RESPONSE OF SOME WHEAT VARIETIES TO INOCULATION BY CEREALIN (*BACILLUS POLYMYXA*) USING N-15 DILUTION TECHNIQUE

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Abstract

A field experiment was conducted in the Agricultural Research Station at Mallawy (El-Minia Governorate) to estimate the amount of nitrogen fixed by Cerealin (*Bacillus polymyxa*) as a nitrogen fixer on two wheat varieties (*Triticum aestivum*), Sakha 69 and Benisuef using N-15 dilution technique.

Data showed that inoculation of wheat by *B. polymyxa* had a significant effect at 1% (LSD = 1.03) on grain yield (Sakha 69) when combined with the nitrogen fertilizer at the rate of 178 kg N/ha. Grain yields were 6.48 and 5.2 ton/ha for the inoculated and uninoculated treatments, respectively. A similar trend was observed for wheat variety Benisuef as 5.45 and 4.51 ton/ha were respectively obtained. The amount of nitrogen fixed varied appreciably among wheat varieties. The values of % Nitrogen derived from air (%Ndfa) observed with wheat varieties Sakha 69 and Benisuef inoculated by *B. polymyxa* at the rate of 178 kg N/ha were 10.82 and 7.9%, respectively.

INTRODUCTION

Biological nitrogen fixation contributes to productivity both directly, where the fixed nitrogen is harvested in grain or other food for human, or indirectly, by contributin to the maintenance or enhancement of soil fertility in the agricultural system by adding nitrogen to the soil. The association of *Bacillus Polymyxa* with the roots of wheat is well established (Chanway *et al.*, 1988). Rennie and Thomas (1987) and Omar *et al.* (1991) showed that the *Bacillus* supplied more nitrogen to wheat plants under temperate conditions than *Azospirillum brasilense* and that the amount of associative nitrogen fixed was dependent on the host plant genome. Response of the host genome on the *A. brasilense* have also been reported (Millet *et al.*, 1984).

The earlist appication of N-15 in nitrogen fixation studies was by Burris and Miller (1941). This method has been used to provied direct evidence for nitrogen fixation since the N-15 concentration in plants exposed to N-15 is greater than the 0.3663% (natural abundance) if fixation occurs. The extent to which N-15 is detected in the plant provides an estimate of the proportion of the plant nitrogen that was derived from flxation, and is thus a direct method for quantifying nitrogen fixed.

The present study aimed to evalute amount of nitrogen fixed in wheat by the inoculant Cerealin which contains *B. polymyxa* by using N-15 dilution technique using Egyption wheat varieties.

MATERIALS AND METHODS

At Mallawy Agricultural Research Station, El-Minia Governorate an experiment was conducted to evalute the response of two varieties of wheat to inoculation by *Bacillus polymyxa* under gradient of nitrogen levels using N-15 dilution technique. The experiment was sep up as a complete randomized block design with four replications. Two wheat varieties Sakha 69 and Benisuef were used in this experiment. Mallawy soil was of a pH 7.9, total nitrogen nitrogen 0.148% (as determined by Bremner, 1965), organic matter, 1.94% (as determined by Walkley, 1946). The seeds were inoculated shortly before planting by Cerealin a biofertilizer containing nitrogen fixing bacteria (*B. polymxa*) 10^9/g according to Omar and Hassan (1993). Uninoculated treatments were also included.

Each plot recieved super phosphate at a rate of 350 kg/ha and plaughed in the soil. Potassium sulphate was added at the rate of 48 kg K_2O/ha. In combination with inoculation, 3 levels of nitrogen fertilizer in the form of ammonium sulphate (20.5% N) were applied 0, 89 and 178 kg N/ha. Nitrogen labelled as ammonium sulphate $(NH4)_2\ SO_4$ containing 1% N-15 enriched, 7.4% atom excess was added to the plots. N-15 was determined by emission spectronetry. Percentage of nitrogen derived from fertilizer (% Ndff), % nitrogen derived from soil (% Ndfs) and % nitrogen derived from air (% Ndfa) were calculated according to Fried and Middelboe (1977).

Grain and straw yield recorded after harvesting the plots. Other characteristics were calculated from sampling of 5 m^2. Nitrogen content of grains was determined using the micro-Kjeldahl method.

RESULTS AND DISCUSSION

The importance of nonsymbiotic and associative nitrogen fixation in soils can only be realized if the nitrogen from nitrogen fixation ultimately becomes available

to plants under cultivation. To evalute the role of Cerealin (*B. polymxa*) as seed inoculant to satisfy some of the nitrogen requirement of growing plants, a field experiment was conducted at Mallawy and two cultivars of wheat were tested by using N-15 dilution technique under 3 levels of nitrogen fertilizers.

Data presented in (Table 1) show that regardless of seed inoculation, there is an obvious response of both wheat cultivars towards the level of nitrogen fertilizer applied. Grain yield of Sakha 69 cultivar in the inoculated treatment as compared with uninoculated increased by 6 and 25% due to increasing the dose of nitrogen fertilization from 83 to 178 kg N/ha in respective order. The corresponding percentages of the other wheat cultivar Benisuef were 21 and 2%. The amount of nitrogen fertilizer needed to obtain 5.20 ton of wheat grain/ha in uninoclated treatment amounted to 178kg N/ha (Table, 1). The same yield could be obtained in the inoculated treatment with much less nitrogen fertilizer, i.e. 83kg N/ha. This means that 95 kg N/ha can be saved by inoculation. The difference in nitrogen yield in the grain was also significant between the two treatments inoculated and uninoculated (Table 1).

