

RESPONSIVENESS OF DIFFERENT WHEAT GENOTYPES TO NITROGEN BIOFERTILIZERS

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ABSTRACT

This work aimed to study nitrogen response and sufficiency indices using some Egyptian bread wheat varieties and their F₁ hybrids in order to detect the existed genetic variabilities and genetic behaviours for grain yielding under *Azospirillum* and *Anabeana* biofertilizers.

Genotypes exhibited variations in grain yielding under the effect of both biofertilizer forms. Mean performance showed that Sids 1 and Sids 7 produced higher grain yielding under *Azospirillum* and *Anabeana* inoculation. Also, F₁ hybrids resulted from hybridization between Sakha 8 × Sids 7 appeared high grain yielding under both biofertilizer forms. This indicated the superiority of these hybrids in response to biofertilizers. *Azospirillum* spp existed higher genotypic variations than *Anabeana oryza*. In addition, estimates of generation means appeared significant effects on grain yielding under the effect of *Anabeana* biofertilizer.

Response to biofertilizers and N sufficiency in F₁ hybrids reflected the possess of different genes to their hybrids, which varying in their actions and interactions with biofertilizers which affect on expression of the genes related to grain yielding. On the other hand, biofertilizer supply forms reflecting some sort of genotype- environment interaction in some traits related to nitrogen fixation.

Keywords: Additive genes, *Anabeana*, *Azospirillum*, biofertilizers, bread wheat.

INTRODUCTION

Nitrogen (N) fertilization plays a central role for improving the yield in wheat plants. High N use efficiency (NUE) is desired to protect ground and surface waters (Salvagiotti *et al.* 2009). The genetic informations about the Egyptian wheat varieties can help to explain the genotypic variations existed for their responses to N biofertilization.

A wide literature exists on (NUE) and its components has been reviewed before by Parry and Reynolds, 2007; Dawson *et al.*, 2008; Bradley and Kindred, 2009; Abedi *et al.*, 2011; El-Sayed *et al.*, 2013 and Peter *et al.*, 2014. *Azotobacter chroococcum* strains achieved positive effect on the yield and N concentration in grains (Kizikaya, 2008).

Nitrogen response index (NRI), is an indicative of the percentage increase in yield that could be obtained via N fertilization, *i.e.* determining the actual response of a given wheat genotype to applied N. But N sufficiency index of the studied wheat genotypes expressed as N response index but in inverse trend.

This investigation aimed to study the effect of genotypic variations in Egyptian wheat genotypes, parents and their F₁'s, on N response and N sufficiency under biofertilization with *Azospirillum* and *Anabeana*, as well as, to examine the genetic components of generation means under each N treatment.

MATERIALS AND METHODS

I- Genetic materials:

Three Egyptian bread wheat (*Triticum aestivum*, L.) varieties were used in this study. These varieties were supplied from Wheat Research Section., Agriculture Research Center., Ministry of Agriculture, Giza, Egypt. The pedigree and origin of these varieties are shown in Table 1.

Table 1. Breeding history and pedigree of wheat varieties used in this study.

Entry	Name	Pedigree
1	Sakha 8	Indus 66/Norteno "S" Pk 3418-6 _s -lsw-Os
2	Sids 7	Maya "S"/Mon "S"// CMH 74 A59 2/3/sakha8*
3	Sids 1	HD2172/Pavon "S"//1158.57//Maya 74 "S"

These varieties were found to be different in their response to nitrogen reaction according to the results of Seham Mohamed (2002).

This investigation was carried out at the Experimental Farm of the Faculty of Agriculture, Zagazig University during the winter growing seasons of 2011 and 2012. The parental wheat plants were crossed in 2011 using Sakha 8 (P₁) as a common female parent, as well as, Sids 7 (P₂) and Sids 1 (P₃) as male parents. Biofertilization tests were done in 2012, using parental and F₁ grains grown in plastic bages. Each bag filled with 5 kg of mixture of clay soil and sand (2:1). Nitrogen content in the soil was 17.5 ppm. Three treatments were applied, no nitrogen supply as a control, *Anabaena oryzae* and *Azospirillum* spp. The bags were arranged in a complete randomized block design using three replicates. Each genotype represented by nine bags *i.e.* three bags for each biofertilization treatment. In each bag, five grains were planted and after two weeks the seedlings were thinned to three seedlings.

