FACULTY OF AGRICULTURE

Minia J. of Agric. Res. & Develop.
Vol. (43), No.2, pp 179- 192 , 2023

RESPONSE OF SOME CULTIVARS WHEAT (*TRITICUM AESTIVUM L.*) TREATED WITH *AZOTOBACTER VINELANDII* MUTANTS UNDER MINIA REGION CONDITIONS, EGYPT

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Received : 16 July 2023 Accepted: 27 July 2023

ABSTRACT

This study aim evaluation response nine wheat cultivars to treat by with three strains of Azotobacter bacteria (Azotobacter wild type, histidineless and histidineless × threonineless) in two seasons 2020/2021 and 2021/2022. Results confirmed that, treated wheat cultivars by Azotobacter histidineless (M2) recorded the highest mean on growth characters: Plant height (104.05 and 106.40 cm), number of tillers/plant (9.43 and 9.74), spike length (11.11 and 11.10 cm), number of spikelets / spike (20.46 and 20.67), 1000 grain weight (56.41 and 56.81 g), biological yield (6.96 and 7.14 ton/fed) and harvest index (38.68 and 40.65%) in seasons 2020/2021 and 2021/2022, respectively. While Sakha 95 cultivar was recorded the highest all growth character means : plant height (106.30 and 107.33cm), number of tillers/plant (9.88 and 11.33) and spike length, but Misr 1 cultivars was recorded the lowest plant height (91.26 and 91.26 cm), number of tillers/plant (8.04 and 9.05) and spike length (9.01 and 9.05 cm), biological yield (7.48 and 6.84 ton/fed) and Harvest index (41.67 and 42.75%) in seasons 2020/2021 and 2021/2022, respectively. Finally, we can summarize the results to the treatment of Sakha 95 wheat cultivars by Azotobacter histidineless record the best yield of wheat.

Keywords: Azotobacter, wheat, cultivars, mutants

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered to be one of the most important crops in the world and especially in Egypt, came between four crops namely rice, maize and barely **(FAO, 2016)**. Wheat (*Triticum aestivum* L.) is considered that the most important humans food which provide about 20% for over one third of world people and about 30% from cereal food **(Namvar and Khandan 2013)**. The gap between wheat production and consumption in Egypt which need to exerted efforts for increasing wheat production **(Attia and Barsoum, 2013)**. Biofertilizer has a direct effect on yield components characters (**Zaki et al., 2012 and Zaki et al., 2016**). Biofertilizers inoculation significantly increased most growth and yield parameters, yeast had superiority on Azotobacter. Moreover, mixed inoculums, generally, had more favorable effect on the majority of studied parameters than single inoculants **(El-Sirafy *et al.*, 2006; Bahrani and Pourreza, 2010; Nawab *et al.*, 2006**. *Azotobacter* biofertilizer has an important role in fixing atmospheric nitrogen in rhizosphere zone of wheat and many other crops and fertility of soil **(Venkatashwarlu, 2008; Rehman *et al.*, 2017)**. Azotobacter is the most important free living organism able to fix molecular nitrogen. Extensive researchers were carried out in different countries concerning their distribution, densities and capacity of nitrogen fixation.

The extensive bacteriological studies of soils of the Nile valley provide their richness in free living Azotobacter bacteria, and it's well established that they play an important role in the replenishment of soil nitrogen **(Abd E-Malik and Ishac, 1980).** The effect of grain or soil inoculation by Azotobacter spp, on plant growth had been studied by several authors e.g. **(Badawy and El-Shafey, 1974)**. On the other hand, the nitrogen content of soil or plant had been studied after inoculation with *Azotobacter vinelandii* **(Fayez, 1980)**.

MATERIALS AND METHODS

This study was carried out Farm of Faculty of Agriculture, Minia University, EL-Minia Governorate, Egypt, during two seasons of 2020/2021and 2021/2022 to study the response of wheat cultivars to strains of *Azotobacter vinelandii*. The experimental design was split plot. Nine wheat cultivars Misr 1, Misr 2, Misr 3, Sakha 95, Sids 1, Sids 12, Shandaweel 1, Giza 168 and Giza 171.

