

Influence of Low-N Stress, Genotype and Their Interaction on Grain Yield and Quality Traits of F₁ and F₂ Diallel Crosses of Wheat (*Triticum Aestivum*L)

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Abstract: Developing high yielding varieties of bread wheat under low soil-N conditions is an important goal for plant breeder in order to overcome the negative impacts of using high rates of N fertilizers. This will lead to a significant reduction in nitrogen fertilizer use. The objective of this investigation was to study the effects of low-N environment (E), genotype (G) and G x E interaction on grain yield and quality traits of wheat F₁ and F₂ diallel crosses and their contrasting parents in N use efficiency. Genetic materials were evaluated at two seasons (2007/2008 and 2008/2009) in a split-plot design with lattice arrangement, using three replications. Main plots were assigned to N levels (0 and 75 kg N/fed), while sub-plots were devoted to genotypes. Data were analyzed across the two seasons. Low-N caused significant reductions in all studied grain yield components and grain protein content (GPC) in parents, F₁'s and F₂'s. The lowest reduction occurred in harvest index (HI), while spikes/plant (SPP) showed the greatest reduction, indicating that SPP is the most determinant component of GYPP. The first three parents L25, L26 and L27 showed significantly higher means than the second three parents Gem7, Gem 9 and Gz168 for most studied grain yield components and GPC trait. The F₁ and F₂ crosses involving one or more of the first three parents showed higher values of one or more of grain yield component traits than crosses that involved parents of the second group. In general, F₁ crosses showed higher means for all studied grain yield and quality traits than their parents. The rank of crosses in F₁ and F₂ generation for most studied traits was changed from one environment (N-level) to another, indicating a significant G x N interaction. Some F₂- progenies under N-limited environment exhibited higher values of GYPP and HI, suggesting transgressive effects in these characteristics, and that selection practiced in such F₂ populations could be effective in developing low-N tolerant genotypes.

Keywords: *Triticum aestivum*, nitrogen rate, grain protein content, yield components, harvest index, G X E interaction.

I. INTRODUCTION

Wheat (*Triticum aestivum* L.) is an important source of both carbohydrates and protein in human and livestock nutrition. It is one of the most important cereal crops globally as well as in Egypt. Wheat yield depends upon the environment, genotype, and their interactions. Low-N availability in soils in Egypt is an important yield-limiting factor frequently found in farmers' fields, since the smallholder farmers cannot afford additional inputs. Scientists try to release wheat cultivars with low-input of fertilizer and decrease of pollution risk to ecosystem (Le Gouis et al., 2000). In order to enhance the efficiency of crop production system while reducing the agricultural pollutions, plant breeders would have to introduce varieties which minimize pollution risks and maximize yield potential. Therefore, development of cultivars that could absorb nitrogen more effectively and use it more efficiently for grain production will lead to a significant reduction in nitrogen fertilizers (Le Gouis et al., 2000).

Variation for quantitative characters is under the control of many genes and the contribution of the genes can differ among environments (Basford and Cooper, 1998; DeLacy *et al.*, 1996 and Meseke *et al.*, 2006). This conditional contribution of genes is the basis of genotype-by-environment (G x E) interactions. Genetic variation for grain yield under low-N conditions has been studied on wheat (Ortez-Monasterio *et al.*, 1997; Van Sanford and Mackown, 1986 and Dhugga and Waines, 1989). Le Gouis *et al.* (2000) confirmed that there is a genetic variability for grain yield at a low N level and that the genotype X N level interaction is significant. Genotype by environment interaction is often described as inconsistent differences from one environment to another (Meseke *et al.*, 2006). The increase in

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grain yield by increasing N-levels may be due to the improved growth which may account for the superiority of yield components and grain yield. In most of the wheat breeding programs, the materials in the segregating generations are grown under high fertility conditions till homozygosity is nearly attained and progenies are ready for bulking. Soil fertility as an environmental factor may differ from soil to another and might affect the assessment of characters in breeding programs, especially nitrogen levels.

Breeding for high grain yield under low-N in wheat has produced good results in some European countries (Good et al., 2011). There has been a 56% decrease in total fertilizer use between 1987 and 2007, including a significant decrease in N application per hectare. The objective of this study was to determine the effect of low soil-N on wheat grain yield and quality attributes, evaluate a number of wheat diallel crosses and their parents for genetic variability in such traits under low-N and also to identify the most promising genotypes to be involved in breeding programs for tolerance to low level of nitrogen fertilizer to sustain clean environment and hoping high grain yield with less nitrogen fertilizer to decrease costs in farmers fields.

II. MATERIALS AND METHODS

This study was carried out at Giza Research Station of the Agricultural Research Center(ARC), Giza Egypt (30° 02'N latitude and 31° 13'E longitude with an altitude of 22.50 meters above sea level), in 2005/2006 season and at Noubarya Research Station of the ARC, Noubarya, Egypt (30° 66'N latitude and 30° 06' E longitude with an altitude of 15.00 meters above sea level), in 2006/2007, 2007/2008 and 2008/2009 seasons.

2.1. Plant Materials

Six bread wheat genotypes (*Triticum aestivum* L.) were chosen for their divergence in nitrogen use efficiency to be used as parents of diallel crosses, based on previous field screening carried out by Wheat Res. Dept., Field Crops Res. Inst., ARC, and Egypt. Three of them were promising breeding lines of high yield under low-N (L25, L26 and L27) and three were commercial local cultivars of low yield under low-N (Gemmeiza 7; Gem7, Gemmeiza 9; Gem9 and Giza 168; Gz168).

2.2. Making The F1 And F2 Diallel Crosses

In season 2005/2006, a half diallel of crosses involving the six parents (without reciprocals) was done at Giza Agric. Res. Stat., Agric. Res. Center, to obtain the F₁ seeds of 15 crosses. In summer 2006, a part of F₁ seeds was sown in greenhouse of Wheat Res. Dept. under controlled conditions to obtain the F₂ seeds. In season 2007/2008, the half diallel of crosses was again done to increase quantity of F₁ seeds and in summer 2007 the F₁ seeds were again sown in the greenhouse under controlled conditions to obtain more seeds of 15 F₂ crosses.

