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# Gains from Selection for High Grain Yield under Contrasting N Environments in $F_2$ Populations of Wheat Diallel Crosses

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**Abstract:** Breeding of low-N tolerant cultivars of wheat is one approach to reduce N fertilizer input, while maintaining acceptable yields. The objective of this investigation was to develop new bread wheat genotypes (transgressive segregants) of high grain yield under low-N stress conditions. Seventy five$F_3$ families were selected for high grain yield from$F_2$populations of diallel crosses among 6 parents under low-N and high-N and evaluated for grain yield and nitrogen use efficiency (NUE) traits in their $F_3$progenies compared with their parents under both high and low N conditions, using a split plot design in lattice (9 x 9) arrangement with three replications. The best $F_3$ families (4) that exhibited the highest grain yield and NUE under low-N as well as under high-N and exceeded significantly their better parents in the respective crosses were identified. They were all selected under low-N conditions and were significantly superior over their respective better parents. Actual significant superiority over the better parent in grain yield/plant ranged from 21.5% for SF11 to 33.7% for SF13 under low-N stress and from 14.2% for SF14 to 25.3% for SF11 under high-N conditions. Actual gain from selection for high yield in the best $F_3$ selected families is higher than corresponding expected genetic gains under both low-N and high-N.Superiority in grain yield over better parent were attributed to their high superiority in number of spikes/plant reaching to 80.1%, number of grains/ spike reaching to 31.2% and 100-grain weight reaching to 50.9% under low-N target environment. Selection in $F_2$ populations under low-N for high grain yield caused simultaneously a superiority in NUE, which reached to 30.4% under low-N and 22.7% under high-N environment. Moreover, superiority of the best selectants in grain yield and NUE traits was associated with superiority in grain protein concentration in most cases, which reached to 45.9 and 47% superiority for SF13 under low-N and high-N, respectively over the better parent.

**Keywords:** Transgressive segregation, Bread wheat, NUE, Target environment, Expected genetic advance, Low-N tolerance

## I. Introduction

Wheat (*Triticum aestivum* L.) is one of the oldest and most important cereal crops in Egypt. Across the last five years, the average annual production of wheat in Egypt was about 8 million tons with an average grain yield of 18.0 ardab / feddan (6.43 t/ha). Egypt is one of the largest countries that import wheat to satisfy local consumption. Wheat imports of Egypt in 2011 were about 9.8 million tons, with a cost of about 3.2 billion US$ (FAOSTAT, 2011). There is a need of increasing local wheat production to reduce the import cost through the development of new high yielding cultivars with tolerance to abiotic stresses, and adoption of the recommended cultural practices for growing such cultivars.

Nitrogen (N) is one of the major inputs in wheat production systems. But, 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. Today, elevated nitrogen level in water, as result of leaching, is an important component of agricultural pollution (Mariotti, 1997) causing major problems in marine ecosystems and eutrophication of freshwater (London, 2005).Moreover, N fertilization increases emissions of the greenhouse gas nitrous oxide ($N_2O$) from agricultural soils (Bouwman *et al.*, 2002). Volatile ammonia emissions from fertilizer contribute to deposition of N in unmanaged ecosystems (Vitousek *et al.*, 1997).

While wheat yields often increase at higher N rates, there can also be negative environmental consequences associated with high N inputs to agriculture. Based on these essential economic and ecological grounds, an increased interest is being shown worldwide in wheat cultivars that are more efficient in utilizing soil resources

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and better fitted to water and nutrient limitations (El Bassam, 1998; Good *et al.*,2004; Fageria and Baligar, 2005; Phillips and Wolfe, 2005; Muurinen *et al.*, 2006; Hirel *et al.*, 2007; Lammerts van Bueren *et al.*, 2008 and Sylvester-Bradley and Kindred ,2009). Among cereals, hexaploid wheat is commonly identified as a species with higher requirements for nutrients, especially nitrogen. Thus, breeding wheat cultivars with improved adaptation to less favorable, but more optimized N fertilization regimes has gained importance. In Egypt, such breeding strategies are also justified by problems of nitrogen that is a major constraint limiting grain production.

Progress in breeding wheat better adapted to less favorable N fertilization is still restricted for several reasons. Wheat breeders are frequently skeptical not only because of the morpho-physiological complexity of the matter, but mainly due to limited data on both the variation among available wheat collections and the genetics of key characters involved. Hence, several important queries remain to be resolved, especially in regard to the most effective selection schemes, desirable plant ideotype for low input ecosystems, appropriate selection criteria and features of the selection environment necessary for such breeding programs (e.g. Ceccarelli, 1996; Dawson *et al*., 2008 and Wolfe *et al.*, 2008). Furthermore, modern Egyptian wheat cultivars are phenotypically different but, in essence, represent a limited gene pool. The majority of them were developed under favorable or even luxurious fertilization regimes used at most breeding stations without or with scarce selection pressure for components of nutrient use efficiency. On the contrary, beneficial plant characteristics for low-input ecosystems may be different from those present in modern, high-yielding wheat cultivars (El Bassam, 1998 and Murphy *et al.*, 2007). This raises concerns for breeders as to whether the range and spectrum of genetic variation in nutrient efficiency among modern wheat cultivars is sufficiently wide under sub-optimal habitats to guarantee progress in breeding more efficient wheat cultivars better adapted to less favorable fertilization practices.

Hybridization between the wheat cultivars and lines is carried out to increase genetic variability. 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.

The chief role of hybridization is to create hybrid populations with wide genetic variation from which new recombination of genes may be selected (Singh, 2000). Transgressive segregation is a phenomenon specific to segregating hybrid generations and refers to the individuals that exceed parental phenotypic values for one or more characters (Rieseberg *et al.*, 1999). Observations on transgressive segregants were previously explained by many researchers (Voigt and Tischler, 1994 and Al-Bakry *et al.*, 2008). Selection from segregating generations of wheat hybrid combinations succeeded to develop new genotypes that possess adaptive traits of some abiotic stresses, such as drought tolerance (Al-Naggar *et al* 2004, 2012 and Al-Bakry and Al-Naggar 2007,Farshadfar *et al* 2011, Al-Naggar and Shehab-Eldeen 2012 and Tharwat, *et al.* 2013).

The main objective of the present investigation was to develop new wheat genotypes (transgressive segregants) of high grain yield under low-N stress conditions. The detailed objectives were: (i) to estimate expected genetic advance from selection in each $F_2$ of the 15 diallel crosses, (ii) to evaluate 75 selections from $F_2$ cross combinations along with their parents for grain yield and nitrogen use efficiency traits under contrasting N environments and (iii) to estimate the actual progress from the best $F_3$ families in such traits compared with their better parents.

## 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, 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 $F_1$ seeds of 15 crosses. In summer 2006, a part of $F_1$ seeds was sown in greenhouse of Wheat Res. Dept. under controlled conditions to obtain the $F_2$ seeds. In season 2007/2008, the half diallel of crosses was again done to increase quantity of $F_1$ seeds and in summer 2007 the $F_1$seeds were again sown in the greenhouse under controlled conditions to obtain more seeds of 15 $F_2$ crosses.

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

In the seasons 2007/2008, 2008/2009, parents (6), $F_1$'s (15) and $F_2$'s (15) were sown on 17$^{th}$ 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 sup plots. Each parent or $F_1$ was sown in two rows and each $F_2$ 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 $m^2$ for parent or $F_1$ and 3.6 $m^2$ for $F_2$. All other agricultural practices were done according to the recommendation of Ministry of Agriculture for growing wheat in Noubarya region.