Each wheat cultivar treated with three Azotobacter strains (Azotobacter wild type M1), Azotobacter histidineless (M2) and Azotobacter histidineless × threonineless (M3). Main Azotobacter bacteria concentrate was 10^6-10^7 cell/ml which diluted with water by 1 bacteria cell : 99 distilled water **(Sanjay and Asmita, 2018)**.

Soil analyzing:

This study was carried out in faculty of Agriculture land, soil contained from a clay loam texture. The physical and chemical properties were determined according to method of **Avery and Bascomb (1982)** in seasons 2020/2021 and 2021/2022.

Bacteria (Azotobacter) preparation:

Strains: A wild type strain of *Azotobacter vinelandii*, four mutants and five intraspecific hybrids previously isolated, developed and tested for nitrogen fixation in *Hordium vulgare* **(Abdel-Rahem et al. 1995)** were used in the present work. These strains are, Azotobacter wild type, histidineless and histidineless × threonineless. This is strains the highest growth on media.

- Medium: a. complete media (CM): was prepared according to **Strandberg and Wilson (1968)**. b. Media free nitrogen : was prepared according to **Mckenney and Melton, (1986).**

- Preparation of bacterial culture for field inoculation: three flasks, each containing 500 ml liquid complete medium, were inoculated with one of the three strains under study and then incubated at 30 ºC on a shaker for 48 hr. The condensed growth in each flask (10 cell/ml) was diluted in 5.0 L distilled water.

The experimental design was randomized complete block design in split plot arrangement with three replicates was used .The cultivars arranged in the main plot and Azotobacter strains were allocated in sub – plot .Each plot consisted of 15 row, 3m long and 15cm between rows .

Characters studied:

At harvest, ten inner rows from each plot were harvested and five plants were taken randomly to estimate the following data:

Plant height(cm.): measured at harvest from soil surface to the tip of the spike of the main stem.

Number of tillers/plants.

Spike length (cm.): measured at harvest from the main stems, which were used for estimation of plant height.

Number of spikelets/spikes: determined as number of fertile and sterile spikelets of ten spikes from each plot at harvest.

1000 grain weight (g.): determined from the three random samples each contained 1000 grains, taken from each plot, then the main of grain index was recorded.

Biological yield (ton/fed.): ten inner rows of 5.25m^2 of each plot harvested and weighted in kg., then transformed into ton /fed.

Harvest Index: was calculated using the following formula:

Harvest Index= (grain yield/biological yield) ×100.

Statistical analysis: The collected data were statistically analyzed according **to Mcintosh (1983) and Gomez (1984).** The treatment means were compared using LSD test according to **Steel and Torrie (1980)**

RESULTS AND DISCUSSION

Results pointed out that significantly affected by treated the nine different wheat cultivars with three strains of Azotobacter bacteria (Azotobacter wild type, histidineless and histidineless × threonineless) compared with control.

Data in Table (2) and Fig. (1) showed that treated wheat cultivars by Azotobacter histidineless recorded the highest mean for Plant height (104.05 and 106.40 cm), number of tillers/plant (9.43 and 9.74) and spike length (11.11 and 11.10 cm) on seasons 2020/2021 and 2021/2022, respectively. This is data are agreement with **Sharma, (1987) & Attia, M.A. and M.S.Barsoum (2013) and Zaki, et al. (2012)** who found that Azotobacter treatments in wheat results in increased plant height, tillers, and ear length of wheat.

On the other hand Sakha 95 cultivar was recorded the highest mean 106.30, 9.88 and 11.33 on season 2020/2021and 107.88, 11.33 and 11.33 on season 2021/2022 for Plant height, number of tillers/plant and spike length. While the lowest mean plant height was recorded in Misr 1 cultivar 91.26 and 91.62 cm for seasons 2020/2021 and 2021/2022, respectively. Also, the lowest mean number of tillers as 8.04 in Misr 1,2 cultivar (8.04), while the shorter mean spike length was 9.01 and 9.05cm in Misr 1 cultivar for seasons 2020/2021 and 2021/2022, respectively. In addition to, results showed significantly affect between wheat cultivars growth characters, which Sakha 95 and Shandaweel 1cultivers significant differences compared with other cultivars.