Two associative rhizosphere bacteria, *Azospirillum brasilense* and *Anabeana oryza* were used for inoculation treatments. Efficient strains of these rhizobacteria were kindly provided by unit of Biofertilizers, Faculty of Agriculture, Zagazig University. *Azospirillum* inoculum was prepared using four days old cultures in a liquid medium. Cultures were incubated at 28°C under static conditions. The *Anabeana* inoculum was prepared using seven days old cultures in a liquid medium, cultures were incubated at 28°C in a rotary shaker. The growth media for both *Azospirillum* and *Anabeana* were done according to Knowles (1982). Cell density of each inoculating bacteria form both cultures was adjusted to 6.0×10^8 cells/ml.

II- Methods:

Grains of the studied wheat genotypes were surfacely sterilized with acidified 0.01% HgCl₂ for 5 min and then washed thoroughly several times with sterilized distilled water.

Sterilized grains of each genotype (parent or hybrid) were divided into three groups. The control grain were surfacely heat-sterilized inoculum. The other two groups were inoculated by soaking for 20 min in each bacterial

liquid culture using Arabic gum as adhesive agent. The inoculated grains and controls grains were left for air drying before sowing in the field. The bacterial count per grain at the time of inoculation was ranged from 20×10^4 to 30×10^4 cells per grain.

Inocula solutions were added once at sowing and then individual plants from each replicate per treatment were harvested at maturity to be measured grain yield. Grain yield was used in calculating N response index (NRI), and N sufficiency index according to Singh and Arora (2001).

$$\text{N response index (NRI)} = \text{GY}_f/\text{GY}_o,$$

$$\text{N sufficiency index (NSI)} = \text{GY}_o/\text{GY}_f$$

Where:

GY_o = grain yield/ plant of control in gm.

GY_f = grain yield/ plant of N fertilized treatment in gm.

The obtained data was statistically analysed using analysis of variance according to Sokal and Rohlf (1995). Means and their standard errors were calculated. The genetic components of generations means under each biofertilization treatment were determined according to Kearsey and Pooni (1996).

RESULTS AND DISCUSSION

Data in Table 2 illustrate mean performance of grain yield by parental genotypes and their F_1 hybrids under two biofertilization forms. The analysis of variance are presented in Table 3.

The grain yield of wheat genotypes (parents and F_1 hybrids) was varied between both biofertilization, which showing higher yield over control, as well as, exhibiting significant variations. Higher grain yield was clearly observed under biofertilization of *Azospirillum* rather than *Anabeana*. Plant genotypes showed lower values in grain yielding with *Anabeana* rather than higher values exhibited with *Azospirillum*. For instance, Sakha 8 showed the lowest value under *Anabeana* and highest value with *Azospirillum* if compared with all the other parents. On the other hand, Sids 1 plants exhibited higher grain yielding when they are biofertilized with *Anabeana* and *Azospirillum*. Genotypic differences appeared herein between the parental genotypes may be due to their differences in responses to biofertilization owing to their different genetic backgrounds which affect on root exudates, as well as, the activity of biofertilization in the rhizosphere (Seham Mohamed 2002).

Table 2. Mean performance of grain yield (gm/plant) under different biofertilizer forms.

Genotype	Control	<i>Anabeana</i>	<i>Azospirillum</i>
Sakha8	1.577	1.640	3.640
Sids1	4.400	4.890	5.483
Sids7	3.000	3.497	3.663
Sakha8 x Sids1	2.940	2.603	4.240
Sakha8 x Sids7	4.133	5.110	5.613
L.S.D at 5%	0.621	1.020	0.974

Table 3. Mean squares from the analysis of variance for grain yield produced under different biofertilizers forms.

S.O.V	D.F	Control	<i>Anabeana</i>	<i>Azospirillum</i>
Reps	2	1.462	2.222	3.618
Genotypes	4	3.790*	6.582*	2.782
P	2	5.979*	7.976*	3.355
F ₁	1	2.136	9.425*	2.829
PxF ₁	1	1.067	0.947	1.589
Error	8	0.579	1.555	1.422

* = Significant at 5%.