2.3. Field Evaluation of 6 Parents, 15 F1's and 15 F2's

In the seasons 2007/2008, 2008/2009, parents (6), F₁'s (15) and F₂'s (15) were sown on 17th of November each season in the field of Noubarya Res. Stat., under two levels of nitrogen fertilizer; the low level was without fertilization (LN) and the high level was 75 kg Nitrogen/ feddan (HN); this is the recommended level of Ministry of Agriculture. This level of nitrogen fertilizer (168 kg Urea/fed) was added in two equal doses, the first dose was added just before the sowing irrigation and the second dose just before the second irrigation (21 days after irrigation). In this experiment, a split plot design in lattice (6x6) arrangement was used with three replications. The two levels of nitrogen were allotted to the main plots and the genotypes to the sub plots. Each parent or F₁ was sown in two rows and each F₂ was sown in four rows; each row was three meter long; spaces between rows were 30 cm and 10 cm between plants, and the plot size was 1.8 m² for parent or F₁ and 3.6 m² for F₂. All other agricultural practices were done according to the recommendation of Ministry of Agriculture for growing wheat in Noubarya region. All other agricultural practices were followed according to the recommendations of ARC, Egypt. The soil analysis of the experimental soil at Noubarya Research Station, as an average of the two growing seasons, indicated that the soil is sandy loam (67.86% sand, 7.00% silt and 25.14%), the pH is 8.93, the EC is 0.55 dSm⁻¹, the soluble cations in meq l⁻¹ are Ca²⁺ (5.30), K⁺ (0.70), Na⁺ (0.31), Mg²⁺ (2.60) and the soluble anions in meq l⁻¹ are CO₃²⁻ (0.00), HCO₃⁻ (2.10), Cl⁻ (5.30) and SO₄²⁻ (1.51).

2.4. Data Collection

The following characteristics were measured on a random sample of 10 plants of each genotype of parents and F₁'s and 30 plants of F₂'s. **1. Number of spikes/plant (SPP):** Number of fertile spikes per plant. **2. Number of grains/spike (GPS):** Number of grains per spike. **3. 100 grain weight (100GW)** in g measured as weight of 100 grains taken from each guarded plant. **4. Grain yield/ plant (GYPP)** in g measured as weight of the grains of each

individual plant. **5. Harvest index (HI%)** according formula: $HI = 100 (GYPP / BYPP)$, where BYPP= biological yield/plant. **6. Grain protein content (GPC)** measured as follows: $GPC\% = N_g \times 5.7$ according to AACCC (2000), where N_g is grain nitrogen content. Grain N_g was determined using Kjeldahl procedure according to A.O.A.C. (1990).

2.5. Biometrical Analysis

The analysis of variance (ANOVA) of the split plot design was performed on the basis of individual plot observation using the MIXED procedure of SAS ® (Littell et al., 1996). Combined analysis of variance across the two seasons was also performed if the homogeneity test was non-significant. Moreover, each environment (HN and LN) was analyzed separately across seasons as lattice design using GENSTAT 10th addition windows software. Least significant differences (LSD) values were calculated to test the significance of differences between means according to Steel *et al.* (1997).

III. RESULTS AND DISCUSSION

3.1. Analysis of Variance

Combined analysis of variance across years (Y) of the split plot design for the studied 36 wheat genotypes (6 parents, 15 F₁'s and 15 F₂'s) under two levels of nitrogen is presented in Table (1). Mean squares due to years were highly significant for five studied traits and non significant for only one trait (harvest index; HI), indicating significant effect of climatic conditions on most studied traits, namely spikes/plant (SPP), grains/ spike(GPS), 100 grain weight (100GW), grain yield/ plant (GYPP) and grain protein content (GPC).

Results in Table (1) also exhibit that mean squares due to nitrogen levels (N) were highly significant for all studied traits, indicating that the N level has an obvious effect on all grain yield traits and grain protein content of studied wheat genotypes. Mean squares due to genotypes (G) were highly significant for all studied traits, indicating that wheat genotypes used in this study were significantly ($P \leq 0.01$) different for all studied yield and quality traits. The observation of considerable variation for grain yield traits under low-N conditions indicates that significant genetic variation exists in bread wheat cultivars of Egypt. Thus, the best genotypes under low-N can be used for developing wheat varieties with higher grain yield, and suitable for low input (low-N) wheat production system. Mean squares due to the interaction N x Y were significant or highly significant for number of grains / spike (GPS), and harvest index (HI), grain protein content (GPC) and grain yield / plant (GYPP) and non significant for SSP and 100GW traits.

Table1. Combined analysis of variance of split plot design for wheat studied traits of 36 genotypes (6 parents, 15 F₁'s and 15 F₂'s) under two levels of nitrogen across two years

SV	df	MS					
		SPP	GPS	100GW	GYPP	HI%	GPC
Year	1	128.3**	22.7**	1.4**	70.0**	0.19	871.95
Rep /Y	4	0.03	1.6	0.3	2.4	7.8	43.75
Nlevels (N)	1	1775.5**	9116.2**	41.7**	2907.3**	1169.1**	55094.1**
N x Y	1	3.3	259.6**	0.63	7.4*	129.8**	2162.37**
Error N	4	0.75	2.4	0.09	0.9	1.3	66.53
Genotypes (G)	35	19.5**	1093.4**	9.04**	81.7**	1487**	2140.0**
G x N	35	13.9**	226.8**	0.79**	35.5**	175.2**	534.6**
G x Y	35	8.1**	33.4**	0.18*	12.8**	57.6**	119.3**
G x Y x N	35	9.4**	31.8**	0.20*	17.8**	54.8**	73.8**
Error	280	1.02	1.9	0.09	1.9	6.8	27.7

* And** indicate significant at 0.05 and 0.01 probability levels, respectively.