In season (2007/2008), 75 individual $F_2$ plants were selected ( the best three plants of each $F_2$ cross under high and the best two plants under low-N) for high grain yield/ plant and other favorable traits (45 from HN and 30 from LN) and harvested individually in the field of Noubarya Res. Sta.

## 2.4. Field Evaluation of Selections and Their Parents

In the season 2008/2009, 81 genotypes (6 parents and 75 $F_3$ selected families) were sown on 21$^{st}$ of November, 2008 under two levels of nitrogen fertilization. A split plot design in three replications in lattice arrangement (9x9) with three replications (0 and 75 kg N/fed) using Urea fertilizer as in the previous experiment. The two levels of nitrogen were assigned to the main plots and the genotypes to the sup plots. Each entry was sown in two rows; each row three meter long with spaces between rows of 20 cm and between hills of 30 cm; the plot size was 1.8 $m^2$.

Available soil nitrogen in 30 cm depth was analyzed immediately prior to sowing and N application at the laboratories of Water and Environment Unit, ARC, Egypt in the two seasons. Soil nitrogen was found to be 55 and 57 kg N/ fed in the seasons 2007/2008, 2008/2009, respectively. Available soil nitrogen after adding nitrogen fertilizer was therefore 55 and 130 kg N/fed in the first season and 57 and 132 kg N/fed in the second season for the two treatments, i.e. LN and HN, respectively. The available nitrogen to each plant (including soil and added N) was calculated for each environment to be 0.79, 1.85 g/plant in 2007/2008 season and 0.81 and 1.89 kg/fed in 2008/2009 season, with an average across the two seasons of 0.80 and 1.87 g/plant for the two environments LN and HN, respectively. 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% clay), the pH is 8.93, the EC is 0.55 $dSm^{-1}$, the soluble cations in meq $l^{-1}$ are $Ca^{2+}$ (5.30), $K^+$ (0.70), $Na^+$ (0.31), $Mg^{2+}$ (2.60) and the soluble anions in meq $l^{-1}$ are $CO_3^{2-}$ (0.00), $HCO_3^-$ (2.10), $Cl^-$ (5.30) and $SO_3^{2-}$ (1.51). All other agricultural practices were followed according to the recommendations of ARC, Egypt.

## 2.5. Data Collection

A random sample of 10 plants of each genotype of parents and $F_1$'s and 30 plants of $F_2$'s was used to collect data for 14 traits: **days to 50% heading (DTH)** as number of days from sowing date to the date at which 50% of main spike awns/ plot have completely emerged from the flag leaves, **days to maturity (DTM)** measured as number of days from sowing date to the date at which 50% of main peduncles/ plot have turned to yellow color (physiological maturity), **plant height (PH)** measured as plant length from the soil surface to the tip of the spikes, excluding awns, **number of spikes/plant (SPP)** as number of fertile spikes per plant, **number of grains/ spike (GPS), 100 grain weight (100GW)** measured as weight of 100 grains taken from each guarded plant, **grain yield/ plant (GYPP)** measured as weight of the grains of each individual plant, **biological yield/ plant (BYPP)** measured as weight of the grains and stem of each individual plant and **harvest index (HI%)** according formula: HI= 100 (GYPP/ BYPP). At physiological maturity stage, five random guarded plants were removed from each plot by cutting at the soil surface. The plants were bulked as one sample per plot. They were separated into straws (including leaves, stems and spike residues) and grains. Samples were oven dried at 70°C to a constant weight and each part was weighed separately. Samples were ground in powder and nitrogen of straws (N straw) and grains ($N_g$) was determined using Kjeldahl procedure according to A.O.A.C. (1990). Total plant nitrogen ($N_t$) was calculated as follows: $N_t = N_g + N_{straw}$. Data were collected for: **nitrogen use efficiency (NUE) g/g**= (GYPP / $N_s$), **nitrogen uptake efficiency (NUPE)% =**100 ($N_t$ / $N_s$), **nitrogen utilization efficiency (NUTE)** (g/g)= (GYPP/$N_t$), **nitrogen harvest index (NHI%)=** 100($N_g$/ $N_t$), and **grain protein content (GPC)** measured as follows: **GPC%=** $N_g$ x 5.70 according to AACC(2000), where GYPP is grain yield/ plant in gram, $N_t$ is total nitrogen in the whole plant (grains and straw), $N_s$ is available nitrogen in the soil for each plant, and $N_g$ is grain nitrogen content. Nitrogen efficiency parameters were estimated according to Moll *et al.* (1982).

## 2.6. 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). Moreover, each environment (HN and LN) was analyzed separately as lattice design for the purpose of determining genetic parameters using Genestat10$^{th}$ addition windows software. Least significant differences (LSD) values were calculated to test the significance of differences between means according to Steel *et al*. (1997).

## 2.7. Estimating Genetic Parameters for Each F2 Cross

Genotypic ($\delta^2_g$) and phenotypic ($\delta^2_{ph}$) variances of each of the studied $F_2$ cross were estimated separately. Phenotypic variance of each parent and $F_1$ was considered as environmental variance according to Shin (1968), while that of the $F_2$ cross was assumed to include both genetic ($\delta^2_g$) and environmental ($\delta^2_{ph}$) variances. Therefore, $\delta^2_g$ of each $F_2$ cross was calculated using the formula: $\delta^2_g$ of $F_2$ = $\delta^2_{ph}$ of $F_2$ – ($\delta^2_{ph}$ of $P_1$ + $\delta^2_{ph}$ of $P_2$ +$\delta^2_{ph}$ of $F_1$ )/3. Heritability in the broad sense ($h^2_b$) for each $F_2$ was estimated as follows: $h^2_b$ = 100 ($\delta^2_G/\delta^2_{ph}$). Expected gain from selection (GA) for each $F_2$ was estimated using $h^2_b$ as follows: GA = 100 $h^2_b$ k $\delta_{ph}$ / x, where k = 2.64 for the 1 % selection intensity used in this study.

# III. Results and Discussion

### 3.1. Expected selection gain in each $F_2$ cross

Phenotypic and genotypic variances, heritability in the broad-sense and expected genetic advance (GA%) from selection using 1% selection intensity in each $F_2$ generation of the studied 15 diallel crosses for grain yield/plant under high and low N are presented in Table (2). Results in this table indicates that $F_2$ crosses differ in magnitude of genotypic and phenotypic variances as well as in heritability and expected estimate of selection gain for GYPP.

Based on the genetic parameters presented in Table (2) under high-N, the best five $F_2$ populations for practicing selection for improving GYPP are L2 x Gem9, L2 xGem7, Gem 7x Gz168, L1 x Gem9 and L3 x Gem 9, where genetic advance from selection for high GYPP reached 12.23% in Gem 7x Gz168 (Table 2).

Under low–N environment, the most suitable crosses for practicing selection, *i.e*. those exhibited high estimates of genetic variance, heritability and genetic advance from selection for high yield were L1 x Gem9, L2 x L3, L3 x Gem7, L1 x Gz 168 and L3 x Gem 9, where genetic advance from selection for high GYPP reached 21.28% in L1 x Gem9(Table 2). It is therefore expected that low-N tolerant transgressive segregants could be released from the $F_2$ generation of these crosses under the respective soil-N environment.