The expression of grain yielding by F₁ hybrids appeared the same trend as their parental genotypes, showing lower values under *Anabeana* biofertilization form and higher values under *Azospirillum*. Grain yield was increased in F₁ hybrids than their common parent, Sakha 8. This agreed with Zhao *et al.* (2009) who found varietal differences in N accumulation and partitioning to the grain among oat cultivars grown in pots and subjected to five N fertilization regimes. Peter *et al.* (2014) studied twenty varieties of wheat (*Triticum aestivum* L.) that were grown with low and high supplies of nitrogen (N) in the field experiment and found significant genetic variation in grain yielding as a crop performance.

Nitrogen response index and N sufficiency index are presented in Table 4. Regarding N response index, it is an indicative of the percentage increase in yield that could be obtained via N fertilization, *i.e.* determining the actual response of a given wheat genotype to applied N. Plant genotypes were nearly similar in N response index under both biofertilizer forms. For instance all parental genotypes were similar in response index under the effect of biofertilization with *Anabeana*. But under *Azospirillum*, Sids 1 and Sids 7 were similar in response index and both were decreased than Sakha 8 which was high in its response. It is of interest to note that biofertilization response indices by *Azospirillum* was greater than that observed by *Anabeana*. The results also indicated that F₁ hybrids, as well as, their corresponding parents were differed to both biofertilization forms. On the other hand, F₁ plants resulted from the cross between Sakha 8 x Sids 1 showed lower response index if compared with their parents under the effect of *Anabeana* form, while the opposite trend was obtained under the effect of *Azospirillum* form.

Worthily, sufficiency index is simply the inverse of biofertilizer response index, mathematically, but theoretically has different concept, it is bound directly to the actual biofertilizer applied without recognizing yield potential (Vervel *et al.*, 1997).

Table 4. Response index and sufficiency index of grain yield (gm) under different biofertilizer forms.

Genotype	Response index		Sufficiency index	
	<i>Anabeana</i> /Control	<i>Azospirillum</i> /Control	Control / <i>Anabeana</i>	Control / <i>Azospirillum</i>
Sakha8	1.040	2.309	0.961	0.433
Sids1	1.111	1.246	0.899	0.802
Sids7	1.1656	1.221	0.858	0.819
Sakha8 × Sids1	0.885	1.442	1.129	0.693
Sakha8 × Sids7	1.236	1.358	0.809	0.736

Therefore, biofertilizer sufficiency index of the wheat genotypes studied herein expressed as biofertilizer response index was higher under the effect of *Azospirillum* than that under *Anabeana* supply forms. Wheat genotypes, parents or F₁ hybrids, that showed higher biofertilizer response exhibited lower N sufficiency. These results agreed with Raun and Johnson (1999), who reported that biofertilizer response index ranged from 1.5 to 4.1 in wheat. These lower values may be due to the lower yielding potentials of the unfertilized treatment. So, when biofertilizer response index is low, there is little hope to identify N improved strategies because of non responsiveness. Furthermore, Halvorson *et al.* (2000) obtained lower values of response index and stated that it is not possible to identify the requirement for changing N management.

Estimates of yield components based on generations means of grain yielding for each biofertilizer supply form reflected different environmental interactions. The estimates of genetic components for biofertilizer response index and sufficiency index under biofertilization supply forms are presented in Table 5.

Table 5. Genetic components of generation means for biofertilizer response and sufficiency index over wheat crosses.

	Response index				Sufficiency index			
	<i>Anabeana</i>		<i>Azospirillum</i>		<i>Anabeana</i>		<i>Azospirillum</i>	
	Sakha8 × Sids1	Sakha8 × Sids7	Sakha8 × Sids1	Sakha8 × Sids7	Sakha8 × Sids1	Sakha8 × Sids7	Sakha8 × Sids1	Sakha8 × Sids7
m	1.076	1.103	1.778	1.765	0.930	0.910	0.618	0.626
a	0.591*	0.125*	1.686*	1.698*	0.534*	0.532	0.586*	0.602
d	-0.190	0.133*	-0.335	-0.407*	0.199	-0.101*	0.076	0.110*
d/a	-0.321	1.064	-0.199	-0.239	0.372	-0.189	0.129	0.183
d-a	-0.781	0.008	-2.020	-2.105	-0.335	-0.633	-0.510	-0.492

m = The average of generation means .

a = The additive gene effects.

d = The dominance gene effects.

d/a = Degree of dominance.

d-a = Heterosis.

