Preliminary Studies on The Response of Two Local Wheat Cultivars to Seed Bacterization

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ABSTRACT

Preliminary studies on the response of Sidi El-Masri and El-Muhktar wheat cultivars to seed bacterization with a mixed culture of *Azotobacter* species were found promising. Tube culture data obtained from five weeks seedings showed an increase of 22 and 26% in the mean weight of roots and 24 and 32% in the mean weight of shoots in inoculated seeds of El-Mukhtar and Sidi El-Masri wheat cultivars, respectively.

The nitrogen fixing potential of the bacterial inoculum was found to be as important as any factor that might be accounted for in increasing plant growth of inoculated seeds. The increase in the percent total nitrogen of plant tops that was found to be 32 and 34% for El-Mukhtar and Sidi El-Masri, respectively, and the calculated S/R values for the latter cultivar confirm the above conclusion.

INTRODUCTION

Seed bacterization of field crops as a practice was originally developed in the Soviet Union in 1928. Several genera of soil bacteria were employed as seed inoculants, among which are *Azotobacter, Bacillus, Clostridium,* and *Pseudomonas.* Each organism was proved to promote better growth conditions throughout the life span of the crop being associated with. The effect was observed in more germination rate of seeds, longer stems, larger leaves and early flowering and fruiting. (9)

Inoculation of seeds or seedlings of wheat with a mixed culture of *Azotobacter* had been investigated and an increase of 12-13% in yield was reported at a fertilizer rate below 22 kgN/ha (7). Much higher values (24 and 38%) of yield increase of wheat as a result of seed bacterization were observed (1,3). Rovira (6) in a pot experiment conducted to compare the response of wheat to soil inoculation with *Azotobacter, Clostridium,* or *Bacillus,* reported an increase in yield in response to all three inoculants in loamy soils, but only to *Azotobacter* and *Clostridium* in sandy soils. The use of the same three genera as inoculants of wheat in a field experiment were found to give significant increases in yield in 18 out of 84 field trials. (5)

Although the nitrogen-fixing potential of *Azotobacter* as the sole reason for most of

the beneficial effects obtained has been debated by several investigators (9), and although certain bacterial growth-promoting substances such as indole acetic acid (IAA) and gibberelic acid (GA) have been implicated (2, 4, 8), one cannot totally ignore the value of certain species of *Azotobacter* which were found to fix up to 50 KgN/ha/ year thus supplementing the requirement for this important plant nutrient, particularly when no or low nitrogen fertilization is practised.

The objectives of this work were to obtain preliminary data concerning the response of the two wheat cultivar: Sidi El-Masri and El-Mukhtar to seed bacterization with a mixed culture of three *Azotobacter* isolates selected out of more than 35 local cultures for their highest nitrogen fixing ability.

MATERIALS AND METHODS

1. Preperation of the bacterial inoculum:

Three isolates of *Azotobacter* spp. designated as: ORS-2, YEM-3 and BLM-2 were obtained from local soils and tested for their nitrogen fixing potentials which averaged 18.5, 12.8 and 11.4 mgN/gm CHO consumed, respectively. Each of the three cultures was inoculated into several 250ml flasks containing 100ml of a nitrogen-free sucrose mineral salt medium. After two weeks of incubation at 28 C, the cells were harvested by centrifugation and then suspended in sterilized 10ml increments of a quarter strength Ringer's solution. The three isolates were later mixed together and used for inoculating wheat seeds.

2. Wheat seed treatments:

Wheat seeds of two local cultivars, Sidi El-Masri and El-Mukhtar were obtained from the Agriculture Research Center in Tripoli and tested in the laboratory for their viability. Both gave, approximately, 100% germination. Selection of the most suitable method for seed bacterization was carried out by mixing the seeds after being soaked in Ringer's solution containing the bacterial inoculum with natural gum of an almond tree, peat or wheat flour. Wheat flour gave much better adhesive results and allowed more germintation and establishment of the inoculum by reducing fungal colonization of the seeds.