**Table1.** *Estimates of some genetic parameters for grain yield/plant of each $F_2$ cross under high and low levels of nitrogen*

| $F_2$ crosses | $\delta^2_g$ | $\delta^2_P$ | $h^2_b$ | GA% | $\delta^2_g$ | $\delta^2_P$ | $h^2_b$ | GA% |
|---|---|---|---|---|---|---|---|---|
| | **High-N** | | | | **Low-N** | | | |
| **L25 X L26** | 3.63 | 25.96 | 13.99 | **7.25** | 1.45 | 24.97 | 5.79 | **3.06** |
| **L25 X L27** | 3.44 | 23.94 | 14.35 | **7.75** | 0.13 | 26.09 | 0.50 | **0.26** |
| **L25 X Gem 7** | 2.69 | 23.33 | 11.52 | **6.30** | 1.13 | 23.88 | 4.73 | **2.56** |
| **L25 X Gem 9** | 3.37 | 22.97 | 14.66 | **8.07** | 5.14 | 15.97 | 32.21 | **21.28** |
| **L25 X Gz 168** | 0.47 | 27.08 | 1.74 | **0.88** | 1.75 | 21.75 | 8.05 | **4.56** |
| **L26 X L3** | 2.18 | 28.97 | 7.52 | **3.69** | 6.65 | 20.25 | 32.84 | **19.27** |
| **L26 X Gem 7** | 5.01 | 23.95 | 20.91 | **11.28** | 0.85 | 23.51 | 3.63 | **1.98** |
| **L26 X Gem 9** | 3.88 | 25.45 | 15.22 | **7.97** | 0.10 | 22.04 | 0.47 | **0.27** |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **L26 X Gz 168** | 1.61 | 31.84 | 5.06 | **2.37** | 1.16 | 24.03 | 4.81 | **2.59** |
| **L27 X Gem 7** | 0.30 | 29.74 | 1.01 | **0.49** | 3.32 | 19.62 | 16.91 | **10.08** |
| **L27 X Gem 9** | 4.39 | 24.07 | 18.22 | **9.81** | 1.51 | 20.07 | 7.52 | **4.43** |
| **L27X Gz168** | 2.51 | 26.21 | 9.56 | **4.93** | 0.17 | 23.39 | 0.71 | **0.39** |
| **Gem 7 X Gem9** | 0.12 | 25.41 | 0.49 | **0.25** | 1.17 | 19.18 | 6.10 | **3.68** |
| **Gem 7 X Gz168** | 4.77 | 21.97 | 21.71 | **12.23** | 0.75 | 18.25 | 4.12 | **2.55** |
| **Gem 9 X Gz168** | 1.97 | 23.88 | 8.27 | **4.47** | 0.74 | 20.16 | 3.66 | **2.15** |

### 3.2. Actual gain from selection experiment

Selection for desirable plant types in wheat could be practiced in heterogeneous populations resulting from segregating generations following hybridization. Selection for qualitative characters is simple and quick, but that for quantitative character such as low-N tolerance is often difficult and time consuming (Singh, 2000). Rajaram *et al*. (1997) stated that the CIMMYT's approach to wheat breading for low-N tolerance depends on growing the segregating wheat generations under limited N conditions, and the characters that are important for selection of individual plant for low-N stress situations include relative higher yield.

In the present study, 75 plants with desirable traits related to low-N tolerance were selected from $F_2$ populations of diallel crosses between six wheat cultivars and lines, 45 of which were selected under high-N and 30 under low-N environment in 2007/2008 season. Progenies of these selections (75 $F_3$ families) were evaluated in the field in 2007/2008 season along with their six parental cultivars and lines (L25, L26, L27, Gem7, Gem9 and Gz 168) for 14 traits under low-N (0 kg N/fed) and high-N (75 kg N/fed) conditions.

#### 3.2.1. Analysis of variance

Analysis of variance of the split plot experiment that included two N levels in the main plots and 81 wheat genotypes in the sub-plots (75 selected $F_3$ families and 6 parents) for studied characters is presented in Table (3).

**Table2.** *Analysis of variance of split plot design for 81 genotypes including 75 selected families (45 from high-N and 30 from low-N) and 6 parents in (2008/ 2009 season*

| SV | df | MS | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | **DTH** | **DTM** | **PH** | **SPP** | **GPS** | **100GW** | **GYPP** |
| Rep | 2 | 32.4** | 50.4** | 136.6** | 1.4 | 26.1 | 1.0** | 7.5 |
| N levels (N) | 1 | 2802.2** | 3303.1** | 3997.8** | 3224.2** | 14072.0** | 264.1** | 9473.6** |
| Error N | 2 | 0.35 | 2.4 | 44.4 | 20.7 | 3.7 | 0.08 | 1.1 |
| Genotypes G) | 80 | 35.7** | 26.6** | 305.9** | 28.6** | 182.3** | 1.4** | 26.5** |
| G x N | 80 | 50.8** | 48.4** | 45.9** | 17.7** | 57.9 | 0.9** | 25.1** |
| Error | 320 | 8.2 | 8.3 | 13.0 | 2.8 | 67.6 | 0.25 | 3.3 |
| | | **BYPP** | **HI%** | **NUE** | **NUPE** | **NUTE** | **NHI** | **GPC** |
| Rep | 2 | 20.0 | 63.1** | 9.1 | 2.4 | 0.01 | 0.6 | 66.6** |
| N levels (N) | 1 | 3303.2** | 14409.5** | 12109.0** | 3023.3** | 2.5** | 46.5** | 46250.6** |
| Error N | 2 | 28.2 | 4.2 | 3.9 | 1.9 | 0.02 | 10.6 | 1.7 |
| Genotypes(G) | 80 | 51.6** | 59.6** | 20.0** | 67.5** | 0.19** | 15.4** | 745.7** |
| G x N | 80 | 25.7** | 79.8** | 18.8** | 14.8** | 0.08** | 75.0** | 228.1** |
| Error | 320 | 7.3 | 11.5 | 3.3 | 0.7 | 0.01 | 4.78 | 21.8 |

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

Results indicated than mean squares due to N levels and those due to genotypes were highly significant for all studied traits, suggesting the significant effect of both nitrogen level and genotype on such traits. Mean squares due to genotypes x nitrogen levels interaction were highly significant for all studied traits, indicating that performance of the studied genotypes in this experiment varied with nitrogen status of the soil, confirming the results of previous workers (Gorny *et al*., 2011, Al-Naggar *et al*.,2004, 2007, 2010, 2014 and 2015 ). The significant G×N interaction for grain yield was also a good evidence for varying responses of these wheat genotypes at various N levels (Earl and Ausubel, 1983 and Austin *et al*., 1980).

#### 3.2.2. Comparison of the performance of selection groups

The highest yielding eight families selected from 45 families under high N and six families selected from 30 families under low N were identified. Means of studied traits of three groups representing the best 8 $F_3$ families selected under HN, the best 6 $F_3$ families selected under LN and the six parental cultivars and lines evaluated under low-N and high-N are presented in Table (4).

On average, under high-N conditions, the group of the best 6 $F_3$ families selected from low-N environment showed the highest mean grain yield per plant (29.98 g), while the group of 6 parents exhibited the lowest grain yield (27.06 g/plant) (Table 3). In the same manner, under low-N (Table 3) the highest average grain yield / plant (25.90 g ) was observed for the group of 6 $F_3$ families selected from low-N environment, but the lowest average (21.53 g/ plant) was achieved by the group of 8 $F_3$ families selected under high-N environment.

