



Mode of inheritance of low-N tolerance adaptive traits in wheat (*Triticum aestivum* L.) under contrasting nitrogen environments

Ahmed M. M. Al-Naggar¹, Reda Shabana¹, Mosaad M. Abd-El-Aleem² and Zainab El-Rashidy²

¹ Cairo University, Faculty of Agriculture, Dept. of Agronomy, Giza, Egypt. ² FCRI, Agricultural Research Centre (ARC), Dept. Wheat Research, Giza, Egypt

Abstract

Because of essential economic and ecological concerns, there is increased interest worldwide in developing wheat cultivars that are more efficient in utilizing nitrogen (N) and better suited to N limitations. The objective of the present investigation was to get information on the type of gene action controlling the inheritance of wheat low-N tolerance traits in order to start a breeding program for improving such traits. Six parents of contrasting low-N tolerance were crossed in a diallel fashion. Evaluation of 6 parents, 15 F₁ crosses and 15 F₂ crosses was done using a randomized complete block design with three replications under two levels of soil N, *i.e.* low-N (0 kg N/ha) and high-N (180 kg N/ha). The magnitude of dominance variance in F₂'s for all studied traits was much greater than that of additive variance under both high N and low N, suggesting that selection should be postponed to later segregating generations in order to eliminate masking effects of dominance variance and take advantage of the additive variance for the improvement of nitrogen use efficiency and grain yield traits. Narrow-sense heritability (h²_n) in F₂'s was generally of higher magnitude under low-N than high-N, suggesting that it is better to practice selection for studied nitrogen efficiency and grain yield traits under low-N conditions to obtain higher values of selection gain.

Additional keywords: gene action; NUE; NUPE; non-additive; heritability; selection gain

Abbreviations used: 100-GW (100-grain weight); BYPP (biological yield/plant); GCA (general combining ability); GPS (grains/spike); GYPP (grain yield/plant); HI (harvest index); HN (high nitrogen); LN (low nitrogen); NUE (nitrogen use efficiency); NUPE (nitrogen uptake efficiency); NUTE (nitrogen utilization efficiency); SCA (specific combining ability); SPP (spikes/plant)

Authors' contributions: Conceived and designed the experiments, and supervised the work: AMMA, RS and MMA. Performed the experiments: ZER. Analyzed the data: AMMA and ZER. Contributed reagents/materials/analysis tools: MMA, AMMA, RS and ZER. Managed the literature searches: AMMA and RS. Wrote the paper: AMMA. All authors read and approved the final manuscript.

Supplementary material (Tables S1, S2, S3) accompanies the paper on SJAR's website

Citation: Al-Naggar, A. M. M.; Shabana, R.; Abd El-Aleem, M. M.; El-Rashidy, Z. (2017). Mode of inheritance of low-N tolerance adaptive traits in wheat (*Triticum aestivum* L.) under contrasting nitrogen environments. Spanish Journal of Agricultural Research, Volume 15, Issue 2, e0702. <https://doi.org/10.5424/sjar/201715210808>

Received: 26 Nov 2016. **Accepted:** 09 May 2017

Copyright © 2017 INIA. This is an open access article distributed under the terms of the Creative Commons Attribution (CC-by) Spain 3.0 License.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Ahmed Medhat M. Al-Naggar: medhatalnaggar@gmail.com

Introduction

Because of essential economic and ecological concerns, there is increased interest worldwide in cereal cultivars that are more efficient in utilizing soil resources and better suited to water and nutrient limitations (El Bassam, 1998; Fageria & Baligar, 2005; Hirel *et al.*, 2007; Sylvester & Kindred, 2009). Among cereals, bread wheat (*Triticum aestivum* L.) 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.

Although numerous reports on genotypic variation in components of N efficiency already suggest potential applications of this genetic knowledge for wheat improvements (Ortiz-Monasterio *et al.*, 1997; El Bassam, 1998; Le Gouis *et al.*, 2000; Gorny *et al.*, 2006; Kichey *et al.*, 2007; Baresel *et al.*, 2008; Barraclough *et al.*, 2010), relatively fewer attempts have been made to breed wheat for these traits (Van Ginkel *et al.*, 2001; Wolfe *et al.*, 2008). Progress in breeding bread wheat better adapted to less favorable nitrogen 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 traits involved. Hence, several important questions remain to be resolved, especially in regard to the most effective selection schemes, desirable plant ideotypes for low input ecosystems, appropriate selection criteria and features of the selection environment for such breeding programs (e.g. Ceccarelli, 1996; Dawson *et al.*, 2008; 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 (El Bassam, 1998; Murphy *et al.*, 2007). The manner in which target traits are inherited has, of course, major consequences for the whole breeding strategy. However, our understanding of the inheritance of the components of N efficiency in wheat, as in other cereals, is still extremely limited. There is some information regarding various characteristics decisive for the uptake efficiency in juvenile wheat plants (Gamzikova, 1992; An *et al.*, 2006; Gorny *et al.*, 2006; Laperche *et al.*, 2006), but the limited data may lead to uncertain conclusions on N efficiency over the whole growing season. However, the genetic control of whole-season N efficiency has rarely been examined in wheat (Gorny *et al.*, 2011). In earlier investigations (Le Gouis *et al.*, 2002), both additive and non-additive genetic effects were crucial for agronomic nitrogen use efficiency (NUE) components in F₁ hybrids between modern French cultivars. In contrast, additive gene action was only important for NUE components among F₂ progenies of wheat of Turkish origin (Yildirim *et al.*, 2007). In Poland, Gorny *et al.* (2011) reported that under high N-fertilization, the efficiency components were inherited in a manner favorable for wheat selection

(preponderance of additive effects). However, they found that the enhanced contribution of non-additive gene effects and increased dominance under N-limited conditions could impede wheat selection to improve the N efficiency and adaptation to less luxurious fertilization regimes. To the best of our knowledge, scarce information in wheat for the major physiological measures of N efficiency, *i.e.* nitrogen uptake efficiency (NUPE) and nitrogen utilization efficiency (NUTE) when whole-season indices and conventional genetic/breeding approaches are considered, and this may have an impact on current breeding methods and goals. Thus, the objectives of this study were (i) to assess the modes by which low-N tolerance adaptive traits are inherited (ii) to evaluate the effects of varied nitrogen levels on combining ability, heritability and expected genetic advance from selection for such traits and (iii) to identify the most promising genotypes to be included in breeding programs to improve tolerance to low rate of nitrogen fertilizer.