Data showed that the additive component was significant in most F₁ crosses. The significance of additive gene effects for biofertilizers response

index and biofertilizers sufficiency index may give some promising opportunities to genetically improvement biofertilizer efficiency in nitrogen fixation with wheat. This agreed with Johnson and Raun (2003), who mentioned that improving of biofertilizer response index could lead at the same time to improving N use efficiency.

On the other hand, dominance component was the average of dominance gene effects over all loci, [d] values were significant and negative in the crosses between Sids 1 x Sakha 8, Sids 7 x Sakha 8 under *Azospirillum*, as well as, Sids 1 x Sakha 8 under *Anabeana*. Such significances in both crosses appeared to be due to the involvement of the common parent Sids 1. Also, these negative estimates of [d] suggested the dominance gene effects of the lower parent controlling these traits.

The results obtained herein related the response of wheat genotypes to biofertilizer supply forms suggested that the additive gene effects were more operating than the dominance gene effects. The F₁ cross results from Sids 7 x Sakha 8 was exhibited significant additive and dominante gene effects for N response index and N sufficiency index.

It is interest to note that the estimation of additive and dominance gene effects appeared to be biased by some sort of epistatic effects. This may be lead to a kind of discrepancy in the importance of additive and dominance gene effects for N response and sufficiency indices in wheat genotypes used in this study.

Expression of heterosis values related to N response and sufficiency index in F₁ plants over their better parents under both N supply forms of biofertilization are presented in Table 4. Negative heterotic effects were detected in most F₁ plants under both N supply forms. Likewise, the F₁ cross "Sakha 8 x Sids 1" exhibited significant negative heterosis for N response and sufficiency under N supply forms, while "Sakha 8 x Sids7" only exhibited significant positive heterotic effects for N response unser *Anabeana*.

Therefore, genetic improvement of biofertilizer response and sufficiency index could be achieved through selection method which given a good attention.

In conclusion, further attentions must be needed and directed towards genetic studies, integrating with soil biofertilization and plant nutrition to warding wheat populations originated from diverse germplasm to give more clear picture in genetic behavior of biofertilizer N response in wheat.

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استجابة تراكيب وراثية مختلفة من القمح للتسميد النيتروجيني الحيوي

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استهدف هذا البحث دراسة المكونات والدلائل الوراثية لكفاءة الإستجابة والكفاية للنيتروجين في بعض أصناف قمح الخبز المصرية وهجن الجيل الأول الناتجة عنها وذلك لاختبار سلوكهم الوراثي في إنتاج الحبوب تحت تأثير صورتين من التسميد النيتروجيني الحيوي باستخدام الأيزوسبيرليم والأنبينا.

أظهرت التراكيب الوراثية إختلافات في إنتاج الحبوب تحت تأثير كلتا الصورتين من التسميد النيتروجيني، كما أظهرت المتوسطات أن الأصناف سدس1، سدس7 قد أنتجا محصول حبوب مرتفع تحت تأثير كلاً من الأيزوسبيرليم والأنبينا. كذلك انتيجت نباتات الجيل الأول الناتجة عن التهجين بين سحا8 x سدس7 محصول حبوب مرتفع تحت تأثير كلتا الصورتين من التسميد النيتروجيني الحيوي. عكست النتائج تفوق هذه الهجن في الاستجابة للتسميد الحيوي بالأيزوسبيرليم فأظهرت تفوقاً كهجن عالية الكفاءة في الإستجابة للتسميد الحيوي النيتروجيني. كما أوضحت الأيزوسبيرليم إختلافات وراثية أكثر من الأنبينا. وقد أظهرت دراسة متوسطات الأجيال وجود تأثير معنوي علي إنتاج الحبوب تحت تأثير التسميد الحيوي بالأنبينا.

عكست الإستجابة للمخصبات الحيوية وكفاءة تثبيت النيتروجين في هجن الجيل الأول مرور جينات مختلفة من السلالات الأبوية إلى الهجن، تختلف هذه الجينات في طبيعة فعلها الجيني وفي تفاعلها مع المخصبات الحيوية مما يؤثر على تعبير الجينات ذات الصلة بإنتاج الحبوب. وقد أظهرت أنماط التسميد الحيوي المستخدمة في هذه الدراسة حدوث تفاعل بين التراكيب الوراثية مع البيئة المحيطة بالنسبة لبعض الصفات المتعلقة بتثبيت النيتروجين.