Also, data in Table (3) and Fig. (2) revealed that increase mean number of spikelets on spike with treated by Azotobacter histidineless (20.46 and 20.67) however, wheat control record the lowest mean (18.22 and 18.00). On the other side weight of 1000 grain was increased with treated by Azotobacter histidineless 56.41 and 56.81 g for seasons 2020/2021 and 2021/2022, respectively This is data are agreement with **Zaki, et al. (2012) and Sanjay and Asmita, (2018)** . Finally Sakha 95 cultivar was recorded the highest mean of number of spikelets 20.83 and 20.76 between all cultivars Sakha 95 cultivar record the bigger weight for 1000 grain 54.58 and 55.33 g for seasons 2020/2021 and 2021/2022, respectively. Also, results revealed that significantly affect between wheat cultivars number of spikelets and 1000 grain weight characters. These data came in the same direction of **Egamberdieva, et al. (2008)** & **Esmailpour, et al. (2013) and**

Rasool, et al (2013) those found that Azotobacter bacteria play big role in increase of spikelets and 1000 grain weight.

Finally, data in Table (4) and Fig. (3) pointed that Sakha 95 recorded the highest the highest biological (7.48 and 6.84 ton/fed) and Harvest index (41.67 and 42.75%), while for seasons 2020/2021 and 2021/2022, respectively. On the other hand treated with Azotobacter histidineless record the highest biological yield (6.96 and 7.14 ton/fed) and harvest index (38.68 and 40.65%) for seasons 2020/2021 and 2021/2022, respectively, these results are agreement with **Hassanein et al., 2018**

Table (1) Physical and chemical soil properties.

Soil physical properties	Value
Sand	27.3%
Silt	33.2%
Clay	39.5%
F.C.	44.55%
PWP	14.6%
WHC	47. 6%
Soil chemical properties	**Value**
pH	7.5
CaCO3	18.2 g/ kg
Total N	1.75 g/ kg
Total C/N ratio	23.2
EC	1.31
Organic N	0.82 g/ kg
Organic C/N ratio	23.21
Mineral N	56.9 mg/ kg
CEC	39.16 cmolc/ kg

Table (2): Mean plant attributes (Plant height, no. of tillers/plant and spike length) of wheat cultivars treated with strains of *Azotobacter vinelandii* during seasons of 2020/2021 and 2021/2022.