Material 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 the 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 m asl), in 2005/2006, 2006/2007, 2007/2008 and 2008/2009 seasons.

Material

Six bread wheat (*Triticum aestivum* L.) genotypes were chosen for their divergence in tolerance to low nitrogen, based on previous field screening carried out by Wheat Res. Dept., Field Crops Res. Inst., ARC, Egypt (unpublished data and Table 1).

Table 1. Designation, pedigree and tolerance to low nitrogen of the six promising lines and Egyptian cultivars of wheat used for making diallel crosses of this study.

Wheat lines/varieties	Pedigree	Tolerance to low N
Line 25 (L25)	MYNA/VUL//TURACO/3/TURACO/4/Gem7	Tolerant
Line 26 (L26)	MUNIA/CHTO//AMSEL	Tolerant
Line 27 (L27)	Compact-2/Sakha//Sakha61	Tolerant
Gemeiza7 (Gem7)	CMH74A.630/SX//Seri82/3/Agent.	Sensitive
Gemeiza9 (Gem9)	Ald "s"/HUC ,"s; //CMH74A.630/SX	Sensitive
Giza168 (Gz168)	MRL/BUC//Seri	Sensitive

Source: Wheat Res. Dept., Field Crops Res. Inst., ARC, Egypt.

Making the F₁ and F₂ diallel crosses

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

Field evaluation

In the seasons 2007/2008, 2008/2009, parents (6), F₁'s (15) and F₂'s (15) were sown on November 17th of each season in the field of Noubarya Res. Stat., under two levels of nitrogen fertilizer; the low level was without additional fertilization (LN) and the high level was 75 kg Nitrogen/ feddan (HN) (178.5 kg N/ha). The last one is the level recommended by the Ministry of Agriculture of Egypt. 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 the first irrigation). In this experiment, a split plot design in lattice (6×6) 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 20 cm between plants, and the plot size was 1.8 m² for the parents and F₁ and 3.6 m² for the F₂. All other agricultural practices were done according to the recommendation of Ministry of Agriculture for growing wheat in the Noubarya region.

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 (*ca* 131 and 135.7 kg N/ha) in the seasons 2007/2008, 2008/2009, respectively. Available soil nitrogen after adding nitrogen fertilizer was therefore 55 and 130 kg N/fed (*ca* 131 and 309 kg N/ha) in the first season and 57 and 132 kg N/fed (*ca* 135.7 and 314 kg N/ha) 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 g/plant 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 dS/m, the soluble cations in meq/L are Ca²⁺ (5.30), K⁺ (0.70), Na⁺ (0.31), Mg²⁺ (2.60) and the soluble anions in meq/L are CO₃²⁻ (0.00), HCO₃⁻ (2.10), Cl⁻ (5.30) and SO₄²⁻ (1.51). All other agricultural practices were followed according to the recommendations of ARC, Egypt.

Data collection

The following characteristics were measured on a random sample of 10 plants of each parent and F₁ and 30 plants of each F₂ according to Al-Naggar *et al.* (2012):

- 1- Number of spikes/plant (SPP): Number of fertile spikes/plant.
- 2- Number of grains/spike (GPS): Number of grains/main spike.
- 3- 100-grain weight (100-GW): Measured as weight of 100 grains taken from each guarded plant.
- 4- Grain yield/plant (GYPP): Measured as weight of the grains of each individual plant.
- 5- Biological yield/plant (BYPP): Measured as weight of the grains and stem of each individual plant.
- 6- Harvest index (HI%): Calculated from the following formula: $HI = 100(GYPP/BYPP)$. At physiological maturity, 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 AOAC (1990). Total plant nitrogen (N_t) was calculated as follows: $N_t = N_g + N_{straw}$.
- 7- Nitrogen use efficiency (NUE) g/g = $(GYPP / N_s)$.
- 8- Nitrogen uptake efficiency (NUPE) % = $100 (N_t / N_s)$.
- 9- Nitrogen utilization efficiency (NUTE) (g/g) = $(GYPP/N_t)$, where GYPP is grain yield/ plant in grams, 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).

Statistical analyses

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). A 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 a lattice design for the purpose of determining genetic parameters using GENSTAT 10 software. Least significant differences (LSD) values were calculated according to Steel *et al.* (1997).

Genetic analyses

Diallel crosses in F_1 and F_2 generations were analyzed to obtain general (GCA) and specific (SCA) combining ability variances and effects for studied traits according to Griffing (1956) Model I (fixed effect) Method II. Although parents in our study were selected in purpose for the validity of diallel analysis, we further used Hayman's approach (that assumes random model) (Hayman, 1954a,b) to estimate genetic components and ratios which will help us to characterize our genetic material for its proper use in the future breeding programs and we will not generalize our conclusions for other material. The genetic parameters and ratios were calculated according to methods developed by Jinks & Hayman (1953), Jinks (1954), and Hayman (1954a,b) and described by Sharma (2003). Hayman's approach was performed for estimating variance components and ratios in F_2 generation of the same diallel crosses, based on the same assumption proposed in F_1 , but with the following modifications in F_2 for some genetic parameters (according to Sharma, 2003) as follows: $H_1 = 4V_{OL0} + 16W_{OL01} - [\hat{E}(3n-2)/n]$; $H_2 = 16V_{IL1} - 16V_{OL1} - 2\hat{E}h^2 = 16(M_{L1} - M_{L0})^2 - [4\hat{E}(n-1)/n^2]$. Average degree of dominance in F_2 was estimated as $1/4(H_1/D)^{1/2}$. Narrow-sense heritability (h_n^2) in F_2 was estimated using the following equation: $h_n^2 = [1/4D / (1/4D + 1/16H_1 - 1/8F + \hat{E})]$.