3. Tube culture experiment:

The experiment was carried out in large test tubes (20cm in length and 2.9cm in diameter). All tubes contained about 70ml of mineral-salt agar having the following composition: (gm/1) 0.5 K_2HPO_4, 0.2 $MgSO_4.7H_2O$, 0.2 NaCl, 0.1 $CaCl_2$, 0.1 $NaMoO_4.2H_2O$ 0.02 $MnSO_4.2H_2O$, 0.02 $FeCl_2$, and 5.0 agar. Supplementary nitrogen was added as $(NH_4)_2SO_4$ at a calculated rate of 0, 20, 40, and 80 KgN/ha. A total of 32 tubes were used for each wheat cultivar, representing four replicates with regard to nitrogen and inoculum additions. Seeds were placed aseptically in the middle of the agar surface inside the tube. The mouth of all tubes was then covered with a piece of a parafilm. Tubes were incubated at room temperature (dailly average 18 C) on benches facing south-east to insure maximum day-light length. The experiment started on Feb. 10 and terminated on Mar. 15. (Fig. 1-6).

4. Data collection:

After almost five weeks of growth, the plants were collected, shoots were separated

from roots, and the roots were then air-dried at room temperature and weighed. Plant tops were weighed when fresh and then oven-dried (70-80C for one hour) and their total nitrogen content was determined following the Kjeldahal digestion and steam distillation procedure. Shoot to root ratios (S/R) were also computed for Sidi El-Masri cultivar.

RESULTS AND DISCUSSION

Seedlings of the two tested cultivars showed an appreciable increase in the mean weight of roots and shoots as a result of seed inoculation with a mixed culture of *Azotobacter* (Table 1 and 2). That seed inoculation with this bacterium produces a significant effect only when combined nitrogen was added at a rate below 22 KgN/ha (7) could also be observed in this experiment (Table 1 and 2). Both wheat cultivars showed a maximum response to the bacterial inoculum when nitrogen was added at a rate of 20 Kg N/ha. The magnitude of increasing growth were 22 and 26% in the mean weight of roots and 24 and 32% in the mean weight of shoots of El-Mukhtar and Sidi El-Masri, respectively.

Table 1 — Effect of seed bacterization on the mean weight of roots (mg) of five weeks wheat seedlings.*

		Sidi El-Masri		%	El-Mukhtar		%
Treat.	KgN/ha	Uninocul.	Inocul.	increase	Uninocul.	Inocul.	increase
A	0	20.6	22.7	10	20.0	23.6	18
B	20	20.6	26.0	26	22.6	27.5	22
C	40	23.0	23.9	4	22.3	25.3	13
D	80	24.3	23.7	--	23.4	24.4	4

* Average of four replicates.

Table 2 — Effect of seed bacterization on the mean weight of shoots (mg) of five weeks wheat seedlings.*

		Sidi El-Masri		%	El-Mukhtar		%
Treat.	KgN/ha	Uninocul.	Inocul.	increase	Uninocul.	Inocul.	increase
A	0	42.1	48.6	15	46.4	46.9	1
B	20	47.7	62.9	32	56.2	69.6	24
C	40	56.8	59.2	4	60.6	70.3	16
D	80	57.6	57.2	--	61.9	65.4	6

* Average of four replicates.

With that much of an increase in the weight of plants and the accompanied increase in the percent total nitrogen of the plant tops which was found to be 32 and 34% for El-Mukhtar and Sidi El-Masri, respectively (Table 3), one would not attribute that to the effect of growth promoting substances produced by the bacterial inoculum alone, and if it were not for the nitrogen fixing ability of the bacterium that supplemented the

nitrogen requirement for plant growth and development, possible nitrogen deficiency symptoms would have eventually occurred particularly at 20 KgN/ ha as a supplement. The calculated S/R values obtained for Sidi El-Masri cultivar may be in favor of the above conclusion (Table 4).

Table 3 — Effect of seed bacterization on the mean total nitrogen (%) of shoots of five weeks wheat seedlings.*

		Sidi El-Masri		%	El-Mukhtar		%
Treat.	KgN/ha	Uninocul.	Inocul.	increase	Uninocul.	Inocul.	increase
A	0	2.94	2.52	--	2.45	2.61	7
B	20	3.33	4.45	34	3.47	4.59	32
C	40	3.24	3.99	23	2.85	3.36	18
D	80	4.56	3.37	--	3.96	4.01	1

* Average of four replicates.

Table 4 — Calculated S/R values for five weeks seedlings of Sidi El-Masri wheat cultivar.