**Table3.** *Summary of group means of the best 8 $F_3$ families selected under high N, the best 6 $F_3$ families selected under low-N and 6 parents for studied traits under low-N and high-N conditions (2008/2009 season).*

| Group | DTH | DTM | PH cm | SPP | GPS | 100 GW (g) | GYPP | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Mean (g) | Reduction% |
| **Low-N** | | | | | | | | |
| Best 8 $F_3$'s selected under HN | 84.13 | 124.83 | 100.2 | 11.30 | 76.02 | 3.69 | 21.53 | 25.79 |
| Best 6 $F_3$'s selected under LN | 87.83 | 129.17 | 90.05 | 12.07 | 76.30 | 4.11 | 25.90 | 13.60 |
| 6 parents | 86.83 | 125.44 | 78.52 | 8.67 | 70.51 | 4.17 | 23.27 | 14.02 |
| **High-N** | | | | | | | | |
| Best 8 $F_3$'s selected under HN | 89.17 | 130.21 | 91.27 | 18.27 | 86.91 | 5.09 | 29.01 | |
| Best 6 $F_3$'s selected under LN | 90.33 | 131.06 | 82.57 | 14.83 | 84.81 | 4.69 | 29.98 | |
| 6 parents | 89.06 | 132.00 | 83.08 | 11.19 | 80.88 | 4.58 | 27.06 | |
| $LSD_{0.05}$ | 3.87 | 3.9 | 4.88 | 2.26 | 11.4 | 0.66 | 2.46 | |
| | **BYPP(g)** | **HI%** | **NUEg/g** | **NUPE%** | **NUTE g/g** | **NHI%** | **GPC%** | |
| **Low-N** | | | | | | | | |
| Best 8 $F_3$'s selected under HN | 60.37 | 35.70 | 26.47 | 11.90 | 1.31 | 56.05 | 9.43 | |
| Best 6 $F_3$'s selected under LN | 59.72 | 43.40 | 31.50 | 19.55 | 0.95 | 58.85 | 13.88 | |
| 6 parents | 53.95 | 43.16 | 29.48 | 27.01 | 1.05 | 55.06 | 11.10 | |
| **High-N** | | | | | | | | |
| Best 8 $F_3$'s selected under HN | 66.20 | 43.96 | 15.35 | 9.89 | 1.19 | 55.98 | 13.77 | |
| Best 6 $F_3$'s selected under LN | 63.74 | 47.10 | 15.86 | 11.13 | 1.09 | 54.39 | 16.84 | |
| 6 parents | 62.22 | 43.57 | 14.63 | 17.32 | 0.85 | 54.31 | 15.42 | |
| $LSD_{0.05}$ | 3.85 | 3.85 | 4.59 | 2.46 | 3.85 | 4.59 | 2.46 | |

Moreover, yield reduction due to low-N stress was at minimum (13%) for the group of selected families under low-N conditions. On the contrary, maximum reduction (25.79%) due to low-N was exhibited by the group of selected families under high-N conditions. This means that in the present experiment selection practiced in $F_2$ populations under low-N as selection environment was more effective in producing higher yielding genotypes under low-N target environment than when selection was practiced under high-N as selection environment. Selection for high grain yield under low-N was even more efficient than under high-N for the evaluation under high-N target environment.

Published research on abiotic stress, such as low-N stress, shows two contrasting strategies for identifying high yielding genotypes under selection environment. First, genotypes may be evaluated under the conditions in which they will be ultimately produced, namely a certain type of stressed environment, to minimize genotype environment interaction (Shabana *et al*., 1982, Al-Naggar *et al*. 2004, 2007, 2010, 2011, 2009, 2012, 2014 and 2015).

Second, genotypes may be evaluated under optimum conditions that maximize heritability; but in this case problem with genotype x environment may be encountered. Braun *et al.* (1992) has argued for this approach, citing results from 17 years of CIMMYT winter performance trails.

Our results are in favor of the first strategy. The direct selection under abiotic stress would ensure the preservation of alleles for stress tolerance (Langer *et al*.,1979) but direct selection under optimal environment would take advantage of high heritability (Allen *et al*., 1978; Blum, 1988; Smith *et al*., 1990 and Braun *et al*., 1992). A third alternative, which is currently used at CIMMYT, deploys the simultaneous evaluation under both near optimum and stress conditions, with selection of those genotypes that perform well in both environments (Calhoun *et al*., 1994). However, ultimate evaluation of selection must be performed in the target environment prior to recommendation of a cultivar for commercial production. Further selection and evaluation under low-N stress conditions should be continued in the selected superior $F_3$ families derived from the present investigation in order to assure their superiority in low-N tolerance and select the most stable and high yielding families under low-N stress conditions.

Selection in the $F_2$'s under low-N stress (the group of the best 6 $F_3$ families selected under LN) gave the highest means of grain yield/ plant (25.9g), spikes/ plant (12.07), grains/ spike (76.3), nitrogen use efficiency (31.5 g/g), nitrogen harvest index (58.85%) and grain protein content (13.88%) when evaluated under low-N conditions , and the highest means of GYPP (29.98g), harvest index (47.1%), NUE (15.86 g/g) and GPC (16.84%) when evaluated under high-N conditions (Table 3). But it was observed that the group of best 6 $F_3$'s selected under low-N matured later than the group of parents by 3.73 days in average.

On the other hand, the group of $F_3$ families selected under high-N exhibited the highest means of SPP, GPS, 100 GW, plant height, BYPP and NUTE when evaluated under high-N conditions and plant height and BYPP when evaluated under low-N conditions (Table 4).

### 3.2.3. Transgressive segregants

The best four $F_3$ families that exhibited the highest grain yield under low-N as well as under high-N and exceeded significantly their better parents in the respective crosses were identified. They were all selected under low-N conditions and their parents were Gem 7 x Gem9 for SF11, Gem7 x Gz 168 for SF12, Gem9 x Gz168 for SF13 and SF14.

Means of these transgressive segregants (the best four families selected under low-N; SF11, SF12, SF13 and SF14) and their three parents, namely Gem7, Gem9 and Gz168 for all studied traits are presented in Table (5) and seleced traits are illustrated in Figures (1 through 6).

In general , averages of the four transgressive segregants were higher than averages of the three parents for all studied traits under both low-N and high-N conditions, except for 100 GW, NUTE and NUPE under low-N and DTM, NHI and NUPE under high-N (Table 5).

A significant improvement has been occurred due to selection of transgressive segregants in $F_2$ of three crosses (Gem7 x Gem9, Gem7 x Gz168 and Gem9 x Gz 168) under low-N environment in the studied traits under low-N target environment for grain yield (32.3%), NUE (28.9%), SPP (72.2%), GPS (29.8%), BYPP (21.8%), NHI (19.8%), GPC (22.4%) and HI (7.6%). Moreover, the reduction in grain yield due to low-N was reduced (favorable) from an average of 22.3% for parents to 15.38% for the best four $F_3$ families (Table 5).

The best four selected $F_3$ families (SF11, SF12 , SF13 and SF14) showed on average a significant superiority in most studied grain yield and nitrogen use efficiency traits under both low-N and high-N conditions. These superior families are the results of transgressive segregation and may be considered promising families to produce promising pure lines, after generations of homozygosity, of tolerance to low-N conditions and high grain yield.