Moreover, mean squares due to genotypes x nitrogen levels, *i.e.* G x N were significant ($P \leq 0.01$ or 0.05) for all studied traits, indicating that genotype ranks differ from one nitrogen level to another and that selection can be done under a specific soil nitrogen environment as proposed by Al-Naggar *et al* (2006, 2009, 2010 and 2015 a, b, c). The significant GxN interaction for grain yield was also a good evidence for varying responses of these wheat genotypes at various N levels (Earl and Ausubel, 1983; Austin et al., 1980). The interactions G x Y and G x Y x N were also significant ($P \leq 0.01$ or 0.05) for all studied traits, indicating that genotype ranks differ from one combination of Y x N to another.

Combined analysis of variance of a lattice design for all studied traits under each environment (high N and low N) across two seasons is presented in Tables (2 and 3), respectively.

Table2. Partitioning genotypes degrees of freedom and their interaction with years into their components under high N conditions

SV	df	MS					
		SPP	GPS	100GW	GYPP	HI%	GPC
Years (Y)	1	45.3**	64.3**	0.08	15.9**	60.0**	2919.1**
Error Y	4	0.5	2.2	0.083	1.1	1.4	56.01
Genotypes (G)	35	10.9**	601.4**	5.1**	60.0**	135.5**	1375.8**
Parents (P)	5	6.7**	977.1**	3.3**	36.7**	54.4**	1100.34**
F ₁ 's (F ₁)	14	13.5**	489.9**	4.0**	52.6**	129.1**	1029.03**
F ₂ 's (F ₂)	14	8.4**	48.8**	3.0**	8.2**	23.1**	2191.74**
P vs F ₁	1	155.5**	1973.4**	39.8**	553.6**	1535.5**	245.46**
F ₁ vs F ₂	1	72.0**	824.0**	14.1**	100.2**	728.6**	346.8**
G x Y	35	12.1**	19.6**	0.2**	7.9**	28.3**	164.1**
P x Y	5	1.3**	6.9**	0.44**	4.6*	14.4*	10.57
F ₁ x Y	14	3.3**	6.8**	0.17*	5.4**	15.0**	120.7**
F ₂ x Y	14	6.7**	35.3**	0.13	12.5**	49.0**	366.61**
P vs F ₁ x Y	1	39.5**	60.9**	0.22*	52.4**	138.5**	153.53**
F ₁ vs F ₂ x Y	1	47.9**	398.4**	0.67**	98.8**	475.1**	44.0**
Error	140	0.4	1.8	0.11	1.9	6.3	18.4

* And** indicate significant at 0.05 and 0.01 probability levels, respectively

Table3. Partitioning genotypes degrees of freedom and their interaction with years into their components under low N conditions

SV	df	MS					
		SPP	GPS	100GW	GYPP	HI%	GPC
Years (Y)	1	86.3**	218.1**	2.0**	61.6**	69.9**	112.7
Error Y	4	0.31	1.9	0.27	2.2	7.7	54.9
Genotypes (G)	35	22.4**	718.8**	4.8**	57.2**	188.3**	1298.8**
Parents (P)	5	27.5**	1284.1**	2.6**	106.0**	115.9**	1837.2**
F ₁ 's(F ₁)	14	10.0**	553.7**	2.5**	53.2**	209.1**	1072.2**
F ₂ 's(F ₂)	14	23.9**	623.4**	2.3**	46.5**	217.9**	1302.4**
P vs F ₁	1	21.8**	1331.5**	2.2**	172.7**	88.9**	538.4**
F ₁ vs F ₂	1	195.0**	975.3**	2.5**	94.1**	122.7**	226.6**
G x Y	35	5.5**	45.7**	0.18**	22.7**	84.1**	28.6
P x Y	5	0.69*	87.8**	0.09	3.1	15.3	68.1**
F ₁ x Y	14	6.6**	56.4**	0.07	3.9	18.7*	14.7
F ₂ x Y	14	6.7**	20.4**	0.30**	8.2**	23.1**	54.2**
P vs F ₁ x Y	1	88.9**	349.6**	0.44**	39.3**	186.0**	39.5
F ₁ vs F ₂ x Y	1	0.9**	502.4**	3.2**	521.9**	1608.3**	13.9
Error	140	0.34	2.0	0.07	1.8	7.3	37.3

* and** indicate significant at 0.05 and 0.01 probability levels, respectively

Mean squares due to genotypes, parents, F₁'s and F₂'s under the two levels of nitrogen were highly significant for all studied traits. Significant differences among parents of diallel crosses in all studied traits are pre-requisite for performing the diallel analysis for estimating the inheritance of studied traits under different N- application rates.

Mean squares due to parents vs. F₁'s and F₁'s vs. F₂'s were highly significant for all studied traits under the two levels of nitrogen, indicating the presence of significant heterosis and the presence of inbreeding effects for all studied traits. Mean squares due to the interaction G x Y were highly significant for all studied traits under the two levels of nitrogen. Mean squares due to the interaction P x Y under high level of nitrogen (Table 2) were significant or highly significant for 6 studied traits and non significant for DTH and BYPP. Mean squares due to the interactions F₁'s x Y

and F₂'s x Y under high-N were significant or highly significant for all studied traits, except 100GW for F₂'s x Y, which was not significant. Mean squares due to the interactions F₁'s x Y and F₂'s x Y were significant or highly significant for all studied traits under low N, except for 100GW and GYPP for F₁'s x Y. Mean squares due to the interactions P's vs F₁'s x Y and F₁'s vs F₂'s x Y under the two levels of nitrogen were significant and highly significant for all studied traits. The significance of the interactions P's vs F₁'s x Y and F₁'s vs F₂'s x Y indicates that heterosis and inbreeding effects differ from season to season in all studied traits.

