Improving Bread Wheat Productivity and Reduce Use of Mineral Nitrogen by Inoculation with *Azotobacter* and *Azospirillum* Under Arid Environment in Upper Egypt

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Abstract

The effect of integrated use of mineral N fertilizer (Urea) and biofertilizer (*Azotobacter* and *Azospirillum*) on grain yield, grain yield attributes and harvest index of wheat was assessed. Two field experiments were carried out on a sandy soil in the Experimental Farm of the Faculty of Agriculture, South Valley University at Qena Governorate, Egypt. The recommended N (230 kg N ha^{-1}) and biofertilizer (*Azotobacter* and *Azospirillum*) were applied alone and in various combinations among them. A randomized complete block design, with three replications, was used in this study.

Treatments significantly affected plant height, spike length, number of spikelets/spike, kernel weight /spike, 1000-kernel weight, grain and straw yields and harvest index. The highest values of such traits were obtained in treatment T_{11} (75% mineral N + biofertilizer with *Azotobacter* and *Azospirillum*). However, T_{12} (50% mineral N + biofertilizer with *Azotobacter* and *Azospirillum*) resulted also higher values for the above mentioned traits comparing with T_1 (100% nitrogen and uninoculated) but the differences among the two treatments almost did not attain the statistical differences.

From this study, it can be concluded that the biofertilizers (double-inoculation of *Azotobacter* and *Azospirillum*) of efficient strains could save 25 or 50 % of the recommended dose of mineral N.

Keywords: Wheat, Biofertilizers, Azotobacter, Azospirillum, Grain yield.

1. Introduction

The high cost of chemical nitrogenous fertilizers and the low purchasing power of most of the farmers restrict its use in proper amounts, hampering crop production. Besides, a substantial amount of the urea-N is lost through different mechanisms including ammonia volatilisation, denitrification and leaching losses, causing environmental pollution problems [1, 2].

The utilization of biological nitrogen fixation technology can decrease the use of urea-N, prevent the depletion of soil organic matter and reduce environmental pollution to a considerable extent [3, 4]. Also, Use of biofertilizers on Egyptian soils has decreased the pH, which had led to increased availability of trace elements that enhance plant growth. Bio-fertilizers are eco-friendly and have been proved to be effective and economical alternate of chemical fertilizers with lesser input of capital and energy [5].

Biofertilizer contains live or latent cells of efficient strains of nitrogen fixing, phosphate solubilizing or cellulolytic micro-organisms used for application to seed, soil or composting areas to accelerate microbial processes to augment the extent of availability of nutrients.

Nitrogen fixation potential of *Azotobacter* and *Azospirillum* are known. The organic matter rich soils promote the activities of these organisms [6, 7]. Also, free-living nitrogen-fixing bacteria *eg Azotobacter chroococcum* and *Azospirillum lipoferum,* were found to have not only the ability to fix nitrogen but also the ability to release phytohormones similar to gibberellic acid and indole acetic acid, which could stimulate plant growth, absorption of nutrients, and photosynthesis [8].

Many authors have shown the positive effect inoculation of wheat with Azotobacter or/and *Azospirillum* [9, 10, 11, 12]. Tilak [13] reported positive effects of double-inoculation of *Azotobacter* and *Azospirillum* on dry matter of maize and sorghum. Rai and Caur [14] studied *Azotobacter* and *Azospirillum* and double-inoculation and alone inoculation effects on wheat growth and yield. Double-inoculation of *Azotobacter* and *Azospirillum* had positive effects on plant height, spike length, grain yield, biological yield and harvest index in various wheat genotypes.

Present study aims to evaluate the importance of bio-fertilization in the improvement growth and productivity of bread wheat crop as well as the expansion of bio-agriculture to reduce agriculture costs and environmental pollution via lowering mineral fertilizers application.