**Table4.** *Mean performance of the best four selected $F_3$ families under low-N and their three parents evaluated under low-N and high-N for studied wheat traits (2008/2009 season)*

| Genotype | DTH | DTM | PH | SPP | GPS | 100GW | GYPP(g) | Red. % |
|---|---|---|---|---|---|---|---|---|
| **High-N** | | | | | | | | |
| SF11 | 86.00 | 127.33 | 87.30 | 14.13 | 86.29 | 4.59 | 33.93 | |
| SF12 | 88.33 | 128.67 | 91.60 | 15.50 | 83.88 | 3.72 | 31.50 | |
| SF13 | 91.00 | 131.33 | 76.83 | 11.70 | 88.06 | 5.27 | 30.63 | |
| SF14 | 88.33 | 129.00 | 72.97 | 11.63 | 75.81 | 5.06 | 28.62 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Aver. | 88.41 | 129.08 | 82.22 | 13.24 | 83.51 | 4.66 | 31.17 | |
| Gem 7 | 88.33 | 131.00 | 90.38 | 10.50 | 68.41 | 3.77 | 27.07 | |
| Gem 9 | 86.33 | 132.67 | 75.93 | 9.53 | 69.37 | 3.60 | 24.60 | |
| Gz 168 | 85.67 | 129.33 | 80.60 | 11.19 | 69.39 | 4.58 | 25.07 | |
| Aver. | 86.79 | 131.00 | 81.97 | 10.40 | 69.06 | 3.98 | 25.58 | |
| **Low-N** | | | | | | | | |
| SF11 | 87.33 | 126.00 | 93.87 | 12.47 | 69.27 | 3.33 | 24.89 | 26.65 |
| SF12 | 90.67 | 134.00 | 82.17 | 12.90 | 86.61 | 4.78 | 26.66 | 15.37 |
| SF13 | 92.00 | 134.33 | 83.23 | 12.00 | 80.74 | 4.55 | 26.85 | 12.35 |
| SF14 | 90.33 | 131.00 | 85.70 | 12.23 | 71.75 | 5.22 | 26.57 | 7.15 |
| Aver. | 90.08 | 131.44 | 86.24 | 12.40 | 77.09 | 4.47 | 26.25 | 15.38 |
| Gem 7 | 86.67 | 122.00 | 87.40 | 5.97 | 66.00 | 4.78 | 19.91 | 26.44 |
| Gem 9 | 83.33 | 123.67 | 82.07 | 7.07 | 54.48 | 4.55 | 19.53 | 20.61 |
| Gz 168 | 82.67 | 102.33 | 81.77 | 8.57 | 57.75 | 5.22 | 20.09 | 19.84 |
| Aver. | 84.17 | 116.00 | 83.75 | 7.20 | 59.41 | 4.85 | 19.84 | 22.30 |
| LSD $_{0.05}$ | 3.87 | 3.9 | 4.88 | 2.26 | 11.4 | 0.66 | 2.46 | |
| **Genotype** | **BYPP (g)** | **HI %** | **NUE g/g** | **NUPE %** | **NUTE g/g** | **NHI %** | **GPC %** | |
| **High-N** | | | | | | | | |
| SF11 | 64.40 | 52.84 | 17.95 | 11.72 | 1.16 | 55.34 | 16.15 | |
| SF12 | 59.79 | 52.59 | 16.66 | 10.38 | 1.22 | 56.11 | 14.50 | |
| SF13 | 68.05 | 45.02 | 16.21 | 12.74 | 0.97 | 52.45 | 21.79 | |
| SF14 | 64.45 | 44.40 | 15.14 | 11.33 | 1.01 | 48.29 | 19.26 | |
| Aver. | 64.17 | 48.71 | 16.49 | 11.54 | 1.09 | 53.05 | 17.92 | |
| Gem7 | 59.32 | 45.64 | 14.63 | 16.78 | 0.88 | 52.34 | 16.12 | |
| Gem9 | 61.60 | 39.96 | 13.30 | 16.37 | 0.81 | 56.38 | 12.96 | |
| Gz 168 | 54.38 | 46.15 | 13.55 | 14.62 | 0.93 | 53.94 | 14.82 | |
| Aver . | 58.43 | 43.92 | 13.83 | 15.92 | 0.87 | 54.19 | 14.63 | |
| **Low-N** | | | | | | | | |
| SF11 | 60.05 | 41.43 | 30.73 | 17.34 | 1.01 | 63.91 | 13.27 | |
| SF12 | 60.25 | 44.25 | 32.91 | 20.56 | 0.91 | 64.11 | 13.93 | |
| SF13 | 59.80 | 44.93 | 33.15 | 25.27 | 0.92 | 60.70 | 16.64 | |
| SF14 | 57.41 | 46.32 | 32.80 | 20.37 | 0.74 | 66.62 | 13.64 | |
| Aver. | 59.38 | 44.25 | 32.39 | 20.88 | 0.89 | 64.68 | 14.37 | |
| Gem7 | 42.05 | 47.36 | 25.20 | 22.11 | 1.08 | 54.34 | 12.72 | |
| Gem9 | 52.49 | 37.21 | 24.73 | 17.67 | 1.42 | 53.44 | 11.40 | |
| Gz 168 | 51.69 | 38.87 | 25.43 | 25.22 | 1.05 | 54.16 | 11.10 | |
| Aver | 48.74 | 41.15 | 25.12 | 21.67 | 1.18 | 53.98 | 11.74 | |
| LSD 0.05 | 3.85 | 3.85 | 4.59 | 2.46 | 3.85 | 4.59 | 2.46 | |

Transgressive segregation is a phenomenon specific to segregating hybrid generations and refers to the individuals that exceed parental phenotypic values for one or more characters (Rieseberg *et al.*1999). Such plants are produced by an accumulation of favorable genes from both parents as a consequence of recombination.

Observations on transgressive segregation in segregating hybrid generations were previously explained by several research workers (Voigt and Tischler 1994 and Al-Bakry *et al.* 2008).

The selection of new recombinants and transgressive segregants was previously reported in different crops (Vega and Frey 1980, Snape 1982, Rieseberg *et al.* 1999, Al-Bakry *et al.* 2008). Rieseberg *et al.* (1999) assessed the frequency of transgressive segregants in hybrid populations, described its genetic basis and discussed the factors that best predict its occurrence.