Treatment	KgN/a	Uninoculated	Inoculated
A	0	2.0	2.1
B	20	1.8	2.4
C	40	2.5	2.5
D	80	2.4	2.5

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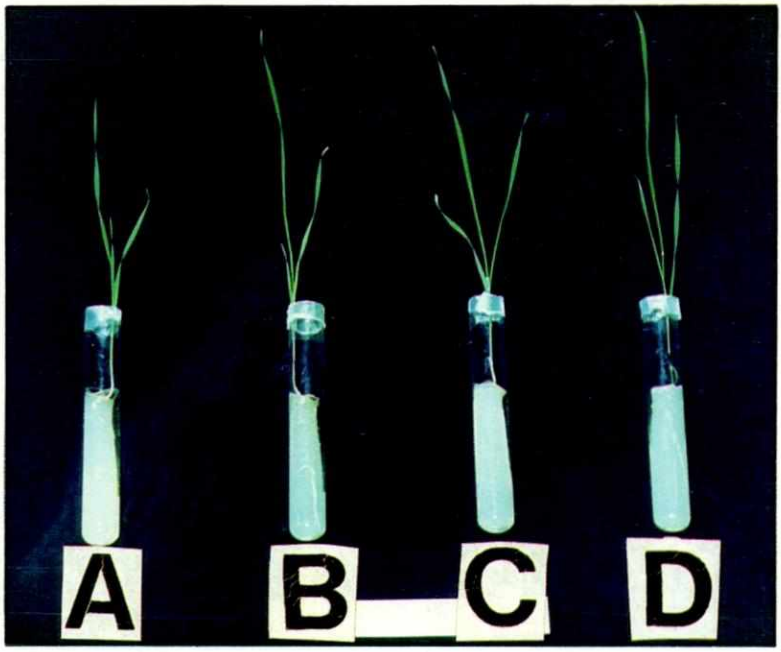

Fig. 1 Response of Sedi El-Masri wheat cultivar growing in mineral-salt agar to nitrogen addition.
(A = 0, B = 20, C = 40, D = 80 KgN/ha)

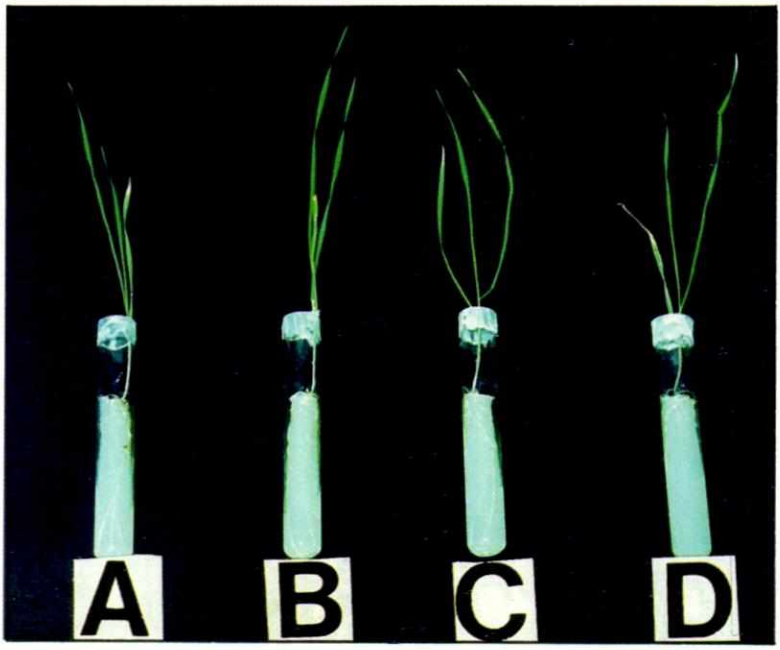

Fig. 2 Response of Sedi El-Masri wheat cultivar growing in mineral-salt agar inoculated with a mixed culture of *Azotobacter* spp. to nitrogen addition.
(A = 0, B = 20, C = 40, D = 80 KgN/ha)