Cultivars	2020/2021														
	Plant height (cm)				Mean	No. of tillers/plant				Mean	Spike length (cm)				Mean
	M1	M2	M3	Control		M1	M2	M3	Control		M1	M2	M3	control	
Misr 1	89.20	95.93	93.10	86.80	**91.26**	7.90	8.67	8.30	7.30	**8.04**	8.50	9.20	10.13	8.20	**9.01**
Misr 2	103.43	108.00	100.33	83.77	**98.88**	7.73	8.57	8.63	7.23	**8.04**	8.93	10.03	10.17	8.17	**9.33**
Misr 3	98.80	101.60	97.43	87.50	**96.33**	8.17	9.13	8.93	7.97	**8.55**	8.50	9.67	10.07	7.93	**9.04**
Sakha 95	109.57	115.07	106.73	93.77	**106.30**	9.70	11.10	10.10	8.70	**9.88**	11.43	12.97	11.90	9.03	**11.33**
Sids 1	95.17	96.90	93.83	87.97	**93.47**	8.33	8.60	8.47	7.87	**8.32**	9.77	12.10	9.93	8.33	**10.03**
Sids 12	98.87	100.43	90.87	90.33	**95.13**	8.20	9.37	8.77	7.63	**8.49**	10.40	10.97	10.70	8.63	**10.18**
Shandaweel 1	107.13	108.03	102.80	92.10	**102.50**	8.70	10.60	9.63	8.27	**9.29**	11.17	12.37	11.10	8.80	**10.86**
Giza 168	101.30	105.73	100.43	88.50	**98.99**	7.93	9.13	8.20	7.77	**8.26**	9.40	11.50	11.60	9.27	**10.44**
Giza 171	104.40	104.77	91.30	89.73	**97.55**	8.50	9.80	9.40	8.13	**8.96**	9.43	11.20	10.63	8.47	**9.93**
G. mean	**100.87**	**104.05**	**97.43**	**88.94**		**8.35**	**9.43**	**8.93**	**7.87**		**9.73**	**11.11**	**10.69**	**8.54**	
LSD 5%	**8.474**	**7.248**	**7.173**	**7.062**		**0.653**	**0.843**	**0.629**	**0.635**		**0.987**	**1.022**	**0.944**	**1.304**	
2021/2022															
Misr 1	89.27	97.30	93.10	86.80	**91.62**	8.70	9.30	9.23	7.60	**9.05**	8.67	9.20	10.13	8.20	**9.05**
Misr 2	98.53	107.73	101.07	84.30	**97.91**	8.60	8.57	9.03	7.47	**9.43**	9.33	10.03	10.17	8.17	**9.43**
Misr 3	97.03	103.97	99.43	88.47	**97.23**	9.13	10.00	9.40	8.30	**9.12**	8.80	9.67	10.07	7.93	**9.12**
Sakha 95	108.57	116.93	111.27	94.77	**107.88**	9.93	11.63	10.00	8.97	**11.33**	11.43	12.97	11.90	9.03	**11.33**
Sids 1	95.57	102.53	98.47	88.17	**96.18**	8.97	8.60	8.53	8.07	**10.08**	9.77	12.10	10.13	8.33	**10.08**
Sids 12	97.77	102.70	100.07	91.00	**97.88**	8.87	9.37	9.17	8.13	**10.18**	10.40	10.97	10.70	8.63	**10.18**
Shandaweel 1	106.40	112.77	108.13	92.17	**104.87**	9.73	10.70	9.77	8.77	**10.86**	11.17	12.37	11.10	8.80	**10.86**
Giza 168	98.27	105.73	103.70	87.63	**98.83**	8.87	9.33	8.63	8.30	**10.44**	9.40	11.50	11.60	9.27	**10.44**
Giza 171	100.27	108.00	102.13	90.20	**100.15**	9.23	10.20	9.67	8.50	**9.93**	9.43	11.20	10.63	8.47	**9.93**
G. mean	**99.07**	**106.40**	**101.90**	**89.30**		**9.11**	**9.74**	**9.27**	**8.23**		**9.82**	**11.10**	**10.70**	**8.54**	
LSD 5%	**8.098**	**7.433**	**7.450**	**7.111**		**0.702**	**0.858**	**0.699**	**0.687**		**1.003**	**0.972**	**0.956**	**1.388**	

M1= Azotobacter wild type
M2= Azotobacter histidineless
M3= Azotobacter histidineless × threonineless

Table (3): Mean plant attributes (No. of spikelets /spike and 1000 grain weight (g) of wheat cultivars treated with strains of *Azotobacter vinelandii* during seasons of 2020/2021and 2021/2022.

Cultivars	No. of spikelets/ spike				Mean	1000 grain weight (g)				Mean
	M1	M2	M3	Control		M1	M2	M3	Control	
2020/2021										
Misr 1	17.60	19.10	18.53	15.93	**17.79**	46.33	52.67	51.00	43.67	**48.42**
Misr 2	18.53	20.67	20.27	18.73	**19.55**	45.00	54.67	49.33	46.33	**48.83**
Misr 3	18.53	18.33	19.67	18.43	**18.74**	45.00	55.67	51.33	48.00	**50.00**
Sakha 95	20.07	22.97	20.97	19.30	**20.83**	50.33	64.00	57.33	46.67	**54.58**
Sids 1	18.73	20.13	19.67	17.87	**19.10**	46.33	54.67	51.67	45.67	**49.58**
Sids 12	18.40	20.10	19.57	18.07	**19.03**	44.33	53.67	51.00	44.33	**48.33**
Shandaweel 1	19.80	21.17	19.90	18.83	**19.93**	48.33	62.00	52.67	45.67	**52.17**
Giza 168	18.83	20.73	19.43	18.63	**19.41**	48.33	52.33	50.33	44.00	**48.75**
Giza 171	19.40	20.93	19.80	18.17	**19.58**	47.33	58.00	50.67	44.33	**50.08**
G. mean	**18.88**	**20.46**	**19.76**	**18.22**		**46.81**	**56.41**	**51.70**	**45.41**	
LSD 5%	**0.753**	**0.754**	**0.842**	**1.088**		**4.367**	**8.206**	**5.803**	**5.282**	
2021/2022										
Misr 1	17.90	19.57	18.53	15.83	**17.96**	46.67	53.33	49.67	45.00	**48.67**
Misr 2	18.67	20.83	19.37	18.53	**19.35**	48.33	55.33	49.00	47.00	**49.92**
Misr 3	18.83	18.73	19.67	18.20	**18.86**	49.33	56.67	52.00	48.00	**51.50**
Sakha 95	20.20	22.97	20.97	18.90	**20.76**	52.00	63.33	58.33	47.67	**55.33**
Sids 1	18.97	20.27	19.67	17.67	**19.14**	48.33	55.33	52.33	46.67	**50.67**
Sids 12	18.60	20.27	19.57	17.93	**19.09**	47.33	54.33	51.67	45.67	**49.75**
Shandaweel 1	19.83	21.4	19.90	18.33	**19.87**	50.33	61.33	54.33	47.00	**53.25**
Giza 168	18.83	20.97	19.43	18.63	**19.47**	47.33	53.33	51.00	45.00	**49.17**
Giza 171	19.40	21.03	19.80	18.00	**19.56**	49.67	58.33	52.33	47.00	**51.83**
G. mean	**19.03**	**20.67**	**19.66**	**18.00**		**48.81**	**56.81**	**52.3**	**46.56**	
LSD 5%	**0.788**	**0.750**	**0.823**	**0.987**		**4.561**	**8.322**	**5.845**	**5.347**	