Results

Analysis of variance

Mean squares due to years were significant ($p \leq 0.01$) for all studied traits except for harvest index (HI), NUE and NUPE (Table 2). Mean squares due to nitrogen levels and genotypes were significant ($p \leq 0.01$) for all studied traits.

Mean squares due to the interaction $N \times Y$, $G \times N$, $G \times Y$ and $G \times N \times Y$ were significant ($p \leq 0.01$ or 0.05) for all studied traits, except $N \times Y$ for SPP, 100-GW, NUPE and NUTE.

Combined analysis of variance for all studied traits under each environment (high-N and low-N) for parents (P's), F_1 's and F_2 's across two seasons (Table 3) showed that mean squares due to P's, F_1 's and F_2 's under each of the two levels of nitrogen were significant ($p \leq 0.01$)

for all studied traits, except NUPE of F_2 's under high-N. Mean squares due to P's vs F_1 's and F_1 's vs F_2 's were significant for all studied traits under both high-N and low-N.

Mean squares due to the interactions $P \times Y$, $F_1 \times Y$, $F_2 \times Y$, P vs $F_1 \times Y$ and F_1 vs $F_2 \times Y$ were significant ($p \leq 0.01$) under high-N and low-N for all studied traits, except $P \times Y$ for NUE, $F_1 \times Y$ for NUPE and $F_2 \times Y$ for 100-GW under high-N only. Mean squares due to the interactions P's vs F_1 's $\times Y$ and F_1 's vs F_2 's $\times Y$ under the two levels of nitrogen were significant ($p \leq 0.01$) for all studied traits.

Mean performance

Means of studied traits of six wheat parents and their 15 F_1 and 15 F_2 diallel crosses under high-N and low-N across two seasons are presented in Table S1 [suppl.]. In general, means of GYPP, GPS, 100 GW, SPP, NUE and NUPE 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 low-N as well as high-N. For crosses, the highest mean of GYPP and NUE under low-N was obtained from L26 \times L27 followed by L25 \times L26 and L25 \times L27 in F_1 and L25 \times L27 followed by L25 \times L26 and L26 \times Gz168 in F_2 generation. On contrary, the three crosses Gem7 \times Gem9, Gem7 \times Gz168 and L27 \times Gem9 in F_1 and F_2 generations showed the lowest GYPP and NUE under low-N.

General combining ability effects

Estimates of GCA effects of parents for studied traits in F_1 and F_2 generations of studied crosses under the two levels of nitrogen across two years are presented in Table S2 [suppl.]. Favorable significant GCA effects were expressed by positive estimates for all studied traits. In general, the best general combiners in F_1 and F_2 generations were L25, L26 and L27 parents for most studied traits under low-N and high-N. On the contrary, the worst general combiners under low-N and high-N in F_1 's and F_2 's were Gem 9, Gem 7 and Giza 168 for most studied yield and NUE attributes.

Specific combining ability effects

Estimates of SCA effects of the F_1 and F_2 crosses for the studied traits under the two levels of N are presented in Table S3 [suppl.]. The best crosses in SCA effects were considered those which exhibited significant positive SCA effects for all studied traits. The rank of F_1 and F_2 crosses for SCA effects changed from under high-N to under low-N conditions. It was observed that the crosses L25 \times L26, L26 \times L27 and L25 \times Gem7 were superior in

Table 2. Significance of mean squares (MS) from combined analysis of variance of split plot design for wheat studied traits of 36 genotypes across two N levels and two years.

SOV	df	MS							
		SPP	GPS	100-GW	GYPP	HI%	NUE	NUPE	NUTE
Years (Y)	1	**	**	**	**	ns	ns	ns	**
N levels (N)	1	**	**	**	**	**	**	**	**
N × Y	1	ns	**	ns	*	**	**	ns	ns
Genotypes (G)	35	**	**	**	**	**	**	**	**
G × N	35	**	**	**	**	**	**	*	**
G × Y	35	**	**	*	**	**	**	**	**
G × Y × N	35	**	**	*	**	**	**	**	**

ns, *, **: not significant, significant at 0.05 and at 0.01 probability levels, respectively. SPP: spikes/plant. GPS: grains/spike. 100-GW: 100-grain weight. GYPP: grain yield/plant. HI: harvest index. NUE: nitrogen use efficiency. NUPE: nitrogen uptake efficiency. NUTE: nitrogen utilization efficiency. SOV: source of variance. df: degrees of freedom.

SCA effects in both F₁ and F₂ under low-N conditions. Under high-N conditions, it was also observed that the crosses L27 × Gem7, L26 × Gem7 and L26 × Gz168 were superior in SCA effects in both F₁ and F₂.

Gene action, heritability and selection gain

Estimates of genetic components and ratios for studied traits in F₁'s and F₂'s under high- and low-N environments across two years are presented in WW. The additive component of variation (D) was significant ($p \leq 0.01$ or 0.05) for all studied traits in F₁'s and F₂'s under both high-N and low-N. The magnitude of additive variance was much higher under low-N than that under high-N conditions in all studied traits, except for NUTE and 100-GW traits for both F₁'s and F₂'s. The dominance genetic component of variation (H₁) in F₁'s and F₂'s was also significant ($p \leq 0.01$ or 0.05) for all studied traits under both high-N and low-N environments, except for 100-GW of F₁'s under low-N. It was also observed that the magnitude of dominance variance was much higher under low-N than under high-N conditions in all studied traits of F₁'s and F₂'s, except for GPS, SPP and 100-GW of F₁'s and GYPP of F₁'s and F₂'s, where the opposite was true.