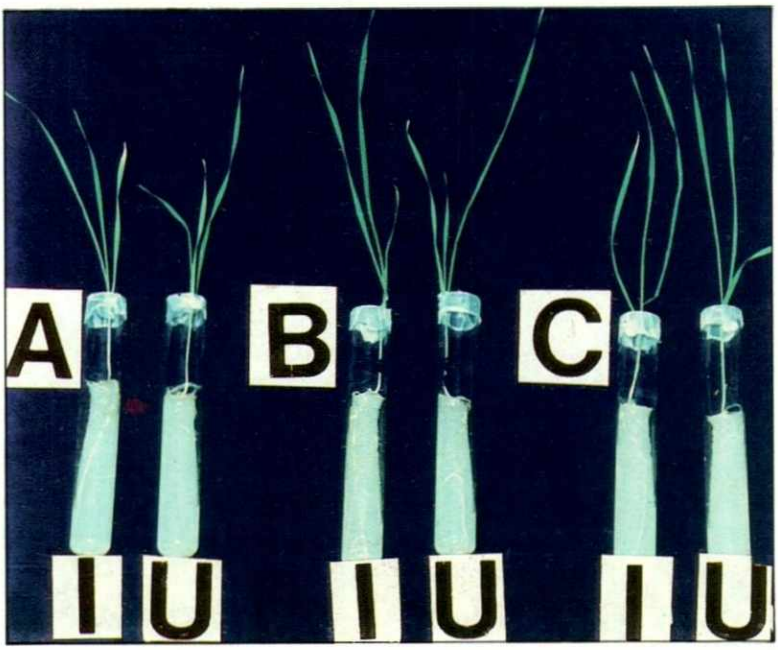

Fig. 3 Response of Sedi El-Masri wheat cultivar growing in mineral-salt agar inoculated (I) and uninoculated (U) with a mixed culture of *Azotobacter* spp. to nitrogen addition.
(A = 0, B = 20, C = 40 KgN/ha)

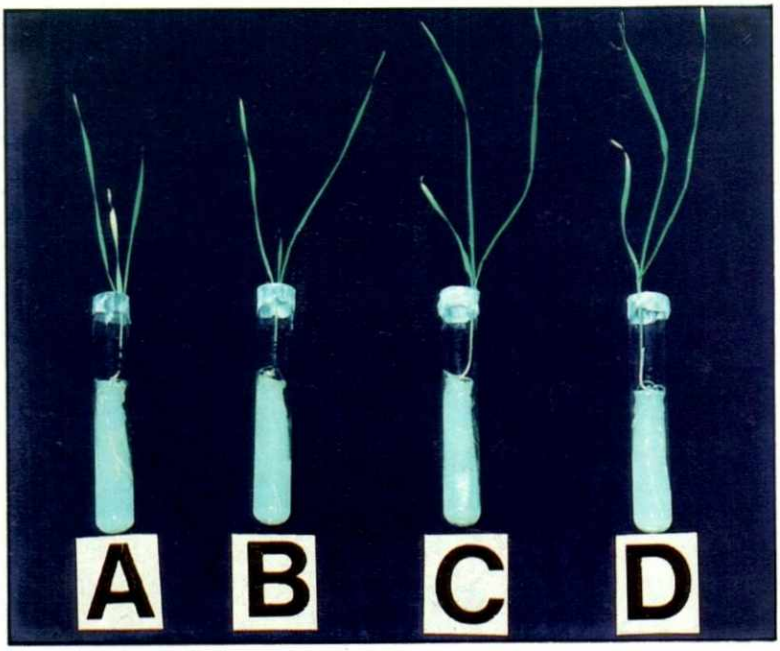

Fig. 4 Response of El-Mukhtar wheat cultivar growing in mineral-salt agar to nitrogen addition.
(A = 0, B = 20, C = 40, D = 80 KgN/ha)