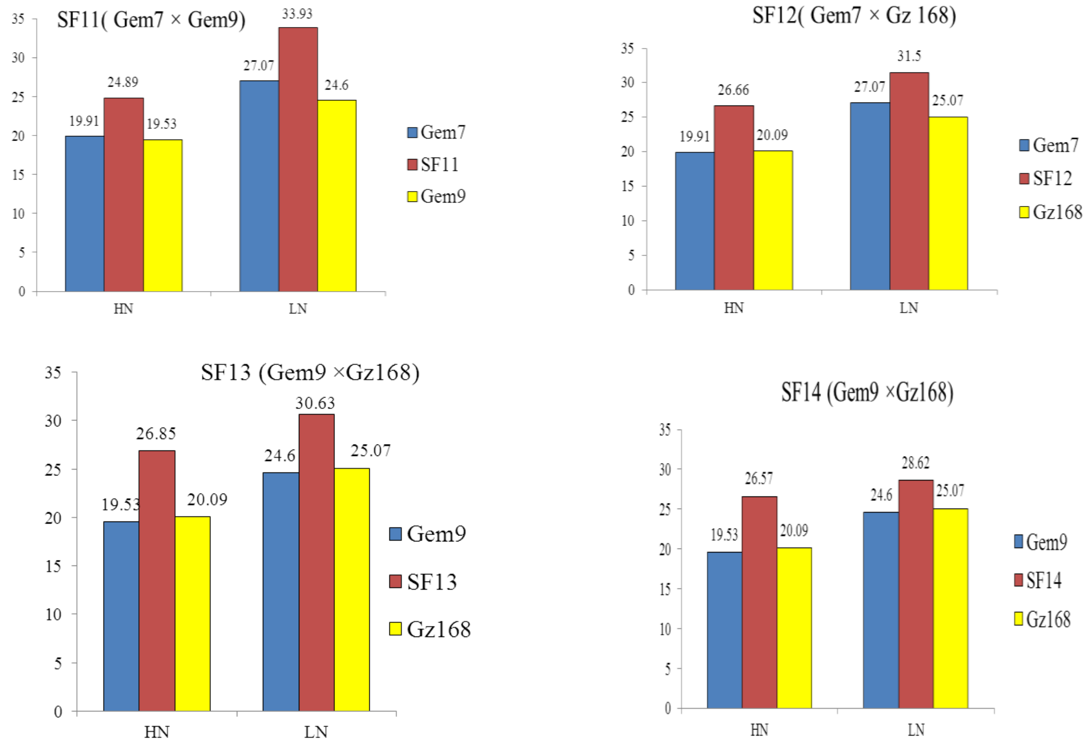


**Fig1** *Mean grain yield/ plant of the four transgressive segregant $F_3$ families SF11, SF12, SF13 and SF14 as compared to their parents under high-N and low-N (LSD.05= 1.57g under HN and 1.53g under LN).*

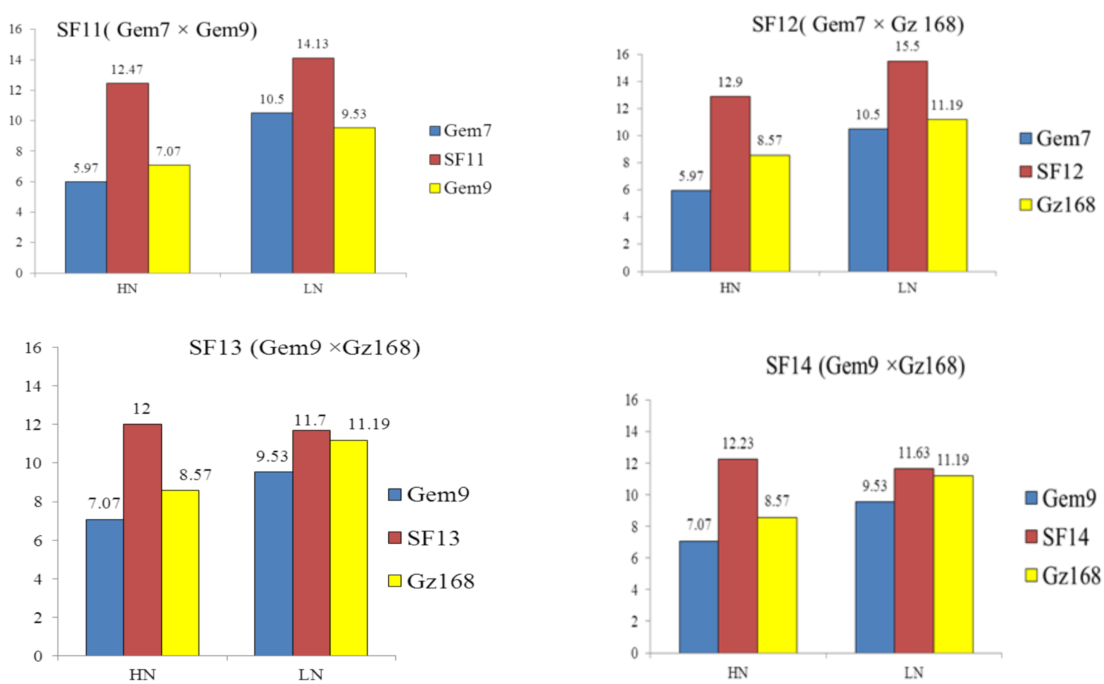


**Fig2.** *Mean number of spikes/ plant of the four transgressive segregant $F_3$ families SF11, SF12, SF13 and SF14 as compared to their parents under high-N and low-N (LSD.05= 0.72 under HN and 0.66 under LN).*

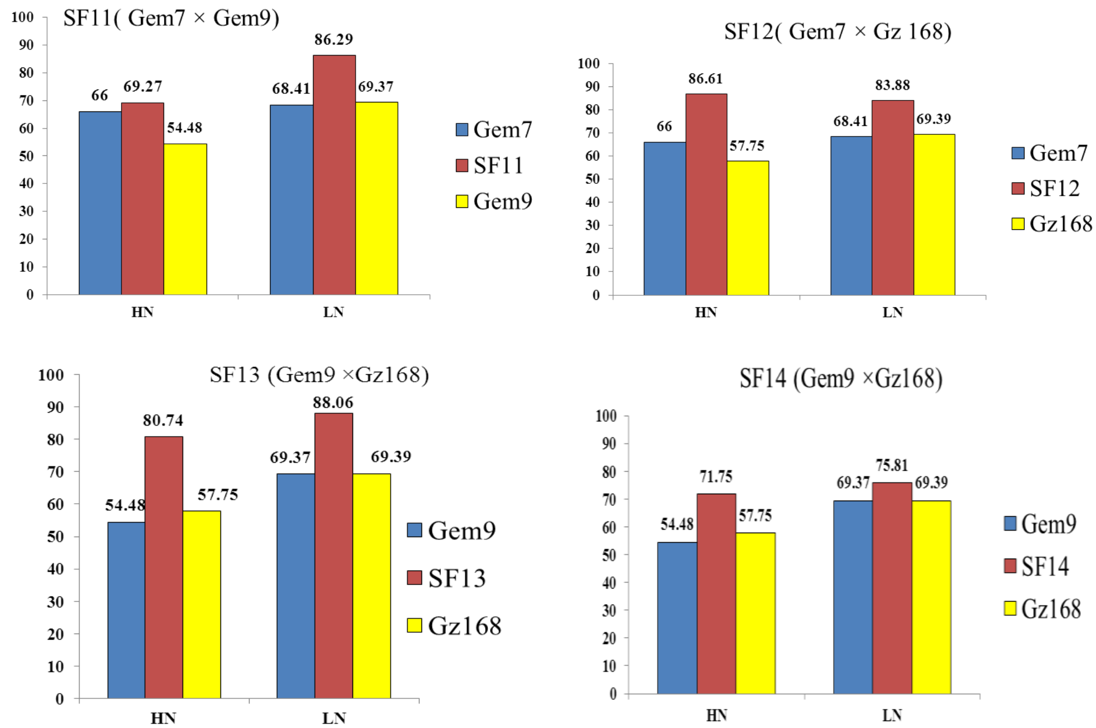


**Fig3.** *Mean number of grains/ spike of the four transgressive segregant $F_3$ families SF11, SF12, SF13 and SF14 as compared to their parents under high-N and low-N (LSD.05= 1.53 under HN and 1.60 under LN).*