M1= Azotobacter wild type
M2= Azotobacter histidineless
M3= Azotobacter histidineless × threonineless

Table (4): Effect of Azotobacter strains (*Azotobacter vinelandii*) on Biological Yield and Harvest index of wheat cultivars during seasons of 2020/2021and 2021/2022.

2020/2021										
	Biological Yield (ton/fed.)				Mean	Harvest index %				Mean
Cultivars	M1	M2	M3	Control		M1	M2	M3	Control	
Misr 1	6.95	8.89	6.78	4.97	**6.90**	37.23	36.36	38.62	35.26	**36.87**
Misr 2	5.86	5.67	5.87	5.33	**5.68**	36.00	36.02	35.26	34.99	**35.57**
Misr 3	6.80	6.45	6.36	4.33	**5.99**	38.26	37.89	37.00	36.25	**37.35**
Sakha 95	7.78	8.75	8.88	4.50	**7.48**	41.82	43.01	42.22	39.62	**41.67**
Sids 1	6.78	7.14	6.82	4.87	**6.40**	35.09	34.25	34.58	35.29	**34.8**
Sids 12	6.17	5.60	6.09	5.65	**5.88**	37.89	37.86	36.36	37.02	**37.28**
Shandaweel 1	7.78	7.50	7.15	5.56	**7.00**	40.21	41.39	40.00	41.42	**40.76**
Giza 168	6.80	7.37	7.00	5.08	**6.56**	39.25	38.99	39.02	37.88	**38.79**
Giza 171	5.87	5.30	5.18	5.67	**5.50**	41.23	42.36	40.06	38.69	**40.59**
G. Mean	**6.76**	**6.96**	**6.68**	**5.11**		**38.55**	**38.68**	**38.12**	**37.38**	
LSD 5%	**0.689**	**0.538**	**0.772**	**0.685**		**5.361**	**6.288**	**8.266**	**5.876**	
2021/2022										
Misr 1	5.10	8.00	6.78	4.97	**6.21**	38.63	39.58	39.02	38.88	**39.03**
Misr 2	5.20	5.30	5.87	5.67	**5.51**	39.36	41.58	39.78	36.69	**39.35**
Misr 3	6.80	6.45	6.36	4.33	**5.99**	41.03	40.25	39.02	37.88	**39.55**
Sakha 95	4.67	10.00	7.15	5.56	**6.84**	44.28	42.35	43.29	41.07	**42.75**
Sids 1	6.78	7.14	6.00	4.87	**6.20**	38.27	39.26	37.78	35.26	**37.64**
Sids 12	4.73	5.60	6.09	5.65	**5.52**	41.78	40.25	36.36	35.36	**38.44**
Shandaweel 1	6.80	7.37	7.00	5.08	**6.56**	41.00	41.58	41.00	40.58	**41.04**
Giza 168	5.31	5.67	6.29	5.33	**5.65**	42.23	41.48	39.00	36.00	**39.68**
Giza 171	7.78	8.75	8.88	5.40	**7.70**	38.25	39.56	37.89	36.78	**38.12**
G. Mean	**5.91**	**7.14**	**6.71**	**5.21**		**40.53**	**40.65**	**39.24**	**37.61**	
LSD 5%	**0.623**	**0.553**	**0.823**	**6.856**		**6.278**	**7.233**	**6.228**	**5.268**	