2. Materials and methods

The field experiments were conducted at the Experimental Farm, Faculty of Agriculture, South Valley University (latitude 26°10′ N, longitude 32°43′ E, Altitude 79 m above sea level), Qena, Egypt during 2010-11 and 2011-12 seasons. The soil of the experimental site is sandy throughout its profile (73.7% coarse sand, 16.8% fine sand, 5.8% silt and 3.7% clay). Its pH value of 7.62, 1.75 EC (dSm^{-1}), 0.45% organic matter content, 0.25% total N, and available P and K of 7.42 and 170 ppm, respectively. The weather is very hot and dry from May to October where temperatures can reach up to 40 °C. On the other hand, the weather is usually warm during winter months and rainfall is rare.

The dose of nitrogen (230 kg N ha^{-1}) was manipulated at various levels in combination with different biofertilizers as per the treatment schedule. The different treatment combination as follows:

T_1- 100% mineral N (MN), T_2- *Azotobacter* (AZB) alone, T_3- *Azospirillum* (AZS) alone, T_4- AZB + AZS, T_5- 75 % MN + AZB, T_6- 50 % MN + AZB, T_7- 25 % MN + AZB, T_8- 75 % MN + AZS, T_9- 50 % MN + AZS, T_{10}- 25 % MN + AZS, T_{11}- 75 % MN + AZB + AZS, T_{12}- 50 % MN + AZB + AZS, T_{13}- 25 % MN + AZB + AZS, T_{14}- Control (without nitrogen and uninoculated). The seeds were inoculated by liquid culture of locally isolated strains of *Azotobacter lipoferum* and *Azospirillum chroococcum* (≈10^7 CFU/ml) which obtained from Biofertilizers Production Unit of Faculty of Agriculture, South Valley University. 1% of carboxy methyl cellulose (CMC) was added to the culture to increase its viscosity to gel form to act as adhesive biostabilizer, the addition of CMC was made just before using.

The experiment was carried out in a randomized block design with three replications. Experimental unit measured 3.0 m in width and 4 m in length. Bread wheat (Giza 168 cv.) was sown on the 10th of November in each season. The other cultural practices were carried out as recommended for the crop.

At harvest time, ten fertile stems were taken at random from each plot for measuring plant height, spike length, number of spikelets/spike and kernel weight /spike. Also, 1000-kerenl weight was estimated for each plot. Meanwhile, grain and straw yields were estimated at plot basis. Harvest index (%) of each plot was calculated by using the following formula:

$$Harvest\ Index\ (\%) = \frac{Grain\ \ yield}{Biolo\ gical\ \ yield}\ x\,100$$

The data were analyzed by analysis of variance (ANOVA) using MSTAT-C statistical software. Treatment means were compared using Duncan's multiple tests [15]. Since data followed the homogeneity test, pooling was carried out over the seasons and mean data are given.

3. Results and discussion

3.1. Yield attributes

Data presented in Table 1 indicated that various studied treatments had a significant effect ($P < 0.01$) on plant height, spike length, number of spikelets/spike, kernel weight /spike and 1000-kernel weight. Table 2 shows that greatest values of such traits were from treatment T_{11} (75% mineral N + biofertilizer with *Azotobacter* and *Azospirillum*). Also, T_{11} significantly increased plant height, spike length, number of spikelets/spike, kernel weight /spike and 1000-kernel weight by 4.1, 13.2, 10.2, 9.6 and 12.0 %, respectively, compared to T_1 (100% mineral nitrogen and uninoculated) and by 30.9, 67.2, 53.5, 100, 76.3%, respectively, compared to T_{14} (without nitrogen and uninoculated). However, T_{12} (50% mineral N + biofertilizer with *Azotobacter* and *Azospirillum*) resulted also higher values for the above mentioned yield components comparing with T_1 (100% nitrogen and uninoculated) but the differences among the two treatments almost did not attain the statistical differences. Meanwhile, T_1 resulted yield and yield components almost significantly higher in their values than those in T_{14} (without nitrogen and uninoculated) treatment. These findings are in agreement with those of Sharief *et al* [16], Elsayed *et al* [17] and El-Garhi *et al* [18].

Inoculation with *Azospirillum* alone (T_3) produced significantly higher plant height (98.4 cm), kernel weight per spike (2.035 g) and 1000-kernel weight (33.33 g) than those of inoculation with *Azotobacter* alone (T_2).