3.2. Effects of low N

A comparative summary of means of all studied traits across all 36 genotypes (6 parents, 15 F₁'s and 15 F₂'s) subjected to two levels of nitrogen conditions and across two years is presented in Table (4) and Fig (1). In general, low N caused a significant reduction in 7 out of 8 studied traits, namely GYPP, SPP, 100GW, GPS and HI. Mean grain yield/plant (GYPP) was significantly decreased due to low-N by an average of 18.96, 21.17, and 15.40% for parents, F₁'s and F₂'s, respectively. Reduction in grain yield of wheat due to low soil nitrogen was reported by several investigators. A positive relationship between N application levels and the grain yield has already been shown in many studies (Austin *et al.*, 1980; Desai and Bahatia, 1979). Significant reduction in grain yield as a result of low-N was associated with significant reductions in all yield components traits, *i.e.* SPP, 100GW and GPS. These reductions were relatively high in magnitude for number of spikes/ plant (SPP) for parents (23.65%), F₁'s (23.99%) and F₂'s (43.52%). This indicates that SPP is the most determining component of grain yield / plant of wheat under low-N stress. The importance of this trait (number of spikes or fertile tillers per plant) in wheat for grain productivity under abiotic stress conditions was previously reported by several investigators (Al-Naggar *et al.*, 2004,2007, 2011, and 2015 a,b,c). Hussain *et al.* (2006) observed that increasing nitrogen application increased the number of fertile tillers per unit area. Geleto *et al.* (1995) reported that grain yield is closely related to the number of spikes per unit area. Fertilized plots produced more spikes than control. Such response can be attributed to the adequate nitrogen availability which might facilitate the tillering ability of plants, resulting in a greater spike population. Ayoub *et al.* (1994) also reported that spike population increased with increase in nitrogen level.

Table5. Means of studied wheat traits under low-N (0 Kg N/fed) and high-N (75 Kg N/fed) and relative reduction compared to high-N combined across parents, F₁'s and F₂'s across two seasons

Traits	Parameter	Parents		F ₁ crosses		F ₂ crosses	
		High-N	Low-N	High-N	Low-N	High-N	Low-N
GPS	Average	80.23	69.81	79.95	71.76	74.48	64.78
	Reduction%	---	13.47**	---	9.80**	---	12.47**
100GW(g)	Average	4.66	4.05	4.33	3.84	3.37	2.61
	Reduction%	---	12.96**	---	10.51**	---	21.72**
SPP	Average	11.88	9.11	12.13	9.14	12.95	7.31
	Reduction%	---	18.96**	---	23.99**	---	43.52**
GYPP(g)	Average	27.53	22.41	29.12	22.83	25.65	21.54
	Reduction%	---	18.96**	---	21.17**	---	15.40**
HI(%)	Average	43.67	40.73	45.11	40.51	43.50	41.37
	Reduction%	----	6.57**	---	8.97**	---	3.96
GPC(%)	Average	16.18	12.12	19.22	13.61	14.04	13.83
	Reduction%	-----	25.06**	-----	29.18**	-----	23.31**

N= nitrogen, * and** indicate significance at 0.05 and 0.01 probability levels, respectively. Reduction%= 100[(HN-LN)/HN]

Moreover, low nitrogen caused a significant reduction in biological yield / plant (BYPP) by 12.49, 12.27 and 11.24%, grain protein content (GPC) by 25.06, 29.18, and 23.31% and harvest index (HI) by 6.57, 8.97 and 3.69% for parents, F₁'s and F₂'s, respectively. It was observed that low-N caused slight but significant earliness of DTH by 0.70, 4.50 and 5.55 days for parents, F₁'s and F₂'s, respectively.

3.3. Effect of genotypes

Means of studied traits of 6 wheat parents and their 15 F₁ and 15 F₂ diallel crosses across studied N levels and across two seasons are presented in Table (6)