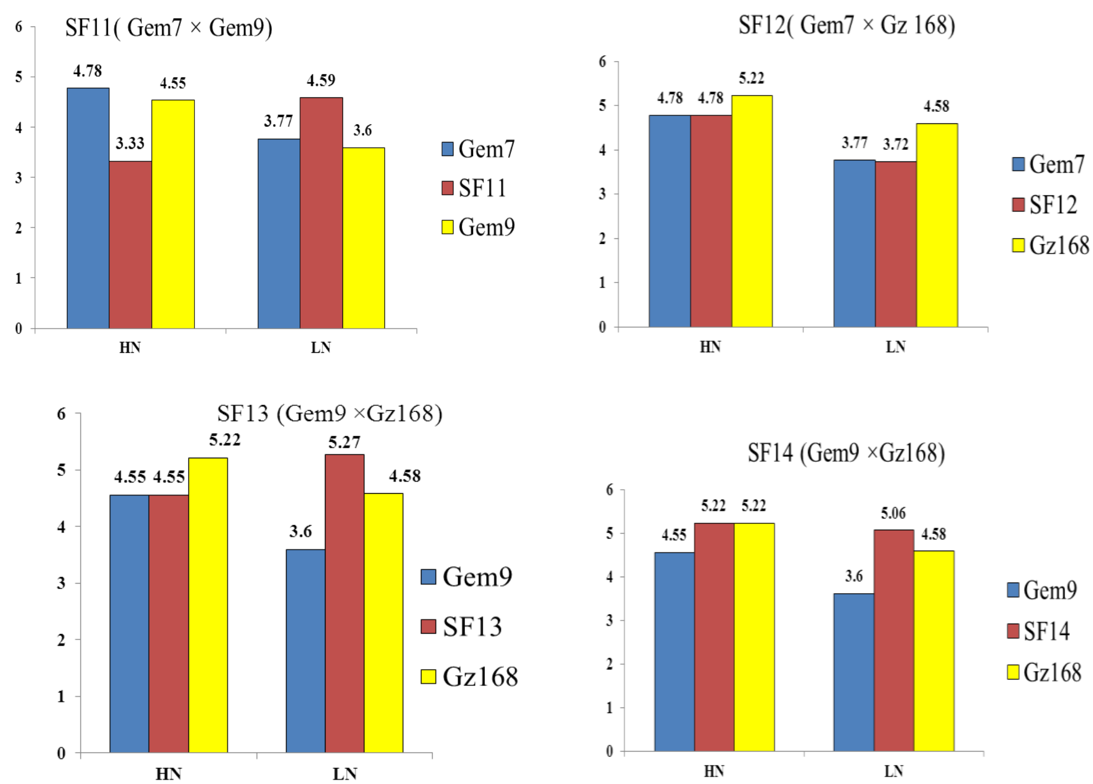


**Fig4**. *Mean 100 grain weight of the four transgressive segregant $F_3$ families SF11, SF12, SF13 and SF14 as compared to their parents under high-N and low-N (LSD.05= 0.38 g under HN and 0.30 g under LN).*

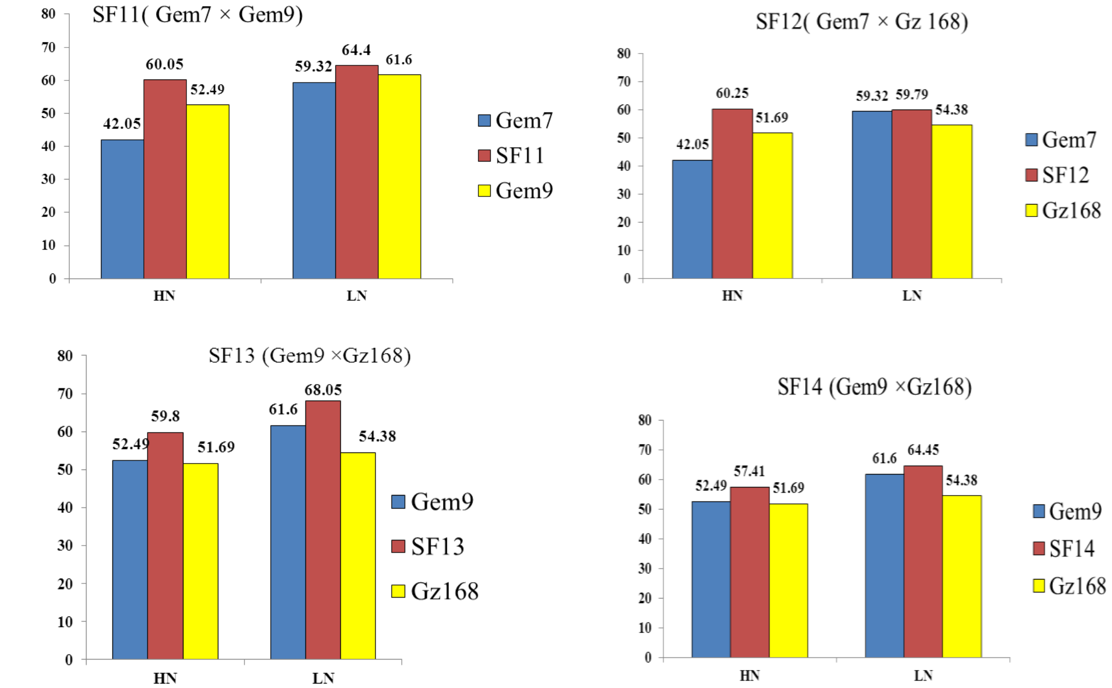


**Fig5.** *Mean biological yield/ plant of the four transgressive segregant $F_3$ families SF11, SF12, SF13 and SF14 as compared to their parents under high-N and low-N (LSD.05= 1.97 g under HN and 1.83 g under LN).*

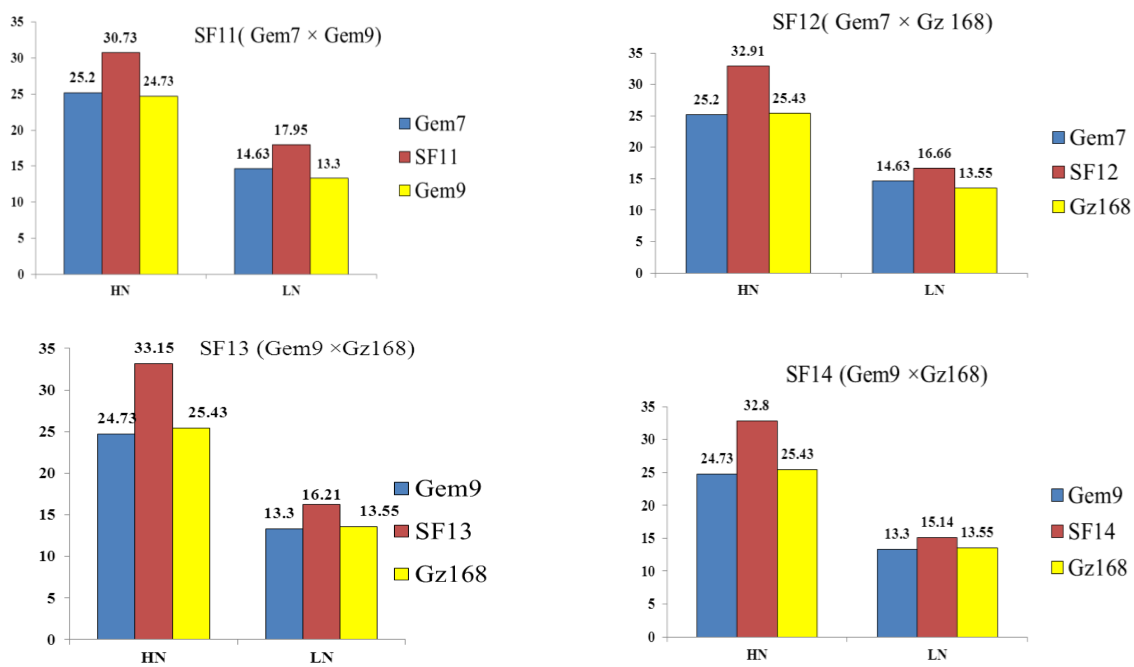


**Fig6.** *Mean NUE of the four transgressive segregant $F_3$ families SF11, SF12, SF13 and SF14 as compared to their parents under high-N and low-N (LSD.05= 0.85 g/g under HN and 1.97 g/g under LN)*