In general, the magnitude of dominance was higher than that of additive variance in both F₁ and F₂ generations under both low-N and high-N for all studied traits, except for NUE, SPP, GYPP and 100-GW of F₁ under low-N.

The average degree of dominance ($(H_1/D)^{1/2}$) in F₁'s was in the range of over-dominance (greater than unity) for four traits (NUPE, NUTE, 100-GW and HI) under the two levels of nitrogen, but showed partial dominance for SPP, GPS, GYPP and NUE traits of F₁'s under low-N. The $(H_1/D)^{1/2}$ in F₂'s was in the range of partial dominance (smaller than unity) for all studied traits under the two levels of nitrogen, except for NUTE under high N, which indicated over dominance.

Broad-sense heritability (h^2_b) in F₁'s and F₂'s for all studied traits in this experiment was of medium to high magnitude and ranged for F₁'s from 61.7% (SPP) to 99.9% (GPS) under high-N and from 41.0% (NHI) to 98.0% (GPS) under low-N and for F₂'s ranged from 40.12% (SPP) to 97.41% (PH) under high-N and from 63.71% (100-GW) to 98.40% (PH) under low-N.

Narrow-sense heritability (h^2_n) in F₁'s was generally of low to medium magnitude and ranged from 8.50% (NUTE) to 56.89% (GPS) under high-N and from 9.00% (HI) to 67.14% (GYPP) under low-N, but in F₂'s was generally of medium to high magnitude and ranged from 2.11% (HI) to 66.4% (BYPP) under high-N and from 6.80% (HI) to 85.00% (NUE) under low-N. For GYPP and NUE traits of F₁'s under low-N, value of h^2_n was 19.70 and 22.43%, respectively.

Expected genetic advance (GA) from selection based on F₁ diallel analysis (using 1% selection intensity) across two years ranged from 2.5% for SPP under high-N to 23.9% for HI under high-N and from 5.42% for HI to 23.26% for GPS under low-N. Moreover, GA based on F₂ diallel analysis ranged from 3.20% for SPP to 20.40% for GPS under high-N and from 7.5% for HI to 28.12% for GPS under low-N. It was observed that magnitude of GA under low-N was generally higher than that under high-N either from F₁ or F₂ analyses in most cases.

Discussion

Breeding wheat cultivars with improved adaptation to less favorable, but more optimized N fertilization regimes has gained importance in Egypt, like in other developing countries. Such breeding strategies are also justified by limited-nitrogen supply that supposes a major constraint for grain production. The NUE and plant adaptation to

Table 3. Significance of mean squares (MS) due to parents, F₁'s and F₂'s and their interaction with years under high N and low-N conditions.

SOV	df	MS							
		SPP	GPS	100-GW	GYPP	HI%	NUE	NUPE	NUTE
High-N									
Parents (P)	5	**	**	**	**	**	**	**	**
F1s (F1)	14	**	**	**	**	**	**	**	**
F2s (F2)	14	**	**	**	**	**	**	ns	**
P vs F1	1	**	**	**	**	**	**	**	**
F1 vs F2	1	**	**	**	**	**	**	**	**
P × Y	5	**	**	**	**	**	ns	**	**
F1 × Y	14	**	**	**	**	**	**	ns	**
F2 × Y	14	**	**	ns	**	**	**	**	**
P vs F1 × Y	1	**	**	**	**	**	**	**	**
F1 vs F2 × Y	1	**	**	**	**	**	**	**	**
Low-N									
Parents (P)	5	**	**	**	**	**	**	**	**
F1s (F1)	14	**	**	**	**	**	**	**	**
F2s (F2)	14	**	**	**	**	**	**	**	**
P vs F1	1	**	**	**	**	**	**	**	**
F1 vs F2	1	**	**	**	**	**	**	**	**
P × Y	5	**	**	**	**	**	**	**	**
F1 × Y	14	**	**	**	**	**	**	**	**
F2 × Y	14	**	**	**	**	**	**	**	**
P vs F1 × Y	1	**	**	**	**	**	**	**	**
F1 vs F2 × Y	1	**	**	**	**	**	**	**	**

ns, *, **: not significant and significant at 0.01 probability level, respectively. For abbreviations of traits see Table 2

less favorable nutrition regimes is complex, involving various mechanisms (Sattelmacher *et al.*, 1994; Coque & Gallais, 2007; Al-Naggar *et al.*, 2008, 2009, 2010; Gorny *et al.*, 2011). Different characteristics, associated with both the N-uptake capacity and efficiency of nitrogen utilization in grain mass formation (NUTE), appear to be critical components of NUE (Moll *et al.*, 1982; Huggins & Pan, 2003).

In the present study, analysis of variance indicated that each of the two factors, genotype and N-level had a marked effect on all studied traits. In that context, the ranks of wheat genotype differ from one nitrogen level to another and from one year to another. Selection for improved performance under a specific soil nitrogen environment is possible as proposed by Al-Naggar *et al.* (2006, 2009, 2010 and 2015 a, b and c). The significant G×N interaction for grain yield was also a good evidence for varying responses of wheat genotypes at various N levels (Austin *et al.*, 1980; Earl & Ausubel, 1983). The interactions G × Y and G × Y × 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 × N to another.

Significant differences among studied parents of diallel crosses in all studied traits are a pre-requisite for performing the diallel analysis for estimating the inheritance of studied traits under different N-application rates. Significance ($p \leq 0.01$) of mean squares due to parents vs F₁'s and F₁'s vs F₂'s for most studied traits under the two levels of nitrogen, indicated the presence of significant heterosis and inbreeding effects, respectively. The significance of the interactions P's vs F₁'s × Y and F₁'s vs F₂'s × Y indicates that heterosis and inbreeding effects differ from season to season in all studied traits due to interactions with the environment.