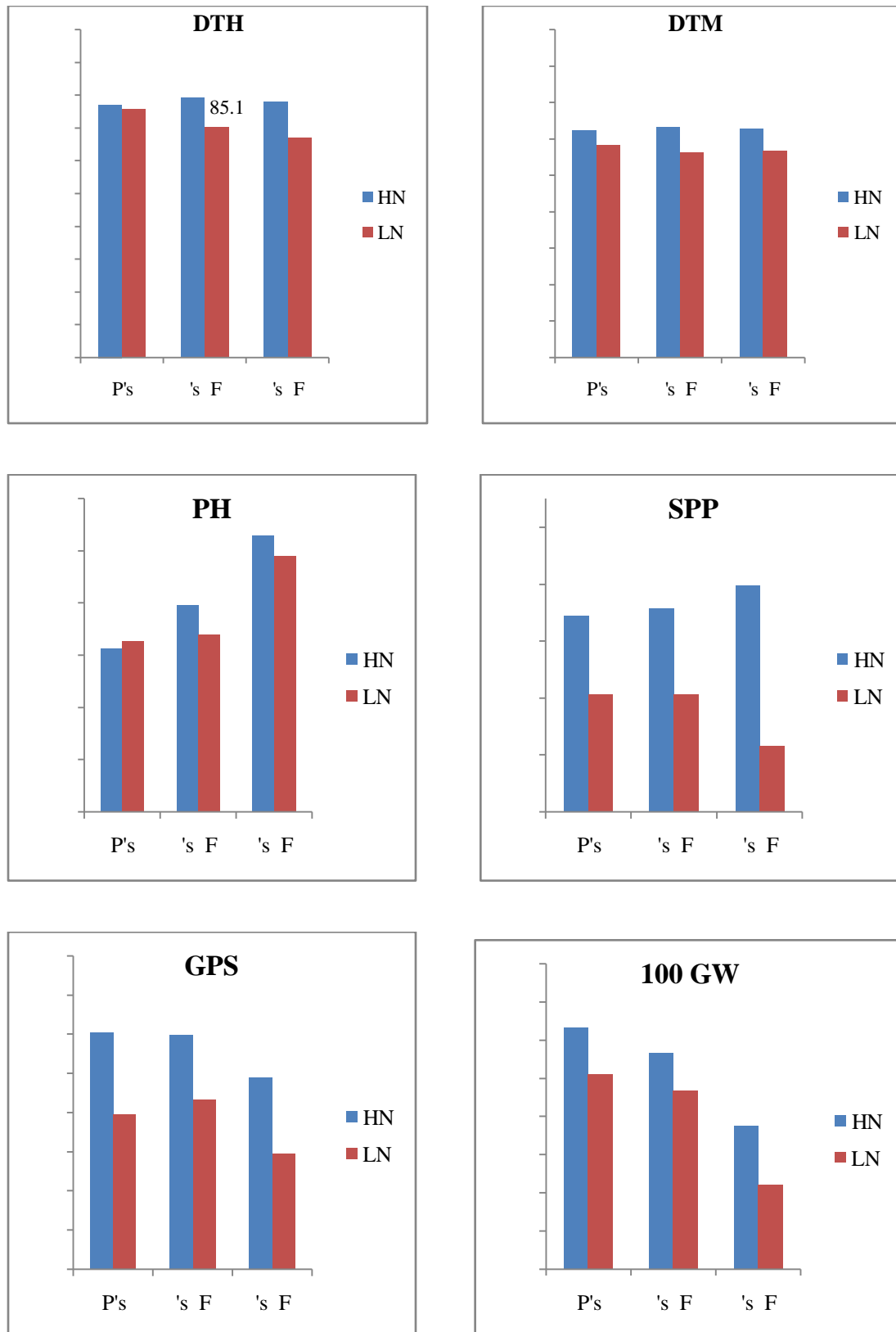


Fig1. Means of DTH, DTM, PH, SPP, GPS and 100GW traits under high-N (HN) and low-N(LN) for parents, F₁'s and F₂'s across two seasons

In general, parents varied in most studied traits, especially in grain yield and nitrogen use efficiency traits, indicating their usefulness as parents of diallel crosses for studying inheritance of these traits. The low mean of DTH and high means of other studied traits were considered favorable. The parental line L26 showed the highest means for GYPP and BYPP (29.16 and 64.67 g, respectively).

Table4. Means of studied traits of wheat parents and their diallel F1 and F2 crosses across two N levels and two seasons

Serial.No	Genotypes	SPP	GPS	100GW(g)	GYPP(g)	HI(%)	GPC %
Parents							
1	L25	12.13	86.15	5.07	25.93	40.40	12.6
2	L26	11.68	82.17	4.80	29.16	45.05	14.9
3	L27	11.53	92.55	5.04	28.07	45.36	13.0
4	Gem 7	8.83	64.87	3.76	22.16	42.83	10.4
5	Gem 9	8.92	60.60	3.69	21.83	37.14	9.1
6	Giza 168	9.89	63.76	3.76	22.68	42.44	10.0
F₁ crosses							
1	L25 X L26	11.73	83.82	5.36	28.90	45.15	12.6
2	L25 X L27	12.61	93.40	5.31	26.00	42.48	12.0
3	L25 X Gem 7	10.22	78.22	4.02	25.06	48.06	12.6
4	L25 X Gem 9	10.78	71.29	3.73	23.42	41.89	13.2
5	L25 X Gz 168	10.83	70.17	3.49	26.55	42.08	14.4
6	L26 X L27	13.08	78.04	5.14	29.84	44.58	15.4
7	L26 X Gem 7	10.54	76.86	3.54	26.08	38.57	12.7
8	L26 X Gem 9	10.21	65.13	3.92	25.90	40.56	12.9
9	L26 X Gz 168	10.43	72.97	3.54	27.81	42.76	12.0
10	L27 X Gem 7	10.11	84.21	3.60	29.24	49.56	10.1
11	L27 X Gem 9	10.27	79.68	4.41	25.15	39.67	13.5
12	L27 X Gz168	11.05	82.29	4.40	27.16	41.75	11.3
13	Gem 7 X Gem9	9.86	63.12	3.51	21.33	37.90	10.4
14	Gem 7 X Gz 168	8.75	69.93	3.84	23.77	42.39	11.0
15	Gem 9 X Gz 168	9.04	68.66	3.50	23.41	44.75	12.3
F₂ crosses							
1	L25 X L26	12.68	77.08	3.92	25.46	42.57	14.2
2	L25 X L27	12.21	84.98	4.03	25.01	44.71	12.4
3	L25 X Gem 7	9.88	79.63	3.26	23.61	46.59	13.0
4	L25 X Gem 9	10.60	67.64	3.49	19.47	36.04	14.1
5	L25 X Gz 168	10.68	67.13	2.42	24.41	38.72	15.2
6	L26 X L27	12.34	72.30	3.47	24.61	39.28	13.9
7	L26 X Gem 7	9.69	76.99	2.50	23.73	38.39	13.8
8	L26 X Gem 9	9.15	58.49	2.93	23.74	42.57	14.7
9	L2 X Gz 168	9.92	63.26	2.88	27.93	48.89	14.9
10	L27 X Gem 7	10.19	67.13	2.97	24.68	46.75	13.4
11	L27 X Gem 9	8.34	77.69	3.53	22.07	37.82	10.8
12	L27 X Gz168	9.33	69.23	2.65	24.80	44.11	9.7
13	Gem 7 X Gem9	9.13	67.98	2.00	22.30	46.31	8.6
14	Gem 7 X Gz 168	8.88	54.21	2.20	20.11	37.70	10.1
15	Gem 9 X Gz 168	8.98	60.72	2.63	22.02	46.12	10.8
	L.S.D. _{0.05} (G)	0.91	2.50	0.44	2.05	3.9	4.00
	(N)	1.30	4.00	0.80	2.50	3.00	8.50
	(GN)	1.50	2.10	0.45	2.04	3.90	5.50

* And** indicate significant at 0.05 and 0.01 probability levels, respectively.

The lowest mean of DTH trait (favorable) was exhibited by the parent Giza 168. On the contrary, the parent Gemmeiza 9 showed the lowest mean for GYPP, HI, GPS, 100GW, SPP and PH (unfavorable).