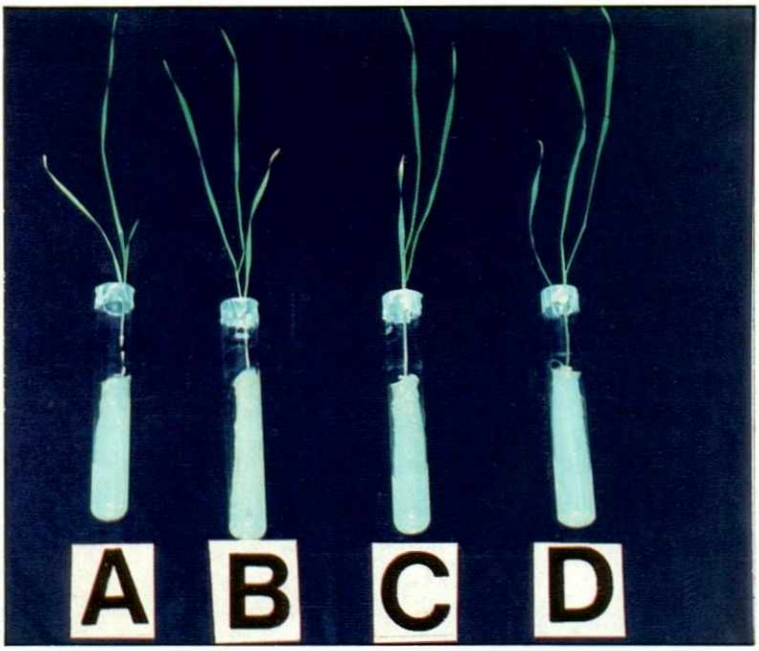

Fig. 5 Response of El-Mukhtar wheat cultivar growing in mineral-salt agar inoculated with a mixed culture of *Azotobacter* spp. to nitrogen addition.
(A = 0, B = 20, C = 40, D = 80 KgN/ha)

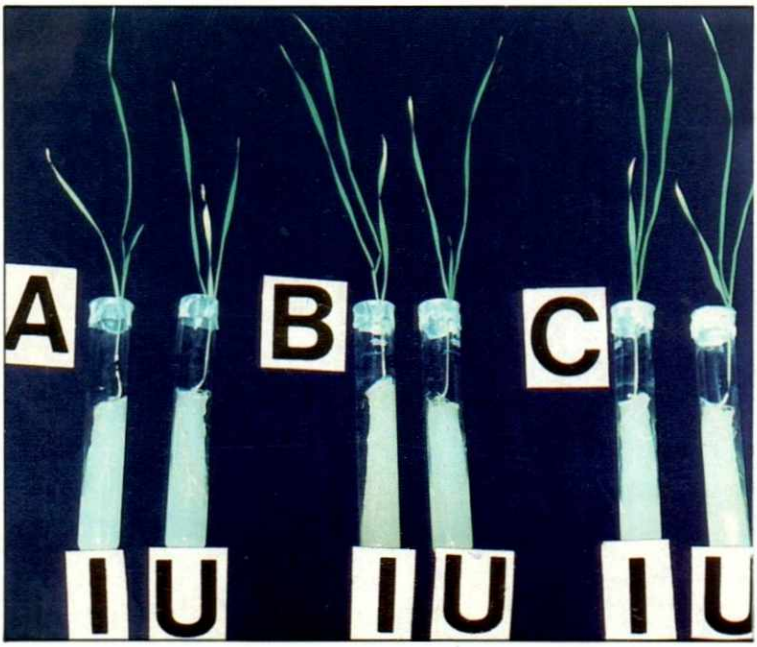

Fig. 6 Response of El-Mukhtar wheat cultivar growing in mineral-salt agar inoculated (I) and uninoculated (U) with a mixed culture of *Azotobacter* spp. to nitrogen addition.
(A = 0, B = 20, C = 40 KgN/ha)

دراسة أولية لمعرفة استجابة بذور صنفين من القمح المحلى للإلقاح البكتيري

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المستخلص

الدراسات الأولية التي أجريت لمعرفة مدى استجابة بذور صنفين من القمح المحلى (سيدي المصري، المختار) للإلقاح البكتيري باستخدام مزرعة خليطة من جنس الأزوتوباكتر كانت مشجعة. أثبتت النتائج المتحصل عليها من زراعة هذين الصنفين في أنابيب ولمدة خمسة أسابيع، أن هناك زيادة في متوسط وزن الجذور بنسبة 22 و 26%، وكذلك زيادة في متوسط وزن المجموع الخضري بنسبة 24 و 32% من الحبوب المعاملة باللقاح البكتيري بالنسبة لصنفي المختار وسيدي المصري، على التوالي.

وجد أن نشاط البكتيريا المستخدمة كلقاح للبذور في تثبيت عنصر النتروجين هو أحد العوامل الأساسية التي قد يساهم بها اللقاح في زيادة نمو النبات. ومما يؤكد ذلك الزيادة في النسبة المئوية للنتروجين الكلي بالمجموع الخضري والتي بلغت 32 و 34% لصنفي المختار وسيدي المصري على التوالي، وكذلك حساب النسبة بين وزني المجموع الخضري الى المجموع الجذري لصنف سيدي المصري.