It is worthy to note that the magnitude of N-induced alterations due to low-N stress in the majority of the N-efficiency components and other studied traits was distinctly dependent upon the genotype, as evident by the significant genotype × environment interactions. These results are consistent with observations previously reported in wheat (El Bassam, 1998; Le Gouis *et al.*, 2000, 2002; Al-Naggar & Shehab-El-Deen, 2012), barley (Ceccarelli, 1996; Gorny & Sodkiewicz, 2001) and maize (Di Fonzo *et al.*, 1982; Medici *et al.*, 2004;

Table 4. Genetic parameters and ratios for studied traits under high-N and low-N in F₁ and F₂ populations of 15 diallel crosses across two seasons.

Parameter ^[1]	High-N		Low-N		High-N		Low-N	
	F ₁	F ₂						
	NUE				NUPE			
D	1.58**	1.58**	26.63**	26.66**	4.64**	4.60**	51.30**	50.19**
H ₁	4.33**	5.44**	7.17**	28.28**	5.89**	20.94**	71.28**	64.78**
(H ₁ /D) ^{1/2}	1.66	0.46	0.52	0.26	1.13	0.53	1.18	0.28
h _b ² %	91.20	81.64	90.75	88.60	96.00	95.03	96.60	88.10
h _n ² %	39.50	42.64	66.90	85.00	40.70	33.37	46.36	72.80
GA%	9.44	7.70	19.62	23.10	11.25	9.52	22.43	27.43
	NUTE				GPS			
D	0.01*	0.01*	0.01*	0.01*	162.81**	162.82**	214.01**	214.00**
H ₁	0.07*	0.09**	0.12*	0.08**	171.19**	185.43**	113.32**	319.15**
(H ₁ /D) ^{1/2}	3.59	1.08	2.85	0.58	1.03	0.27	0.73	0.31
h _b ² %	96.60	91.83	87.30	67.57	99.90	76.87	87.70	87.18
h _n ² %	8.50	20.41	11.11	27.00	56.89	52.90	63.55	63.34
GA%	4.00	6.80	5.63	6.80	15.87	20.40	23.26	28.12
	SPP				100-GW			
D	0.54*	0.58*	3.90**	3.89**	0.46**	0.43*	0.31**	0.31*
H ₂	0.89**	1.18**	0.24**	6.51**	0.93**	1.27**	0.01	1.46**
(H ₁ /D) ^{1/2}	1.37	0.30	0.20	0.32	1.40	0.42	0.01	0.56
h _b ² %	61.70	40.12	66.32	70.00	81.00	65.83	57.50	63.71
h _n ² %	9.20	16.10	54.42	43.76	21.40	30.60	27.43	25.56
GA%	2.50	3.20	23.25	22.00	9.24	12.80	9.90	12.31
	GYPP				HI			
D	5.52**	5.52**	17.12**	17.13**	6.81**	7.34**	17.53*	16.61**
H ₁	15.18**	19.11**	4.63**	18.25**	47.92**	78.83**	90.63**	144.20**
(H ₁ /D) ^{1/2}	1.66	0.47	0.52	0.26	2.65	0.82	2.27	0.74
h _b ² %	91.27	81.76	91.00	88.65	87.30	82.00	93.50	78.20
h _n ² %	20.41	42.70	67.14	75.10	9.60	19.30	15.87	33.38
GA%	5.11	7.70	19.70	23.14	23.90	3.60	5.42	7.50

[1] D: additive variance, H₁: dominance variance, (H₁/D)^{1/2}: degree of dominance, h_b²: heritability in broad-sense, h_n²: heritability in narrow-sense, GA: genetic advance from selection. For abbreviations of traits see Table 2. *,**: significant at 0.05 and at 0.01 probability levels, respectively.

Al-Naggar *et al.*, 2010, 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 in wheat by Brancourt-Hulmel *et al.* (2005), Laperche *et al.* (2006), Dawson *et al.* (2008), Wolfe *et al.* (2008) and in maize by Al-Naggar *et al.* (2010, 2014, 2015a,b,c).

It is worthy to note that the best general combiners under low-N in this study (L25, L26 and L27) showed also high *per se* performance for the most studied grain yield and NUE traits. Based on diallel analyses in both F₁ and F₂, these parents are expected to have more additive genes for the respective traits. The

crosses L25 × L26, L26 × L27 and L25 × Gem7 were superior in both F₁ and F₂ for SCA effects under low-N conditions. These crosses showing high SCA effects under low-N and including at least one parent of high GCA effects are expected to release more transgressive segregants if additive gene effects existed in the high general combiner parent and epistasis acts in the cross in the same direction for decreasing the undesirable traits and increasing the desirable traits. These crosses besides their high *per se* performance, are the best in specific combining ability effects for the respective traits and are therefore good genetic material in their segregating generations for practicing selection for improving adaptive traits to low-N conditions.

Hayman's analysis of the present experiment indicated that both additive and dominance gene effects in F_1 's and F_2 's of this experiment are important for the inheritance of all studied traits under both low-N and high-N, indicating that selection in segregating generations as well as heterosis breeding in wheat could be efficient for improving all studied traits. Results indicated that the dominance gene effects are more important than additive and plays the major role in the inheritance of all studied traits under both low-N and high-N. In this context, Le Gouis *et al.* (2002) reported that in N-limited diallel F_1 hybrids between modern French cultivars found markedly preponderance of additive variance for grain yield, grain N yield and total above ground N than in those grown under high-N nutrition. More recently, a similar preponderance of additive effects for N uptake and NUTE was identified in F_2 and F_3 progenies of factorial hybrids between modern and exotic cultivars of barley grown under reduced N fertilization (Gorny & Ratajezak, 2008). On the other hand, results of Gorny *et al.* (2011) on wheat appear to be in accord with similar N-shortage- induced increases in the importance of non-additive effects for grain yield and components of NUE previously reported in maize (Al-Naggar *et al.*, 2015a,b,c) and those for NUTE in barley (Gorny & Sodkiewicz, 2001). Gorny *et al.* (2011) reported that under high N-fertilization, the efficiency components were incanted in a manner favorable for wheat selection (preponderance of additive effects) however the enhanced contribution of non-additive gene effects and increased dominance under N-limited conditions could impede wheat selection to improve the N efficiency and adaptation to less luxurious fertilization regimes. They concluded that selection methods that eliminate masking non-additive effects and take advantage of the additive variance should be employed to improve those traits. Results of the present study agreed with the conclusion of Gorny *et al.* (2011). The preponderance of dominance (non-additive) effects in this study suggests that it is preferable to postpone the selection to later segregating generations, until completing the homozygosity process and eliminating masking non-additive influences in order to take advantage of the additive variance to improve grain yield and nitrogen use efficiency traits.

