of vegetative growth of wheat crop was increased by the highest rate of nitrogen application. The probable reason might have availability of more nutrients due to which the growing season of wheat crop was enhanced (Li, 2003).

Plant height (cm):

After analysis of data mean table showed that plant heights were significantly affected by nitrogen fixing bacteria and nitrogen ratios (Table 2). All the interactions were found non-significant. Plots treated with *Azospirillum* with 100% nitrogen ratio resulted in taller plants (98.8cm), while shorter plant height (86.9 cm) were recorded in those plots where *Azotobacter* (alone) was applied. A maximum plant height (93.3 cm) was observed in those plants where recommended nitrogen was applied as compared to the control plots (84.3cm). In case of nitrogen ratio higher plant height (97.9cm) was resulted in those plots where 100 % nitrogen was applied as compared to plots treated with 25 % nitrogen (88.5 cm). Among the planned mean comparison control vs rest, nitrogen fixing bacteria (alone) vs nitrogen, nitrogen fixing bacteria (alone) vs mix were significantly affected while nitrogen vs rest was found non-significant. Taller Plants were observed when plots treated with 100% nitrogen plus *Azospirillum*, while shorter plants were recorded from control plots. The possible reason might be that application of high dose of nitrogen increased vegetative growth. However Awasti and Bham (1994) reported same results who concluded that plant height was significantly increased by the highest rate of nitrogen application. It can be clear from the data that those plots which received the highest ratio of nitrogen was resulted maximum plant height, while shorter plants were recorded in control plots.

Leaf area tiller-1:

Analysis of data represented that leaf area tiller^{-1} was significantly influenced by nitrogen ratios, nitrogen fixing bacteria (Table 2 & 3). All the interactions were observed non-significant, while among planned mean all comparison were found significant, while nitrogen vs rest was found non-significant. Plots treated with 100% nitrogen resulted greater leaf area tiller^{-1}(142.1cm^{-2}) as compared to 25% nitrogen applied plots (117.0 cm^{-2}). The mean value showed that plots treated with *Azospirillum* and 100% nitrogen resulted maximum leaf area tiller^{-1}(143.2 cm^{-2}) as compared to control plots (104.1 cm^{-2}). A minimum leaf area tiller^{-1}(104.1cm^{-2}) was recorded in control plots as compared to the other treated or rest plots (126.1 cm^{-2}).

Yasari and Patwardhan (2007) recorded maximum leaf area of canola crop when treated with biofertilizer (Azospirillium and *Azotobacter*) as compared to control plots (only chemical fertilizer). Analysis of data reported that plots treated with 100% nitrogen ratio plus nitrogen fixing bacteria (*Azospirillum*) resulted maximum leaf area tiller^{-1} followed by (*Azotobacter*), whereas minimum leaf area tiller^{-1} was attained in control plots. The suitable reason might be that improvement of leaf area and sustaining leaf photosynthetic activity might be application of high dose of nitrogen. (Deldon, 2001) investigated that the application of high

Table: 2. Days to physiological maturity, plant height (cm), leaf area tiller^{-}(cm^{-2}), grain spike^{-1}, biomass yield (kg ha^{-1}) as effected by nitrogen fixing bacteria with nitrogen ratios.

Treatment s	Physiological maturity	Plant height	Leaf area tiller^{-1}	Grain spike^{-1}	Biomass yield
Nitrogen ratio (%)					
25	173.5d	88.5d	117.0d	45d	8728.9d
50	179.3c	91.9c	126.0c	49c	9159.4c
75	184.0b	95.6b	134.7b	55b	9509.0b
100	187.4a	97.9a	142.1a	62a	10037.3a
LSD (0.05)	**3.02**	**1.60**	**2.19**	**1.61**	**184.8**
Shahkar-2013	177.8	91.6	124.0	49.0	8985.1
Pirsabak-2013	178.3	91.8	124.6	49.8	9085.6
Nitrogen fixing bacteria					
Azosprillium	181.7a	94.3a	130.6a	53.7a	9440.9a
Azotobacter	180.4b	92.7b	129.3b	52.6b	9276.4b
Control vs. Rest					
Control	165.8	84.3	104.1	35.3	7380.1
Rest	179.2	92.3	126.1	50.7	9185.8
Interaction					
NBF X N	Ns	Ns	Ns	Ns	Ns
ABF X V	Ns	Ns	Ns	Ns	Ns

Means in the same category followed by different letters are significantly different at P ≤0.05 levels. Ns = non-significant

Table: 3. Analysis of variance for physiological maturity, plant height (cm), leaf area tiller^{-1} (cm^{-2}), grain spike^{-1} and biomass yield (kg ha^{-1})of wheat as influenced by nitrogen ratio and nitrogen fixing bacteria.