M1= Azotobacter wild type
M2= Azotobacter histidineless
M3= Azotobacter histidineless × threonineless

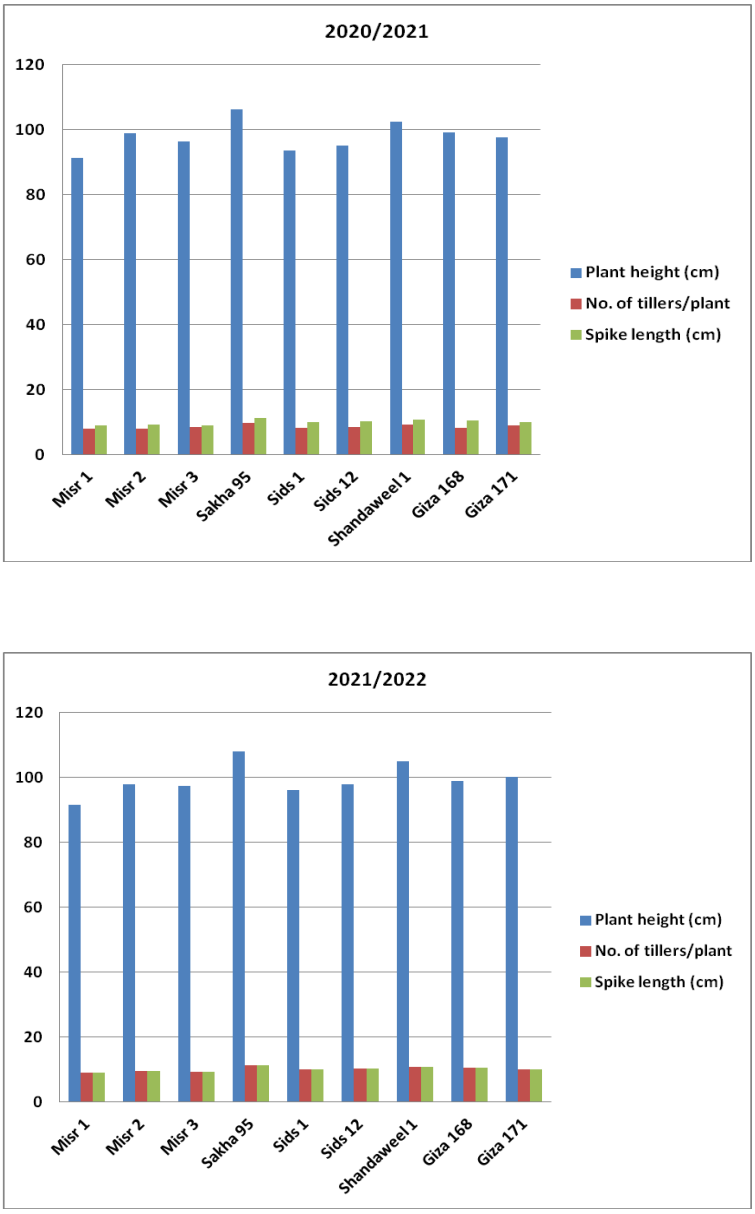

Figure (1): Mean plant attributes (Plant height, no. of tillers/plant and spike length) of wheat cultivars treated with strains of *Azotobacter vinelandii* during seasons of 2020/2021 and 2021/2022.