Broad-sense heritability (h^2_b) in F_1 's and F_2 's for all studied traits in this experiment was of medium to high magnitude, indicating that environment had a small effect on the phenotype of F_1 's and F_2 's for most studied traits. Narrow-sense heritability (h^2_n) in F_1 's was generally of low to medium magnitude, but in F_2 's was generally of medium to high magnitude. The big difference between broad- and narrow- sense heritability estimated from F_1 's and F_2 's in this experiment could be attributed to the high estimates of dominance as compared to additive component.

It is observed that narrow-sense heritability (h^2_n) in

F_1 's and F_2 's of the present study was generally higher in magnitude under low-N than under high-N for most studied traits. Results of this study are in agreement with one group of researchers, *e.g.* Shabana *et al.* (1980), Blum (1988a,b), Hefny (2007), Al-Naggar (2007, 2009, 2010, 2015a,b,c), who support the idea that heritability is higher under stressed than non-stressed environment. On the contrary, other group of investigators reported that heritability is higher under non-stressed environments (Rosielle & Hamblin, 1981; Atlin & Frey, 1990; Banziger & Laffite, 1997; Banziger *et al.*, 1997; Worku, 2005). Our results were in agreement with the first group of researchers. Moreover, the values of expected genetic advance from selection (GA) of this study were higher under low-N than under high-N for most studied cases. It is therefore expected that to improve grain yield and nitrogen use efficiency components in the present germplasm, it is better to practice selection for these traits under low-N conditions to obtain higher values of selection gain.

In this study, for nitrogen use efficiency and grain yield traits under low-N conditions, the best general combiners were L25 and L26 and L27, the best F_1 cross for SCA effects was L25 \times Gz168 and the best F_2 cross was L25 \times Gem 7. These genotypes could be used in the future breeding programs for improving low-N tolerance adaptive traits. The preponderance of dominance (non-additive) effects of this study suggests that selection methods that eliminate masking non-additive effects and take advantage of the additive variance should be employed to improve nitrogen efficiency traits under low-N environment. Therefore, selection should be postponed to later segregating generations under low-N conditions to develop N-efficient genotypes of wheat. The results expected that to improve nitrogen use efficiency components in the present germplasm, it is better to practice selection for studied nitrogen efficiency traits under low-N conditions to obtain higher values of selection gain.

References

- AOAC, 1990. Official Methods of Association of Analytical Chemists. 15th ed. Washington DC, USA, 290pp
- Al-Naggar AMM, El-Kadi DA, Abo-Zaid Zeinab SA, 2006. Genetic parameters of grain sorghum traits contributing to low-N tolerance. *Egypt J Plant Breed* 10 (2): 79-102.
- Al-Naggar AMM, El-Kadi DA, Abo-Zaid Zeinab SA, 2007. Inheritance of nitrogen use efficiency traits in grain sorghum under low- and high-N. *Egypt J Plant Breed* 11 (3): 181-206.
- Al-Naggar AMM, Shabana R, Mahmoud AA, Shaboon SAM, 2008. Genetic improvement of maize for low-soil nitrogen tolerance via S1 recurrent selection. *Egypt J Plant Breed* 12 (2): 255-277.