Sov	Df	Physiological maturity	Plant height	Leaf area tiller^{-1}	Grain spike^{-1}	Biomass yield
Replication	3	12.03	4.84	16.74	3.81	29941.24
Treatments (T)	23	208.67**	82.68**	631.01**	297.32**	2251715**
Varieties	1	5.51 ns	0.86 ns	9.00 ns	13.5 ns	242808.2 ns
NFB	11	401.71**	164.72**	1300.28**	614.31**	4624595**
Control Vs Rest	(1)	1320.79**	467.66**	3570.63**	1746.36**	23910839**
Nitrogen Vs Rest	(1)	15.55 ns	7.41 ns	20.710 ns	18.32 ns	176620.6 ns
NFB (alone)Vs N	(1)	481.33**	185.77**	1636.83**	768**	4356678*
NFB (alone) Vs Mix	(1)	1292.02**	478.65**	5012.57**	2168.40**	11189332**
NFB (alone)	(1)	6.25 ns	3.58 ns	17.85 ns	9 ns	51189.06 ns
Ratio (R)	(3)	585.30**	270.08**	1883.56**	931.30**	4903660**
N X NFB (N)	(3)	0.68 ns	1.53 ns	2.19 ns	1.47 ns	132982.6 ns
V x NFB	11	34.10 ns	8.07 ns	18.29 ns	6.13 ns	61462.67 ns
Error	69	18.42	5.17	9.69	5.26	68714.63
Total	95					

NFB = Nitrogen Fixing Bacteria
ns = non-significant
*** = Significant at 5 % level of probability**
**** = Significant at 1 % level of probability**

dose of nitrogen delayed anthesis and maturity stages which may be respond to enlarge leaf area, canopy of the crop and increased light capturing efficiency.

Grain spike^{-1}:

Data on wheat grains spike^{-1} as affected by different nitrogen ratios and nitrogen fixing bacteria presented in (Table 2). The mean table indicate that the effect of nitrogen ratio and nitrogen fixing bacteria significantly affected grains spike^{-1}. The effect of all interactions was found non-significant. All planned mean comparison except nitrogen vs rest was found significant. In case of nitrogen ratios plot, where 100% nitrogen was applied it resulted in maximum grain spike^{-1} (62.7) while a minimum grain spike^{-1} (45.3) recorded from those plots which were treated with 25% nitrogen. Namvar (2013) concluded that highest number of grains per spike was recorded in 200 kg N ha^{-1} application as compared to control plots. The maximum grains spike^{-1} (62.8) was recorded from those plots where *azospriilium* was used which statistically at par with *Azotobacter* (62.6) while a minimum grains spike^{-1} (35.3) were observed in control plots. Similar trend of results was also observed in case of grains spike and 1000 grain weight (g) of wheat increased significantly when the crop received biofertilizers either alone or combined (Singh and Parsad, 2011) .The results show that urea combined with nitrogen fixing bacteria increased the number of grain spike^{-1} in wheat crop. Maximum number of grain spike^{-1} was recorded when plots treated urea with *Azospirillum* and *Azotobacter*, while minimum number of grains spike^{-1} was attained in control plots. Similar results were reported by Shahzad et al. (2013); Bakht et al. (2010); Fallahi et al. (2008) that nitrogen application increased grain per spike over control. Vessey (2003) reported that grain spike^{-1} was increased due to *Azotobacter sp*. and *Azospirillum* sp. Application which enhanced the available nitrogen in the soil.

Biomass yield (kg ha^{-1}):

Data regarding on biomass yield reported in Table 2. Statistical analysis of the data showed that nitrogen ratios and nitrogen fixing bacteria had significant effect on wheat cultivars. Both of the interaction were found non-significant, while among planned mean comparison control vs rest, BF (alone) vs Nitrogen and BF (alone vs Mix) was found significant whereas Nitrogen vs rest was non-significant. Mean value showed that application of 100% nitrogen resulted maximum biomass yield (10037.3 kg ha^{-1}) as compared to 25% nitrogen treated plot (8728.9 kg ha^{-1}). Yousafi et al. (2014) reported that chemical fertilizers of 100% treatment with the combined *Azotobacter* and Pseudomonas treatment increased biomass yield by 12.9 % compared to chemical fertilizers 100% treatment without inoculation. Among the nitrogen fixing bacteria *Azospirillum* resulted more biomass yield (9440.9 kg ha^{-1}) as compared *Azotobacter* (9276.4 kg ha^{-1}). More biomass yield (9185.5 kg ha^{-1}) was attained from treated plots whereas control plots resulted minimum biomass yield (7380.1 kg ha^{-1}). Namvar and Khandan (2013) observed that usage of 200 kg N ha^{-1} increased the biomass yield by 97.10% as compared to control. The use of nitrogen fixing bacteria and nitrogen fertilizer significantly increased the biomass yield of wheat. The combine application of urea and nitrogen fixing bacteria had higher biomass yield as compare to control plots. El-Lattief (2013) observed that combined application of mineral and biofertilizers were favorable in enhancing biomass yield than using mineral or biofertilizer alone. The ability of nitrogen fixing bacteria to inhibit the nutrient and release slowly which enough for longer time and protect urea from leaching could be the possible reason for increased biomass yield. Kandil et al. (2011) agree with result who reported that great root development and proliferation of plants in response to biofertilizer activities, i. e. *Azotobacter sp.* and *Azospirillum sp.* enhance water and nutrient uptake which result in increasing biomass yield. Namvar et al. (2012) reported that the decrease in biomass production with the application of lower dose of nitrogen was associated with decreases in both radiation use efficiency and radiation interception.

CONCLUSION

It was concluded from the experiment that application of 100% nitrogen plus *Azosprillium* bacteria improved the growth, maturity and biomass of wheat. Pirsabak-2013 cultivar showed superiority over Shahkar-2013 in all studied parameters. Based on results and conclusion the use of nitrogen fixing bacteria (*Azospirillum*) plus 100% nitrogen and Pirsabak-2013 variety was best for maximum plant height, grains spike^{-1} leaf area and biomass production, thus it is recommended for improved crop productivity in agro-climatic region of Peshawar.

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