In general, the first three parents L25, L26 and L27 show significantly higher means than the second three parents Gem7, Gem 9 and Gz168 for GYPP, BYPP, GPS, 100GW and SPP traits, *i.e.* most studied grain yield traits. Such significant differences among wheat parents in this study are prerequisite for the validity of using them as parents of diallel crosses to study the inheritance of these traits. Several investigators reported genotypic variation in grain yield traits in wheat under limited N conditions (Van Sanford and MacKown, 1986; Dhugga and Waines, 1989; Ortiz-Monasterio *et al.* 1997; Le Gouis *et al.* 2000; Austin *et al.* 1977; Foulkes *et al.*, 2006 Barraclough *et al.* 2010, and Al-Naggar *et al.* 2007, 2009, 2010, 2011, 2014, 2015 a,b,c)

The studied diallel crosses varied greatly in all studied traits either in F₁ or F₂ generation (Table 5). The cross Gem 7 x Gem 9 exhibited the lowest means in F₁ for GYPP, HI and GPS and in F₂ for BYPP and 100 GW traits. The cross Gem 9 x Gz 168 showed the lowest mean of BYPP, 100 GW and SPP in F₁ generation. The cross L25 x Gem 9 exhibited the lowest GYPP in F₂. The lowest mean was also shown by L25 x L2 for PH in F₁, L25 x Gz 168 for PH in F₂. Regarding earliness, the cross L2 x Gz 168 was the earliest for DTH in F₁ and F₂.

On the contrary, the highest means were exhibited by the cross L2 x L27 for GYPP and SPP in the F₁, L2 x Gz 168 for GYPP and HI in F₂, L25 x L27 for GPS in F₁ and 100 GW and GPS in F₂, L25 x L2 for 100 GW in F₁ and SPP and DTH in F₂, L27 x Gem7 for HI in F₁ and PH in F₂, L25 x Gz 168 for BYPP in F₂ and DTH in F₁, L2 x Gem 7 for BYPP in F₁ and Gem 7 x Gem 9 for PH in F₁ generation.

It is interesting to mention that the best crosses in GYPP were L2 x L27, L27 x Gem 7, L25 x L2, L2 x Gz 168 and L27 x Gz 168 in F₁ and L2 x Gz 168, L25 x L2, L25 x L27, L27 x Gz 168 and L27 x Gem 7 in F₂, in descending order. The rank of crosses for GYPP differed from F₁ to F₂ generation. The cross L2 x L27 ranked 1st in grain yield in F₁, but ranked 6th in F₂ generation. Moreover, the cross L2 x Gz 168 ranked 4th in F₁, but was in the first place in F₂ generation. It is observed that the three crosses L2 x L27, L27 x Gem 7 and L25 x L2 in F₁ and the crosses L2 x Gz 168, L25 x L2 and L25 x L27 in F₂ were the best for GYPP traits.

In general, F₁ crosses showed higher means for GYPP, HI, SPP, and GPS and lower means for 100 GW than their parents (Table 6), indicating that heterozygotes exhibit better (more favorable) values for most studied wheat traits than homozygotes, which is logic and may be attributed to heterosis phenomenon.

Table 6. Averages of studied traits of wheat parents, F₁ crosses and F₂ crosses across two N levels and two seasons

Genotypes	Traits					
	SPP	GPS	100GW(g)	GYPP(g)	HI(%)	GPC(%)
Parents	10.50	75.02	4.35	24.97	42.20	11.7
F ₁ 's	10.63	75.85	4.09	25.97	42.81	12.4
F ₂ 's	10.13	61.72	2.99	23.60	42.44	12.6

On the contrary, F₂ crosses exhibited lower means for all studied traits, except GPC, than their corresponding F₁ crosses (Table 6), indicating the role of inbreeding depression in most studied traits of wheat, and transgressive effects for GPC trait.

3.4. Genotype X Nitrogen Interaction

Means of each parent, F₁ cross and F₂ cross for studied traits under two nitrogen levels (0 and 75 kg N /Fed) across two seasons are presented in Table (7). In general means of GYPP, GPS, 100GW and SPP of the three parents L25, L26 and L27 were higher in magnitude than those of the three other parents Gem 7, Gem 9 and Giza 168 under both high-N and low-N levels. Reduction in GYPP, due to low-N stress was lower in the first three parents than that in the latter parents. The first three parents (L25, L26 and L27) were therefore considered as low-N tolerant (N-efficient) genotypes and the latter ones (Gem 7, Gem 9 and Giza 168) as low-N sensitive (N-inefficient) parents. These parents are therefore proper genetic material for diallel analysis for studying inheritance of adaptive traits for low-N tolerance in wheat.

The rank of crosses in F₁ and F₂ generation for most studied traits was changed from one environment (N-level) to another. The highest mean of GYPP under low-N was obtained from L26 x L27 followed by L25 x L26 and L25 x L27 in F₁ and L25 x L27 followed by L25 x L26 and L26 x Gz 168 in F₂ generation. These crosses also showed the lowest reduction due to low-N stress, and therefore were considered tolerant (N-efficient) to low-N stress.