- Al-Naggar AMM, Atta MMM, Amein MM, 2009. Maize genotypic differences in nitrogen use efficiency under low soil-N conditions. *Egypt J Appl Sci* 24 (3B): 528-546.
- Al-Naggar AMM, Shabana R, Al-Khalil TH, 2010. Tolerance of 28 maize hybrids and populations to low-nitrogen. *Egypt J Plant Breed* 14 (2): 103-114.
- Al-Naggar AMM, Shehab-El-Deen MT, 2012. Predicted and actual gain from selection for early maturing and high yielding wheat genotypes under water stress conditions. *Egypt J Plant Breed* 16 (3): 73-92. <https://doi.org/10.12816/0003949>
- Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH, 2014. Genetic parameters controlling some maize adaptive traits to elevated plant densities combined with reduced rates. *World Res J Agron* 3 (2): 70-82.
- Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH, 2015a. Regression of grain yield of maize inbred lines and their diallel crosses on elevated levels of soil-nitrogen. *Int J Plant Soil Sci* 4 (6): 499-512. <https://doi.org/10.9734/IJPSS/2015/14228>
- Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH, 2015b. Maize response to elevated plant density combined with lowered N-fertilizer rate is genotype-dependent. *The Crop Journal* 3: 96-109. <https://doi.org/10.1016/j.cj.2015.01.002>
- Al-Naggar AMM, Shabana R, Atta MMM, Al-Khalil TH, 2015c. Response of genetic parameters of low-N tolerance adaptive traits to decreasing soil-N rate in maize (*Zea mays* L.). *Appl Sci Rep* 9 (2): 110-122.
- An DG, Su JY, Liu QY, Zhu JG, Tong YP, Li JM, Jing RL, Li B, Li ZS, 2006. Mapping QTLs for nitrogen uptake in relation to the early growth of wheat (*Triticum aestivum* L.). *Plant Soil* 284: 73-84. <https://doi.org/10.1007/s11104-006-0030-3>
- Atlin GN, Frey KJ, 1990. Selecting oat lines for yield in low productivity environments. *Crop Sci* 30: 556-561. <https://doi.org/10.2135/cropsci1990.0011183X003000030017x>
- Austin RB, Bingham J, Blackwell LT, Evans LT, Ford MA, Morgan CL, Taylor M, 1980. Genetic improvements in winter wheat yields since 1900 and associated physiological changes. *J Agric Sci, Cambridge* 94: 675-689. <https://doi.org/10.1017/S0021859600028665>
- Banziger M, Lafitte HR, 1997. Efficiency of secondary traits for improving maize for low-nitrogen target environments. *Crop Sci* 37: 1110-1117. <https://doi.org/10.2135/cropsci1997.0011183X003700040013x>
- Banziger M, Betrán FJ, Lafitte HR, 1997. Efficiency of high nitrogen selection environments for improving maize for low-nitrogen target environments. *Crop Sci* 37: 1103-1109. <https://doi.org/10.2135/cropsci1997.0011183X003700040012x>
- Baresel JP, Zimmermann G, Reents HJ, 2008. Effects of genotype and environment on N uptake and N partition in organically grown winter wheat (*Triticum aestivum* L.) in Germany. *Euphytica* 163: 347-354. <https://doi.org/10.1007/s10681-008-9718-1>
- Barraclough PB, Howarth JR, Jones J, Lopez-Bellido R, Parmar S, Shepherd CE, Hawkesford MJ, 2010. Nitrogen efficiency of wheat: genotypic and environmental variation and prospects for improvement. *Eur J Agron* 33: 1-11. <https://doi.org/10.1016/j.eja.2010.01.005>
- Blum A, 1988a. Breeding crop varieties for stress environments. *Crit Rev Plant Sci* 2: 199-238. <https://doi.org/10.1080/07352688509382196>
- Blum A. 1988b. Plant breeding for stress environments. CRC Press Inc., Florida, USA, pp: 78-84.
- Brancourt-Hulmel M, Heumez E, Pluchard P, Beghin D, Depatureaux C, Giraud A, Le Gouis J, 2005. Indirect versus direct selection of winter wheat for low-input or high-input levels. *Crop Sci* 45: 1427-1431. <https://doi.org/10.2135/cropsci2003.0343>
- Ceccarelli S, 1996. Adaptation to low/high input cultivation. *Euphytica* 92: 203-214. <https://doi.org/10.1007/BF00022846>
- Coque M, Gallais A, 2007. Genetic variation for nitrogen remobilization and post-silking nitrogen uptake in maize recombinant inbred lines: heritabilities and correlations among traits. *Crop Sci* 47: 1787-1796. <https://doi.org/10.2135/cropsci2007.02.0096>
- Dawson JC, Huggins DR, Jones SS, 2008. Characterizing nitrogen use efficiency in natural and agricultural ecosystems to improve the performance of cereal crops in low-input and organic agricultural systems. *Field Crop Res* 107: 89-101. <https://doi.org/10.1016/j.fcr.2008.01.001>
- Di Fonzo N, Motto M, Maggiore T, Sabatino R, Salamini F, 1982. N-uptake, translocation and relationships among N-related traits in maize as affected by genotype. *Agronomie* 2: 789-796. <https://doi.org/10.1051/agro:19820901>
- Earl CD, Ausubel FM, 1983. The genetic engineering of nitrogen fixation. *Nutr Rev* 41: 1-6. <https://doi.org/10.1111/j.1753-4887.1983.tb07114.x>
- El Bassam N, 1998. A concept of selection for 'low-input' wheat varieties. *Euphytica* 100: 95-100. <https://doi.org/10.1023/A:1018308023391>
- Fageria NK, Baligar VC 2005. Enhancing nitrogen use efficiency in crop plants. *Adv Agron* 88: 97-185. [https://doi.org/10.1016/S0065-2113\(05\)88004-6](https://doi.org/10.1016/S0065-2113(05)88004-6)
- Fischer RA, Maurer R, 1978. Drought resistance in spring wheat cultivars. I. Grain yield responses. *Aust J Agric Res* 14: 897-912. <https://doi.org/10.1071/AR9780897>
- Gamzikova OI, 1992. Genetic aspects in edaphical adaptation of wheat. *Fizjologia Biohimija Kulturnyh Rastienij* 24: 419-428. [in Russian].
- Gorny AD, Sodikiewicz T, 2001. Genetic analysis of the nitrogen and phosphorus utilization efficiencies in mature spring barley plants. *Plant Breed* 120: 129-132. <https://doi.org/10.1046/j.1439-0523.2001.00584.x>
- Gorny AG, Ratajczak D, 2008. Efficiency of nitrogen and phosphorus utilization in progenies of factorial crosses between European and exotic cultivars of spring barley. *J Appl Genet* 49: 349-355. <https://doi.org/10.1007/BF03195633>