### 3.2.4. Superiority of the four best $F_3$ selected families

Superiority of the best $F_3$ selected families over the better parent of their respective crosses was estimated for grain yield and nitrogen use efficiency traits and presented in Table (6).

Practicing selection in the $F_2$ generation of the studied crosses resulted in an actual significant superiority (actual selection gain) over the better parent of the corresponding cross in grain yield / plant ranging from 21.5% for SF11 (Gem7 x Gem9 – LN) to 33.7% for SF13 (Gem9 x Gz168- LN) under low-N stress and from 14.2% for SF14 (Gem9 x Gz168- LN) to 25.3% for SF11 under high-N conditions.

It is clear that the four transgrassive segragants selected under low-N exceeded significantly their better parent under both low-N and high-N target environments, but their superiority in grain yield / plant was higher in magnitude under low-N than under high-N (Table 6).

**Table5.** *Superiority (%) of the four best $F_3$ families selected under low-N over the better parent of their respective crosses for selected traits under low-N conditions*

| Best family | Original $F_2$ cross | GYPP | SPP | GPS | 100GW | BYPP | NUE | NHI | GPC |
|---|---|---|---|---|---|---|---|---|---|
| **Low-N** | | | | | | | | | |
| SF11 | Gem7xGem9 | 21.5** | 80.1** | 5.0* | -7.3 | 14.4** | 21.9** | 17.6** | 4.3* |
| SF12 | Gem7x Gz168 | 32.7 ** | 50.5** | 31.2** | 33.2** | 16.6** | 29.4** | 16.4** | 9.4* |
| SF13 | Gem9 x Gz168 | 33.7 ** | 40.0** | 22.3** | 31.5** | 13.9** | 30.4** | 9.6* | 45.9** |
| SF14 | Gem9 x Gz168 | 32.3** | 42.7** | 24.2** | 50.9** | 9.4* | 27.1** | 20.2** | 19.7** |
| **High-N** | | | | | | | | | |
| SF11 | Gem7xGem9 | 25.3** | 34.6** | 24.4** | 21.8** | 4.6 | 22.7** | -1.8 | 0.2 |
| SF12 | Gem7x Gz168 | 16.4** | 38.5** | 20.9** | 3.1 | 0.8 | 13.9** | 4.0 | -10.5** |
| SF13 | Gem9 x Gz168 | 22.2** | 4.6 | 26.9** | 15.1** | 10.5** | 19.6** | -7.0 | 47.0** |
| SF14 | Gem9 x Gz168 | 14.2** | 3.9 | 9.3 | 10.5** | 4.6 | 11.7** | 14.3** | 30.3** |

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

The highest actual gain from selection for high GYPP reached 33.7% in SF13, which originated from the $F_2$ cross Gem9 x Gz168 under low-N and 25.3% in SF11, which was selected from the $F_2$ population Gem7 x Gem9. Such actual gain from selection for high yield in the best F3 selected families (Table 6) is higher than corresponding expected genetic gains under both low-N and high-N (Table 2).

Superiority of the four selected $F_3$ families (SF11 through SF14) in grain yield over their better parent could mainly due to their high superiority in number of spikes/plant reaching to 80.1% for SF11, number of grains/ spike reaching to 31.2% for SF12 and 100-grain weight reaching to 50.9% for SF14 under low-N target environment.

Selection in $F_2$ populations under low-N for high grain yield caused simultaneous superiority in nitrogen use efficiency, which reached to 30.4% for SF13 under low-N and 22.7% for SF11 under high-N environment (Table 6). Superiority in NUE was close in magnitude to superiority in GYPP under both low-N and high-N environments. Moreover, superiority of the best selectants in grain yield and NUE traits was associated with superiority over better parents in grain protein concentration in most cases, which reached to 45.9 and 47% superiority for SF13 under low-N and high-N, respectively over the better parent (Table 6).

The highest superiority over the better parent in grain yield, nitrogen use efficiency and grain protein content under low-N environment was shown by the best $F_3$ family SF13. But under high-N environment, the $F_3$ family SF11 showed the highest superiority in GYPP, 100 GW and NUE.

The success of this investigation in obtaining new wheat genotypes of higher grain yield quantity and quality and higher nitrogen use efficiency than their better parents under low-N stress conditions could be attributed to the presence of sufficient additive and additive x additive types of genetic variance , amenable for high selection efficiency in the $F_2$ generation of some studied diallel crosses, besides to accumulation of favorable genes of yield and nitrogen use efficiency traits from both parents as a result of recombination and transgressive segregations. This conclusion was previously reported by some investigators. A further improvement of grain protein content under N-limited conditions without substantial depressions in yielding capacity seems to be possible in bread wheat as suggested by Laperche *et al.* (2007). In their recent study, at least some genome regions responsible for wheat performance and protein content (e.g. those from the 1B and 2A linkage groups) were not found to colocalise with other QTLs negatively influenced the yield protein relationship. In other studies, Ayoub *et al.* (1995) and Ortiz-Monasterio *et al.* (1997) reported negative associations of grain yield with protein content. Sinebo *et al.* (2004) found significant negative correlations of NUTE with grain protein content. The selection of new recombinants and transgrassive segregants was previously reported in wheat (Snape 1982, Al-Bakry *et al*., 2008, Al-Bakry and Al-Naggar 2011 and Al-Naggar *et al*., 2013). The superior segregants obtained in this study should be undergone in selfing process to become complete homozygous lines and to be of usefulness in future wheat breeding programs aiming at developing of N efficient varieties of high tolerance to low-N stress conditions.

## IV. Conclusions

This study concluded that selection in segregating generations of wheat crosses for higher tolerance to low-N is more efficient when practiced under N-limited than under high-N conditions. Four transgressive segregants were identified and showed significant superiority in grain yield and nitrogen use efficiency traits over the better parents of their respective crosses under both low-N and high-N conditions. .Actual significant superiority over the better parent in grain yield/plant ranged from 21.5% for SF11 to 33.7% for SF13 under low-N stress. Actual gain from selection for high yield in the best $F_3$ selected families is higher than corresponding expected genetic gains under both low-N and high-N. Most of these transgressive segregants exhibited simultaneously both high grain yield and high grain protein content. These superior segregants could be useful germplasm for future wheat breeding programs in Egypt aiming at improving low-N tolerance.

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