3.2. Grain and straw yields

The effects of studied treatments on the grain and straw yields were significant at 1 % level (Table 1). Means in Table 2 indicates that superiority of grain and straw yields were achieved by application of double-inoculation of *Azotobacter* and *Azospirillum* plus 75% mineral N (T_{11}) with a grain and straw yields of 5.046 and 6.470 tons ha^{-1}, respectively. Meanwhile, double-inoculation of *Azotobacter* and *Azospirillum* plus 50% mineral N (T_{12}) resulted higher value for the studied grain yield (4.684 t ha^{-1}) comparing with T_1 (4.486 t ha^{-1}) but the differences among the two treatments did not attain the statistical differences. Also, T_{12} treatment did not differ significantly with application with 100% mineral N (T_1) concerning the effect of straw yield as its values attained 6.059 and 6.058 t ha^{-1} for the two treatments, respectively.

Source of variance	d.f	Plant height	Spike length	No. of spiklet/ spike	Kernel weight/ spike	1000-seed weight	Grain yield /ha	Straw yield /ha	Harvest index
Year (Y)	1	112.0	5.054	9.11	0.342	58.73	0.238	0.323	0.0082
Rep./Y(Ea)	6	38.04	2.317	8.38	0.048	12.66	0.167	0.218	0.0551
Treatment (T)	13	493.8**	17.15**	48.35**	1.656**	255.9**	4.518**	6.594**	0.176**
Y x T	13	2.288	0.408	6.42	0.057	0.082	0.020	0.161	0.041
Error (Eb)	78	16.311	1.973	3.86	0.0421	9.78	0.102	0.167	0.039

** significant at P < 0.01 level

Table 1. Analysis of variance of measured parameters

Treatments	Plant height (cm)	Spike length (cm)	No. of spike-lets/ spike (g)	Kernel weight/ spike (g)	1000-kernel weight (g)	Grain yield (t/ha)	Straw yield (t/ha)	Harvest index (%)
T_1- 100% mineral N (MN)	112.7bc	12.63 b	22.18 b	2.800 b	41.72 b	4.486 b	6.058 b	42.5 ab
T_2- *Azotobacter* (AZB)	94.3 g	10.33 c	17.93 d	1.799 f	29.78 f	2.993 f	4.389 e	40.5 ce
T_3- *Azospirillum* (AZS)	98.4 f	11.48bc	18.13 d	2.035 e	33.33 e	3.362 e	4.829 d	41.0 bc
T_4- AZB + AZS	100.2 f	11.38bc	20.65bc	2.275 d	37.50cd	3.708cd	5.411 c	40.7 ce
T_5- 75 % MN + AZB	110.8bc	12.00 b	18.75cd	2.747 b	36.89 d	4.422 b	6.059 b	42.2 ab
T_6- 50 % MN + AZB	104.3 e	12.00 b	20.50bc	2.423cd	37.63cd	3.870 c	5.575 c	41.0 bc
T_7- 25 % MN + AZB	100.1 f	11.43bc	18.50cd	2.013 e	33.08 e	3.385de	4.977 d	40.5 ce
T_8- 75 % MN + AZS	111.3bc	12.60 b	21.90 b	2.758 b	41.63 b	4.521b	6.060 b	42.7 ab
T_9- 50 % MN + AZS	108.9cd	12.50 b	21.80 b	2.553 c	40.63bc	3.877 c	5.647 c	40.7 ce
T_{10}- 25 % MN + AZS	105.3de	11.75bc	20.44bc	2.050 e	35.83de	3.481de	5.000 d	41.0 bc
T_{11}- 75 % MN + AZB + AZS	117.8 a	14.30 a	24.45 a	3.070 a	46.73 a	5.046 a	6.470 a	43.8 a
T_{12}- 50 % MN + AZB + AZS	113.6 b	12.80 b	22.25 b	2.850 b	41.75 b	4.684 b	6.059 b	43.6 a
T_{13}- 25 % MN + AZB + AZS	109.5 c	12.25 b	19.25cd	2.288 d	37.50cd	3.955 c	5.557 c	41.6 b
T14- Control (without)	90.0 h	8.55 d	15.93 e	1.520 g	26.50 g	2.545 g	3.978 f	39.0 e

The same letters within columns means not significant differences at 5% level.