The other parameters under investigation (staw yield and weight of 1000 grains), it is evident that no significant differences could be obtained due to the effect of either dose of nitrogen fertilization or to seed inoculation (Table 1).

Results generally pointed out to a beneficial effect of inoculation of grains with *B. polmyxa* and this is known to be attributed to the nitrogen fixation and/or to the production of certain plant growth promoting substances by the bacteria (Jain and Patriquin, 1985). Data presented in Table (2) show that in wheat grains the percentage of nitrogen derived from soil (% Ndfs) and the percentage of nitrogen derived from fertilizer (% Ndff) varied to some extent depending on the plant cultivar as well as on the dose of nitrogen fertilization. Generally, % Ndfs + % Ndff ranged between 44.59 and 50 in both cultivars. The percentage of nitrogen derived from air (% Ndfa) in case of Sakha 69 cultivar (6.0 %) was much higher than that of Benisuef cultivar (3.5%) when grown at the rate of 83 kg N/ha. Similar trend was recorded in case of high nitrogen fertilization dose where the corresponding percentages were 10.82 and 7.9 respectively. These results agree with the finding of Larson and Neal (1978) and Lindberg *et al.* (1985).

Fertilizer Use Efficiency (FUE %) is a quantative measure of the actual uptake of fertilizer nutrient by the plant in relation to the amount of nutrient added to the soil (Zapata, 1990). Regarding to the FUE%, inoculation had no positive effect at the low nitrogen fertilization dose and slightly decreased the value ranging between 21 and 30% under the higher fertilization level. These results are in agreement with

Table 1. Effect of inoculation by *B. polymyxa* on yield of seeds, straw, nitrogen and weight of 1000 grain of two wheat varieties at Mallawy.

Treatment	Yield ton/ha		Nitrogen yield kg/ha	Weight of 1000 graing(g)
	Grains	Straw		
Sakha 69				
0 kg N/ha				
inoc.	3.073	8.820	71.9	46.76
non inoc.	2.949	8.470	51.0	47.85
83 kg N/ha				
inoc.	5.174	9.100	119.0	48.30
non inoc.	4.856	8.940	107.8	46.40
178 kg N/ha				
inoc.	6.482	9.948	186.6	45.63
non inoc.	5.201	9.989	143.5	42.70
Benisuef				
0 kg N/ha	2.910	3.160	71.3	57.00
inoc.	2.950	4.940	57.3	56.90
non inoc.				
83 kg N/ha	4.641	3.92	103.9	56.26
inoc.	4.538	3.55	103.0	54.40
non inoc.				
178 kg N/ha	5.455	9.010	140.7	48.86
inoc.	4.518	9.320	105.2	54.40
non inoc.				

LSD 1.03

Table 2. Percent nitrogen derived from air (% Ndfa) in grains of inoculated wheat.

Treatment	Sakha 69			Benisuef		
	(%Ndff+ %Ndfs)*	% Ndfa**	FUE%***	(%Ndff+ %Ndfs)*	% Ndfa**	FUE%***
83 kg N/ha						
inoc.	46.99	6.0	52	48.23	3.5	48
non inoc.	50.00	-	50	50.00	--	50
178 kg N/ha						
inoc.	44.59	10.8	30	46.02	7.9	26
non inoc.	50.00	--	26	50.00	--	21

* % Ndff Nitrogen derived from fertilizer, % Ndfs Nitrogen derived from soil
** % Ndfa Nitrogen derived from air
*** FUE% Fertilizer Use Efficiency

the finding of Kucey (1988) and Omar *et al.* (1991).

From the previous results, it could be concluded that inoculation of wheat plants with specific nitrogen fixing bacteria is compatible with normal level of nitrogen fertilizer. On the other hand, yield significantly increased at the rate of 178 kg/N/ha through inoculation. In connection with this points it is worth mentioning that Murty and Ladha (1988) and Omar *et al.* (1989) suggested that the increase in mineral uptake by plants could be due to a general increase in the volume of the root system and not to any specific enhancement of the normal ion uptake mechanism.

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إستجابه بعض اصناف القمح للتلقيح البكتيرى باستخدام السيريالين (باسلس بوليمكسا) باستعمال تكنيك النيتروجين المؤشر

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اقيمت تجربة حقلية بمحطة البحوث الزراعيه بملوى - المنيا لتقدير كميه الازورت الجوى المثبته باستخدام لقاح السيريالين (باسلس بوليمكسا) كبكتريا مثبته للازوت الجوى على صنفين من القمح سخا ٦٩ وبنى سويف باستخدام تكنيك النتيروجين المؤشر N - 15.

اظهرت النتائج ان التلقيح البكتيرى على اصناف القمح باستخدام باسلس بوليمكسا له تأثير معنوى على مستوى ١٪ (LSD = 1.280) على محصول الحبوب تحت معدل تسميد معدنى ازوتى ٧٥ كيلو جرام نتيروجين / هكتار والمحصول تراوح بين ٦,٤٨ ، ٥,٢ طن / هكتار للملقح وغير ملقح على التوالى اوضحت النتائج ان كميات الازوت المثبته من الهواء الجوى اعتمدت على صنف القمح المستخدم.

عند استخدام التلقيح البكتيرى لصنفى القمح سخا ٦٩ وبنى سويف فى وجود الجرعه الكامله للازوت المعدنى تراوحت النسبه المئويه للازوت الجوى المثبته عن طريق التلقيح البكتيرى بين ١٠,٨٣ ، ٧,٩ على التوالى.