Table 7. Mean performance of all genotypes under high and low levels of nitrogen across two years for studied traits

	GPS			100GW(g)			SPP		
	High N	Low N	Red%	High N	Low N	Red%	High N	Low N	Red%
Parents									
L25	91.29	81.02	11.24**	5.58	4.57	18.14**	13.43	10.83	19.35**
L2	87.50	76.85	12.18**	5.22	4.37	16.25**	12.43	10.93	12.06**
L27	96.02	89.08	7.23**	5.17	4.92	4.99**	12.22	10.85	11.19**
Gem7	67.80	61.94	8.64**	3.90	3.62	7.14**	11.75	5.90	49.79**
Gem9	69.52	51.68	25.66**	3.99	3.40	14.68**	10.52	7.32	30.43**
Giza168	69.25	58.28	15.84**	4.10	3.42	16.52**	10.93	8.85	19.05**
F₁ crosses									
L25 X L2	90.44	77.21	14.63**	6.35	4.36	31.29**	13.58	9.88	27.24**
L25 X L27	96.12	90.69	5.66*	5.28	5.34	-1.23**	15.12	10.10	33.19**
L25 X Gem 7	85.24	71.20	16.47**	4.54	3.51	22.79**	13.13	7.30	44.42**
L25 X gem 9	65.59	76.98	-17.4**	3.72	3.74	-0.45	12.53	9.03	27.93**
L25 X Gz 168	73.27	67.08	8.45**	3.67	3.31	9.82**	11.82	9.83	16.78**
L2 X L27	77.46	78.62	-1.50	5.51	4.76	13.63**	13.60	12.57	7.60**
L2 X Gem 7	81.41	72.32	11.17**	3.72	3.37	9.20**	11.65	9.43	19.03**
L2 X Gem 9	72.40	57.86	20.08**	4.25	3.60	15.16**	11.38	9.03	20.64**
L2 X Gz 168	86.58	59.36	31.44**	3.81	3.27	14.15**	12.62	8.23	34.74**
L27 X Gem 7	85.30	83.13	2.55**	3.64	3.56	2.38**	11.55	8.67	24.96**
L27 X Gem 9	87.72	71.63	18.35**	4.40	4.42	-0.42	10.65	9.88	7.20**
L27 X Gz168	85.29	79.29	7.04**	4.47	4.33	3.02**	13.22	8.88	32.79**
Gem 7 X Gem9	68.73	57.52	16.32**	3.64	3.38	7.28**	11.65	8.07	30.76**
Gem 7 X Gz 168	69.81	70.05	-0.35	4.41	3.28	25.46**	10.13	7.37	27.30**
Gem 9 X Gz 168	73.85	63.47	14.06**	3.60	3.40	5.64**	9.28	8.80	5.21**
F₂ crosses									
L25 X L2	87.17	66.98	23.17**	4.63	3.21	30.58**	14.72	10.63	27.75**
L25 X L27	92.23	77.73	15.73**	4.35	3.70	15.08**	14.27	10.15	28.86**
L25 X Gem 7	86.88	72.38	16.69**	3.58	2.93	18.06**	12.92	6.83	47.10**
L25 X gem 9	65.77	69.50	-5.67*	3.53	3.45	2.27**	13.88	7.32	47.30**
L25 X Gz 168	67.96	66.31	2.44	2.35	2.49	-5.74**	13.78	7.57	45.10**
L26 X L27	72.21	72.38	-0.23	4.34	2.60	40.15**	13.15	11.53	12.29**
L26 X Gem 7	76.69	77.28	-0.77	2.99	2.00	33.18**	12.63	6.75	46.57**
L26 X Gem 9	65.84	51.14	22.34**	2.92	2.94	-0.46*	12.03	6.27	47.92**
L26 X Gz 168	70.87	55.66	21.47**	3.45	2.31	32.90**	13.32	6.52	51.06**
L27 X Gem 7	77.33	56.94	26.38**	3.36	2.58	23.15**	13.30	7.08	46.74*
L27 X Gem 9	83.33	72.06	13.52**	3.82	3.24	15.03**	11.42	5.27	53.87*8
L27 X Gz168	77.69	60.77	21.78**	3.34	1.96	41.39**	13.62	5.03	63.04**
Gem7X Gem9	61.89	74.07	-19.7**	2.38	1.62	32.10**	13.32	4.95	62.83**
Gem7XGz168	62.25	46.16	25.85**	2.46	1.94	21.11**	11.63	6.13	47.28**
Gem9XGz168	69.02	52.42	24.05**	3.05	2.22	26.98**	10.27	7.68	25.16**
L.S.D. _{0.05} (G)	2.00	2.10		0.49	0.39		0.94	0.87	
(N)			4.00			0.80			1.30
(GN)			2.10			0.45			1.50