Table 2. The associative influence of biofertilizers and reduced doses of mineral nitrogen on wheat yield parameters (data over two seasons).

Application of T_{11} had significantly higher grain and straw yields by 12.5 and 6.8 % relative to T_1 and by 98.3 and 62.4%, respectively relative to $T_{14.}$ Also, T_{12} had significantly higher grain and straw yields by 84.1 and 52.3%, respectively relative to $T_{14.}$ Also it is showed in Table 2 that *Azospirillum* is more effective than *Azotobacter* on grain yield due to more role of *Azospirillum* in up taking nitrogen produced by biological fixing by *Azospirillum* bacteria that finally will cause to more grain yield of plant. The lower values of grain and straw yields (2.545 and 3.978 t ha^{-1}, respectively) were obtained from T_{14} (without nitrogen and uninoculated). It is evident from the data in Table 2 that combined application of mineral and biofertilizers were favorable in enhancing yield than using mineral or biofertilizer alone.

Such increase in yields (grain and straw) and grain yield attributes, due to application of T_{11} or T_{12}, might be due to the role of biofertilizer (*Azotobacter* and *Azospirillum*) in enhancing soil biological activity, which improved nutrient mobilization from organic and chemical sources. Also, the biofertilizer plays a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients and in increasing nitrogen fixer. In this case, Radwan and Hussein [19], Sharief *et al* [16], Elsayed *et al* [17], El-Garhi *et al* [18], Badr *et al* [11] and Bahrani *et al* [12] found positive effect on yield and yield attributes of wheat when inoculated with biofertilizer. In controlled field trials in Iran, Khavazi *et al* [20] found that yield improvements of more than 20% have been observed for wheat as a result of application of *Azotobacter* and *Azospirillum* inoculums.

3.3. Harvest index

Variance analyzing of harvest index, data showed that harvest index was significant influenced by various studied treatments at 1% probability level (Table 1). Application of T_{11} resulted highest value of harvest index (43.8%) and it was followed by T_{12} (43.6%), T_8 (42.7%), T_1 (42.5%) and T_5 (42.2%) without any differences significant among them (Table 2). Meanwhile, the lower value of harvest index (39.0%) was obtained from T_{14}. Thus it is indicated that using bio-fertilizers caused to increasing harvest index due to effect on dry weight and allocating more photosynthetic matters to grain.

The interaction effect of fertilization and year was not significant for all yield attributes traits and grain yield as well as harvest index (Table 1). Such results indicated that fertilization treatments showed similar effects from season to season.

4. Conclusion

In conclusion, the use of biofertilizers became ineludible to minimize the environmental pollution, caused by the chemical ones, and to improve the yield quality of various crops needed at the time being. Although 25 or 50 % of mineral N was replaced by biofertilizers (double-inoculation of *Azotobacter* and *Azospirillum*), the yield and its components of wheat increased compared to that obtained with the recommended dose of mineral nitrogen. Finally, the biofertilizers of efficient strains could save 25 or 50 % of the recommended dose of mineral nitrogen.

5. References

[1] De Datta, S.K. and R.J. Buresh, 1989. Integrated nitrogen management in irrigated rice. Advances in Soil Science 10, 143–169.

[2] Choudhury, A.T.M.A. and I.R. Kennedy, 2005. Nitrogen fertiliser losses from rice soils and control of environmental pollution problems. Communications in Soil Science and Plant Analysis 36, 1625–1639.

[3] Choudhury, A.T.M.A. and I.R. Kennedy, 2004. Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. Biology and Fertility of Soils 39, 219–227.

[4] Kennedy, I.R., A.T.M.A. Choudhury and M.L. Kecskés, 2004. Non-symbiotic bacterial diazotrophs in cropfarming systems: can their potential for plant growth promotion be better exploited? Soil Biology and Biochemistry 36, 1229–1244.