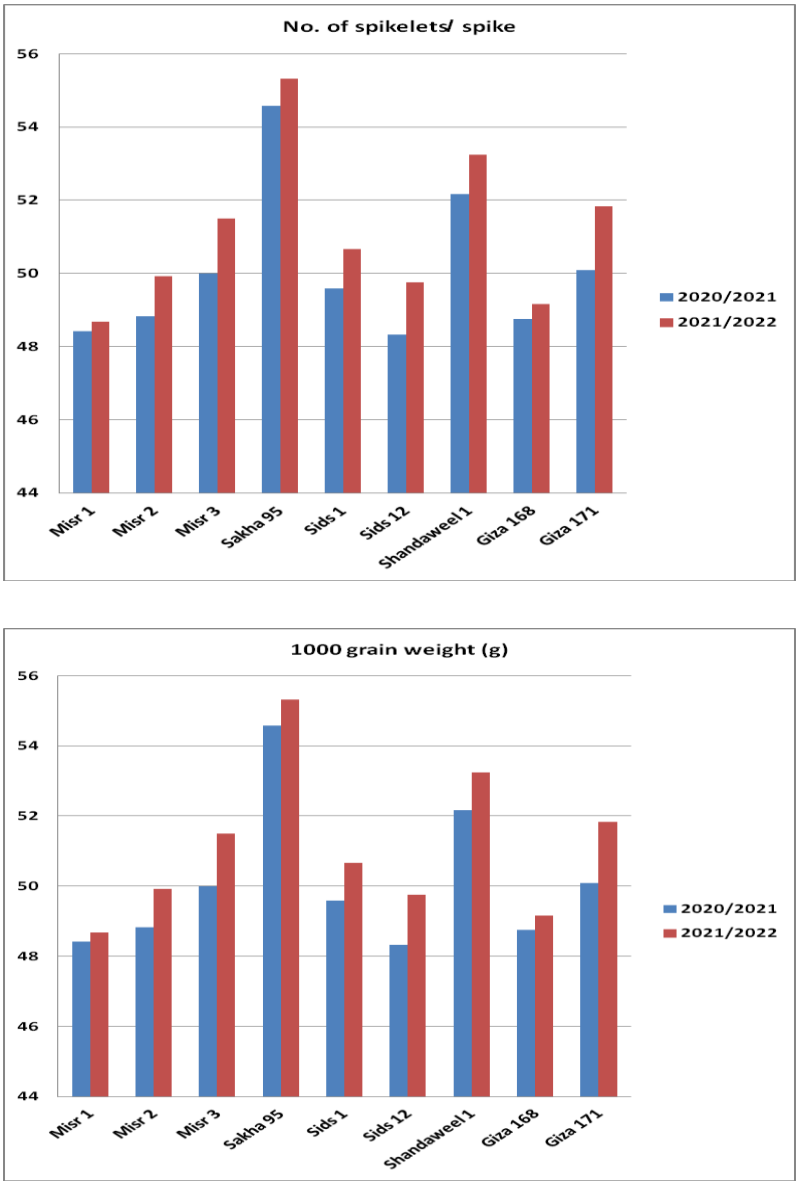

Figure (2): Mean plant attributes (No. of spikelets /spike and 1000 grain weight (g) of wheat cultivars treated with strains of *Azotobacter vinelandii* during seasons of 2020/2021and 2021/2022.