* And** indicate significant at 0.05 and 0.01 probability levels, respectively

Genotypes	GYPP(g)			HI(%)			GPC(%)		
	High N	Low N	Red%%	High N	Low N	Red%	High N	Low N	Red%
Parents									
L25	26.48	25.39	4.1**	39.74	41.06	-3.33	13.6**	11.7**	13.59**
L26	31.42	26.91	14.35**	45.95	44.16	3.89	15.7**	14.2**	9.86**
L27	29.86	26.28	11.99**	45.61	45.11	1.10	14.3**	11.6**	18.81**
Gem 7	25.96	18.37	29.22**	42.84	42.82	0.05	12.3**	8.6*	30.43**
Gem 9	25.76	17.89	30.53**	40.79	33.49	17.88**	11.3**	6.8*	39.52**
Giza 168	25.71	19.65	23.57**	47.12	37.77	19.85**	11.1**	8.9*	19.30**
F₁ crosses									
L25 X L26	30.86	26.94	12.71**	46.32	43.97	5.07**	13.7**	11.5**	16.07**
L25 X L27	25.78	26.23	-1.75	39.92	45.03	-12.8**	13.4**	10.7**	20.85**
L25 X Gem 7	25.62	24.50	4.40**	40.33	55.78	-38.3**	13.8**	11.4**	17.16**
L25 X gem 9	26.79	20.06	25.13**	41.02	42.76	-4.24*	14.1**	12.4**	12.30**
L25 X Gz 168	27.65	25.46	7.94**	42.11	42.04	0.17	15.4**	13.4**	12.89**
L26 X L27	32.16	27.52	14.41**	44.94	44.23	1.58	15.5**	15.3**	1.34
L26 X Gem 7	29.49	22.68	23.11**	40.21	36.93	8.17**	13.6**	11.7**	14.14**
L26 X Gem 9	30.81	21.00	31.84**	45.27	35.86	20.80**	12.7**	13.1**	-3.17
L26 X Gz 168	33.55	22.07	34.23**	49.39	36.13	26.85**	11.5**	12.5**	-8.80**
L27 X Gem 7	34.32	24.16	29.60**	55.52	43.60	21.47**	10.6**	9.7**	9.21**
L27 X Gem 9	29.74	20.56	30.85**	45.70	33.64	26.38**	12.9**	14.2**	-9.79**
L27 X Gz 168	30.59	23.74	22.40**	45.37	38.12	15.97**	12.7**	9.8**	23.05**
Gem7XGem 9	24.88	17.78	28.56**	40.61	35.18	13.39**	12.5**	8.3*	33.44**
Gem7XGz168	28.56	18.99	33.51**	51.73	33.05	36.11**	11.1**	10.8**	2.29
Gem9XGz168	26.09	20.73	20.55**	48.12	41.39	13.98**	15.9**	8.7*	45.50**
F₂ crosses									
L25 X L26	25.96	24.97	3.81**	42.52	42.62	-0.23	16.5**	12.0**	27.53**
L25 X L27	23.94	26.09	-9.02**	41.09	48.33	-17.64**	12.0**	12.7**	-5.45
L25 X Gem 7	23.33	23.88	-2.36	39.97	53.21	-33.1**	13.9**	12.2**	11.89**
L25 X Gem 9	22.97	15.97	30.49**	35.88	36.20	-0.89	15.6**	12.5**	20.10**
L25 X Gz 168	27.08	21.75	19.71**	42.14	35.30	16.23**	17.9**	12.5**	29.91**
L26 X L27	28.97	20.25	30.09**	44.48	34.09	23.37**	16.1**	11.7**	27.33**
L26 X Gem 7	23.95	23.51	1.84	36.58	40.19	-9.85**	14.7**	12.8**	12.75**
L26 X Gem 9	25.45	22.04	13.42**	44.14	40.99	7.15**	14.1**	15.4**	-9.48**
L26 X Gz 168	31.84	24.03	24.52**	54.07	43.71	19.17**	13.8**	15.9**	15.31**
L27 X Gem 7	29.74	19.62	34.04**	56.26	37.25	33.78**	14.4**	12.4**	14.04**
L27 X Gem 9	24.07	20.07	16.61**	40.37	35.27	12.62**	11.5**	10.1**	12.00**
L27 X Gz168	26.21	23.39	10.77**	43.90	44.32	-0.96	10.0**	9.5**	5.50
Gem7X Gem9	25.41	19.18	24.50**	45.78	46.85	-2.34	8.3*	8.8*	-6.97*
Gem7XGz168	21.97	18.25	16.93**	41.39	34.01	17.84**	11.3**	9.0**	19.69**
Gem9X G168	23.88	20.16	15.57**	43.98	48.25	-9.72**	12.7**	8.8*	30.60**
L.S.D. _{0.05} (G)	2.1	2.0		3.8	4.0		4.41	5.47	
(N)			2.5			3.0			10.03
(GN)			2.04			3.9			6.5

* And** indicate significant at 0.05 and 0.01 probability levels, respectively

On the contrary, the three crosses Gem 7 x Gem 9, Gem 7 x Gz168 and L27 x Gem 9 in F₁ and F₂ generations showed the lowest GYPP under low-N, the high reduction due to low-N and therefore were considered sensitive (N-inefficient) to low-N stress.

In general, F₂-means for most characters were within the range of parental genotypes. Some F₂- progenies under N-limited environment exhibited higher values of GYPP, GPC and HI, suggesting transgressive effects in these characteristics. Gorny *et al.* (2011) reported a similar conclusion for grain dry weight produced per unit of N accumulated in grains (G_w/N_g).

It is worthy to note that the magnitude of N-induced alterations due to low-N stress in the majority of studied traits was distinctly dependent upon the genotype, as evident by the significant genotype x environment interactions. These results are consistent with observations previously reported in wheat (El Bassam, 1998, Le Gouis *et al.* 2000 and 2002, Al-Naggar *et al.* 2004, 2007, 2011, 2015 a, b, c), barley (Ceccarelli, 1994 and 1996 and Gorny and Sodikiewicz, 2001) and maize (Di Fonzo *et al.* 1982, Medici *et al.*, 2004, Preseterl *et al.*, 2008, Al-Naggar *et al.* 2011, 2014, 2015a,b,c), corroborating that an evaluation of breeding materials under diverse fertilization regimes is necessary for choice of the most efficient parental forms and/or cross combinations, as suggested by Brancourt-Hulmel *et al.* (2005), Laperche *et al.* (2006), Dawson *et al.* (2008), Wolfe *et al.* (2008) and Al-Naggar *et al.* (2011, 2014, 2015 a and b).

The rank of parents for GYPP was similar in the two N- environments, indicating less effect of interaction between parent and nitrogen level on GYPP. The three tolerant parents showed the highest GYPP under high-N and therefore were considered responsive parents. Moreover, L26 x L27 and L25 x L27 in F₁ and L26 x Gz 168 in F₂ generation had the highest GYPP under high-N and are therefore considered responsive crosses.

IV. CONCLUSION

In this study, non addition of N fertilizer caused in average a significant reduction in grain yield of wheat reaching to 21.17% in F₁ crosses across two growing seasons, but some crosses (L25 x L27 in F₁ generation and L25 x Gem7 and L26 x Gem7 in F₂ generation) did not show any reduction in grain yield due to low-N stress. Wheat genotypes that possess the highest grain yield traits under limited N environment could be identified. These genotypes are the parents L25, L26 and L27, the F₁'s L26 x L27, L25 x L26 and L25 x L27 and the F₂'s L25 x L27, L25 x L26 and L26 x Gz168. They showed the lowest reduction in GYPP due to low-N and were therefore considered tolerant (efficient) to low-N stress. Some F₂- progenies under N-limited environment exhibited increased ability to accumulate protein in their grains and higher values of GYPP as compared with their parents, suggesting transgressive effects in these characteristics and the possibility of obtaining genetic advance from selection for high grain yield and high grain protein content under low-N conditions.

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