[5] Hafeez, F.Y., S. Hameed, A.H. Zaidi, K.A. Malik, 2002. Biofertilizers for Sustainable Agriculture. In: Techniques for Sustainable Agriculture (F. Azam, M. M. Iqbal, C. Inayatullah and K. A. Malik, Editors), pp. 67-73. ISBN, NIAB, Faisalabad, Pakistan

[6] Lakshami, K., N.S. Subba, K.V. Tilak and C.S. Singh, 1972. *Azospirillum*, a new bacterial fertilizer for tropical crops. Sci. Reporter, Council of Scientific and Industrial Research (India), 16:690-692.

[7] Dobereiner, J. and J.M. Day, 1975. Nitrogen fixation in the rhizosphere of tropical grasses. In: Nitrogen fixation by free living microorganisms. W.D.P. Stewart (ed.). Cambridge Univ. Press, London. pp: 39-56.

[8] biofertilizers in Iran. Ministry of Jihad-e- Agriculture, Agricultural Research and Education Organization (AREO) and Soil and Water Research Institute (SWRI), Tehran, Iran, pp: 419.

[9] Fayez, M., N.F. Emam, and H.E. Makboul, 1985. The possible use of nitrogen fixing *Azospirilum* as biofertilizer for wheat plants. Egypt. J. Microbiol., 20(2), 199-206.

[10] Tawfik, M.M. and A.M. Gomaa, 2005. Effect of organic and biofertilizers on the growth and yield of wheat plants Egypt .J. Agrci. Res., 2(2): 711-725.

[11] Abbasdokht, H., 2008. The study of Azotobacter chroococcum inoculation on yield and post harvest quality of wheat. International Meeting on Soil Land Management and Agroclimatology, Turkey, 885-889.

[12] Badr, A. Elham, O.M. Ibrahim and M.F. El-Kramany, 2009. Interaction effect of biological and organic fertilizers on yield and yield components of two wheat cultivars. Egypt .J. Agron., 31(1): 17-27.

[13] Bahrani, A.J. Pourreza and M. Hagh Joo, 2010. Response of Winter Wheat to Co-Inoculation with Azotobacter and Arbescular Mycorrhizal Fungi (AMF) under Different Sources of Nitrogen Fertilizer. American-Eurasian J. Agric. & Environ. Sci., 8 (1): 95-103

[14] Tilak, K.V.B.R., 1992. *Azospirillum* brasilense and *Azotobacter* chrooccocum inoculum effect of mayze and sorghum. Soil Bio. Biochem., 14: 417-418.

[15] Rai, S.N. and A.C. Caur, 1998. Characterization of *Azotobacter* Spp. And effect of *Azospirilum lipoferum* on the yield and N-Uptake of wheat crop, Plant and Soil, 109: 131-134.

[16] Steel, R.G.D. and J.H. Torrie, 1980. Principles and procedures of statistics, 2nd ed. McGraw-Hill, New York.

[17] Sharief, A.E., S.E. El-Kalla, A.A. Leilah and H.E.M. Mostafa, 1998. Response of wheat cultivars to nitrogen fertilizer levels and biological fertilization. J.Agric.Sci. 23(12):5807-5816. Mansoura Univ., Egypt.

[18] El-Sayed, M.Z., A.E. Abd El-Sattar, H.A. Basha and I.M. Abd El-Hammeed, 2005. Improvement of wheat productivity in newly reclaimed soil in Egypt. Annals UMCS, Sec. E.60:113-121.

[19] El-Garhi, A.S., N.A. Atia and Sara E.E. Fouda, 2007. Effect of inoculating N-fixing bacteria (Cerealine) on wheat (Triticum aestivum, L.) growth and nutrient content. Zagazig J. Agric. Res., 34(2): 249-273.

[20] Radwan, S.M.A. and H.F. Hussein, 1996. Effect of bio. and organic fertilization on wheat yield under different weed control treatments. Egyptian J. Appl. Sci., 11:267-281.

[21] Khavazi, K., H. Asadi-Rahmani and M.J. Malakouti, (eds.), 2005. Necessity for the production of biofertilizers in Iran. Ministry of Jihad-e- Agriculture, Agricultural Research and Education Organization (AREO) and Soil and Water Research Institute (SWRI), Tehran, Iran, pp: 419.