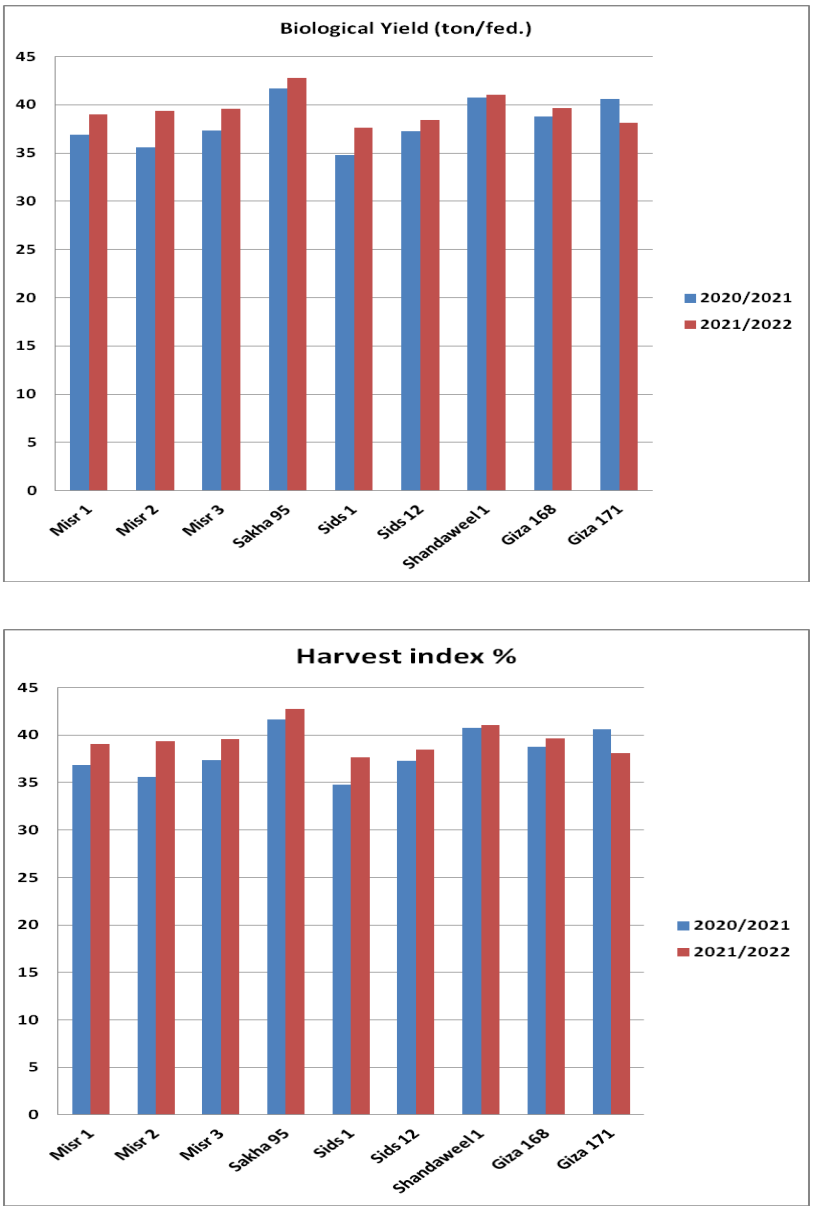

Figure (3): Effect of Azotobacter strains (*Azotobacter vinelandii*) on Biological Yield and Harvest index of wheat cultivars during seasons of 2020/2021and 2021/2022.

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الملخص العربي

استجابة بعض أصناف القمح للمعاملة بطفرات الازوتوبكتر (*Azotobacter vinelandii*) تحت الظروف المناخية بالمنيا , مصر

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هدفت هذه الدراسة إلى تقييم استجابة تسعة أصناف من القمح تم معاملتها بثلاث سلالات من بكتريا الازوتوبكتر (Azotobacter wild type, histidineless and histidineless × threonineless) في موسمين 2021/2020 و 2022/2021. أكدت النتائج أن أصناف القمح المعالجة بواسطة (Azotobacter histidineless) سجلت أعلى متوسط في صفات النمو: ارتفاع النبات (104.05 و 106.40 سم) ، عدد الأفرع / نبات (9.43 و 9.74) ، طول السنبلة (11.11 و 11.10 سم). عدد السنيبلات / سنبلة (20.46 و 20.67) ، وزن 1000 حبة (56.41 و 56.81 جم) ، المحصول البيولوجي (6.96 و 7.14 طن / فدان) ومؤشر الحصاد (38.68 و 40.65٪) في موسمي 2021/2020 و 2021/م2022 ، على التوالى. بينما سجل الصنف سخا 95 أعلى صفات النمو: ارتفاع النبات (106.30 و 107.33 سم) وعدد الأفرع / نبات (9.88 و 11.33) وطول السنبلة ، بينما سجل الصنف مصر 1 أقل ارتفاع للنبات (91.26 و 91.26) سم) وعدد الأفرع / نبات (8.04 و 9.05) وطول السنبلة (9.01 و 9.05 سم) والمحصول البيولوجي (7.48 و 6.84 طن / فدان) ومؤشر الحصاد (41.67 و 42.75٪) في موسمي 2021/2020 و 2021 / 2022 على التوالي. أخيرًا ، يمكننا تلخيص نتائج معاملة أصناف قمح سخا 95 بواسطة Azotobacter histidinless لتسجيل أفضل محصول للقمح.