- Gorny AG, Garczynski S, Banaszak Z, Ługowska B, 2006. Genetic variation in the efficiency of nitrogen utilization and photosynthetic activity of flag leaves among the old and modern germplasm of winter wheat. *J Appl Genet* 47: 231-237. <https://doi.org/10.1007/BF03194628>
- Gorny AG, Banaszak Z, Ługowska B, Ratajczak D, 2011. Inheritance of the efficiency of nitrogen uptake and utilization in winter wheat (*Triticum aestivum* L.) under diverse nutrition levels. *Euphytica* 77: 191-206. <https://doi.org/10.1007/s10681-010-0230-z>
- Griffing B, 1956. Concept of general and specific combining ability in relation to diallel crossing system. *Aust J Biol Sci* 9: 463-493. <https://doi.org/10.1071/BI9560463>
- Hayman BL, 1954 a. The theory and analysis of diallel crosses. *Genetics* 39: 789-809.
- Hayman BL, 1954 b. The analysis of variance of diallel tables. *Biometrics* 10: 235-244. <https://doi.org/10.2307/3001877>
- Hefny MM, 2007. Estimation of quantitative genetic parameters for nitrogen use efficiency in maize under two nitrogen rates. *Int J Pl Breed Genet* 1: 54-66. <https://doi.org/10.3923/ijpb.2007.54.66>
- Hirel B, Le Gouis J, Ney B, Gallais A, 2007. The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. *J Exp Bot* 58: 2369-2387. <https://doi.org/10.1093/jxb/erm097>
- Huggins DR, Pan WL, 2003. Key indicators for assessing nitrogen use efficiency in cereal-based agroecosystems. *J Crop Prod* 8: 157-185. https://doi.org/10.1300/J144v08n01_07
- Jinks JL, 1954. The analyses of continuous variation in diallel cross of *Nicotiana rustica* varieties. *Genetics* 39: 767-788.
- Jinks JL, Hayman I, 1953. The analysis of diallel crosses. *Maize Genetics Cooperation Newsletter* 27: 48-54.
- Kichey T, Hirel B, Heumez E, Dubois F, Le Gouis J, 2007. Wheat genetic variability for post-anthesis nitrogen absorption and remobilisation revealed by ¹⁵N labeling and correlations with agronomic traits and nitrogen physiological markers. *Field Crop Res* 102: 22-32. <https://doi.org/10.1016/j.fcr.2007.01.002>
- Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Le Gouis J, 2006. Estimation of genetic parameters of a DH wheat population grown at different N stress levels characterized by probe genotypes. *Theor Appl Genet* 2: 797-807. <https://doi.org/10.1007/s00122-005-0176-z>
- Le Gouis J, Beghin D, Heumez E, Pluchard P, 2000. Genetic differences for nitrogen uptake and nitrogen utilization efficiencies in winter wheat. *Eur J Agron* 12: 163-173. [https://doi.org/10.1016/S1161-0301\(00\)00045-9](https://doi.org/10.1016/S1161-0301(00)00045-9)
- Le Gouis J, Beghin D, Heumez E, Pluchard P, 2002. Diallel analysis of winter wheat at two nitrogen levels. *Crop Sci* 42: 1129-1134. <https://doi.org/10.2135/cropsci2002.1129>
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD, 1996. SAS system for mixed models. SAS Inst., Cary, NC, USA.
- Medici LO, Pereira MB, Lea PJ, Azevedo RA, 2004. Diallel analysis of maize lines with contrasting responses to applied nitrogen. *J Agric Sci* 142: 535-541. <https://doi.org/10.1017/S002185960400468X>
- Moll RH, Kamprath EJ, Jackson WA, 1982. Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. *Agron J* 74: 562-564. <https://doi.org/10.2134/agronj1982.00021962007400030037x>
- Murphy KM, Campbell KG, Lyon SR, Jones SS, 2007. Evidence of varietal adaptation to organic farming systems. *Field Crop Res* 102: 172-177. <https://doi.org/10.1016/j.fcr.2007.03.011>
- Ortiz-Monasterio JI, Sayre KD, Rajaram S, McMahon M, 1997. Genetic progress in wheat yield and nitrogen use efficiency under four nitrogen rates. *Crop Sci* 37: 898-904. <https://doi.org/10.2135/cropsci1997.0011183X003700030033x>
- Rosielle AA, Hamblin J, 1981. Theoretical aspects of selection for yield in stress and non-stress environments. *Crop Sci* 21: 43-46. <https://doi.org/10.2135/cropsci1981.0011183X002100060033x>
- Sattelmacher B, Horst WJ, Becker HC, 1994. Factors that contribute to genetic variation for nutrient efficiency of crop plants. *Z Pflanzenernahr Bodenk* 157: 215-224. <https://doi.org/10.1002/jpln.19941570309>
- Shabana R, Bailey T, Fery KJ, 1980. Production traits of oats selected under low; medium and high productivity. *Crop Sci* 20: 739-744. <https://doi.org/10.2135/cropsci1980.0011183X002000060015x>
- Sharma RJ, 2003. Statistical and biometrical techniques in plant breeding, 2nd ed. New Delhi, 432 pp.
- Steel RGD, Torrie JH, Dickey D, 1997. Principles and procedure of statistics. A biometrical approach, 3rd ed. McGraw Hill Book Co. Inc., NY, pp. 352-358.
- Sylvester-Bradley R, Kindred DR, 2009. Analysing nitrogen responses of cereals to prioritize routes to the improvements of nitrogen use efficiency. *J Exp Bot* 60: 1939-1951. <https://doi.org/10.1093/jxb/erp116>
- Van Ginkel M, Ortiz-Monasterio JI, Trethowan R, Hernandez E, 2001. Methodology for selecting segregating populations for improved N-use efficiency in bread wheat. *Euphytica* 119: 223-230. <https://doi.org/10.1023/A:1017533619566>
- Wolfe MS, Baresel JP, Desclaux D, Goldringer I, Hoad S, Kovacs G, Loschenberger F, Miedaner T, Ostergard H, Lammerts van Bueren ET, 2008. Developments in breeding cereals for organic agriculture. *Euphytica* 163: 323-346. <https://doi.org/10.1007/s10681-008-9690-9>
- Worwu M, 2005. Genetic and crop-physiological basis of nitrogen efficiency in tropical maize. Ph.D. Thesis. Fac. Agric. Hannover Univ. Germany, 122 p.

- Yildirim M, Bahar B, Genc I, Korkmaz K, Karnez E, 2007. Diallel analysis of wheat parents and their F₂ progenies under medium and low level of available N in soil. *J Plant Nutr* 30: 937-945. <https://doi.org/10.1080/15226510701375531>