



Selection Criteria for High Nitrogen Use Efficiency in Wheat (*Triticum aestivum* L.) Parents and their F₁ and F₂ Progenies

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Authors' contributions

This work was carried out in collaboration between all authors. Author AMMAN designed the study, wrote the protocol, produced the initial draft of manuscript and interpreted the data. Author ZER anchored the field study, gathered the initial data and performed preliminary data analysis. Authors RS and MMAEA managed the literature searches. All authors read and approved the final manuscript

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ABSTRACT

Developing nitrogen use efficient (NUE) genotypes of wheat could reduce crop N fertilizer requirement. However, selection for NUE alone can be difficult and less efficient than associating it with one or more of selection criteria. The aim of this study was to determine selection criteria for improving NUE in wheat genotypes and their F₁ and F₂ progenies under contrasting N environments. The results of this study indicated that high nitrogen uptake efficiency (NUPE), grain yield/plant (GYPP), grains/spike (GPS), spikes/plant (SPP), and grain protein content (GPC) of parents, high NUPE, GYPP, biological yield/plant (BYPP), GPC and GPS of F₁'s and high NUPE, harvest index (HI), BYPP, SPP, GPC and GPS of F₂'s have significant and strong correlation coefficients with high nitrogen use efficiency (NUE). It is observed that NUPE is commonly correlated with NUE in all studied genotypes (parents, F₁'s and F₂'s). Similarly, GPS is also strongly

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correlated with NUE. Moreover, SPP is strongly correlated with NUE in parents and F_2 's. A negative and significant correlation existed between NUPE and NUTE for parents under high-N (-0.82), F_1 's under high-N (-0.73) and low-N (-0.36) and F_2 's under high-N (-0.91) and low-N (-0.81). The traits NUPE, GYPP, SPP and GPS showed high heritability in narrow sense and could therefore be offered to wheat breeders for use in breeding programs as selection criteria for improving nitrogen use efficiency.

Keywords: Correlations; bread wheat; NUE; secondary traits; heritability; N-uptake.

1. INTRODUCTION

Low soil nitrogen is among the major abiotic stresses threatening cereal production and limiting food security and economic growth [1]. The incidence of low-N stress may increase, due partly to global climate changes, declines in soil organic matter and reduction in soil fertility and water holding capacity [2]. One approach to reducing the impact of N deficiency on wheat production may be to select cultivars that are superior in N-use efficiency, either due to enhanced uptake capacity or because of more efficient use of absorbed N in grain production [3].

Nitrogen use efficiency has been described in various ways, but these definitions generally describe two types of efficiency, either uptake efficiency or utilization efficiency. Nitrogen uptake efficiency (NUPE) has been defined as total plant nitrogen content per unit content per unit N available in the soil. Nitrogen utilization efficiency (NUTE) was described by Moll et al. [4] as the grain production per unit N concentration in the plant. They recommended the development of genotypes with both high uptake and high utilization efficiencies.

Various studies worldwide have identified genetic associations between grain yield and NUE components under contrasting N conditions. In general, these studies indicated that NUPE accounts for more of the genetic variation in NUE at low-N than at high N supply, e.g. amongst 10 spring wheat cultivars in Mexico [5] and 20 winter wheat cultivars in France [6]. However, Dhugga and Waines [7] comparing 12 spring wheats in California, found that NUPE was the most important component of NUE at all N levels.

Genetic gains in NUE with breeding under low N supply have been related mainly to improvements in NUPE in spring wheat in Mexico [5] and Finland [8] and to NUTE in winter wheat in France [9] and the UK [10]. In these studies, modern cultivars normally had higher

yields than old cultivars under low N input conditions [5,9,10]. Under high N supply, several studies worldwide concluded that wheat breeding did not result in consistent improvements in NUPE but in improved NUTE associated with higher harvest index, e.g. in Mexico [11], Argentina [12], France [9] and in various countries [13,14]. In contrast, studies in the UK [10], Mexico [5] and Finland [8] found that increases in NUE were explained approximately equally by NUPE and NUTE.

Numerous studies of cultivars and segregating populations have shown inverse relationships between grain yield and grain N concentration (e.g. Kibite and Evans [15] and Triboi et al., [16]. Assuming a constant N, harvest index (the proportion of above-ground N (AGN) at harvest in the grain; NHI), grain yield will be positively associated with AGN and/ or negatively associated with grain nitrogen content (GNC). Under low N supply, genetic gains in yield were positively associated with AGN and NHI and negatively associated with GNC [5,9,10]. Whereas under high N supply, genetic gains in yield were positively associated with AGN [5,9,10], positively [5,9] or neutrally [10] associated with NHI, and negatively [5,9] or neutrally [10] associated with GNC. Essentially, under both low and high N supply, wheat crops with higher NUE compared to current cultivars will require an increase in NUPE to raise AGN and/or an increase in NUTE. The latter may be achieved by raising NHI and/or lowering GNC.

There have to date been relatively few attempts to use 'direct selection' breeding to improve NUE in wheat. CIMMYT in Mexico adopted a strategy to select for grain yield in medium-to-high fertility conditions, since at this fertility level both NUPE and NUTE contribute to the observed variation in NUE, resulting in lines which were more N-efficient [5]. More recently, it has been suggested that this method of selection may not be as efficient as selecting lines under alternating high-low N selection regimes commencing with high N in the F_2 [17].

A selection criterion for a given trait should be of strong association with this trait and of high heritability. Therefore, the objectives of the present study were (i) to evaluate interrelationships among traits for determining the strongest ones with N-use efficiency in wheat parents and their F_1 and F_2 progenies and (2) to estimate heritability and expected genetic advance from selection for such criteria in order to identify those of strong correlation with NUE coupled with high heritability and high selection gain under low-N to be used as selection criteria for NUE.

2. 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.

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).

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. In the seasons 2007/2008, 2008/2009, parents (6), F_1 's (15) and F_2 's (15) were sown on 17th of November each season in the field of Noubarya Res. Stat., under two levels of nitrogen fertilizer; the low level was without fertilization (LN) and the high level was 75 kg

Nitrogen/ feddan (HN); this is the recommended level of Ministry of Agriculture. This level of nitrogen fertilizer (168 kg Urea/fed) was added in two equal doses, the first dose was added just before the sowing irrigation and the second dose just before the second irrigation (21 days after irrigation). In this experiment, a split plot design in lattice (6x6) arrangement was used with three replications. The two levels of nitrogen were allotted to the main plots and the genotypes to the sub plots. Each parent or 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 m² for parent or F_1 and 3.6 m² for F_2 . All other agricultural practices were done according to the recommendation of Ministry of Agriculture for growing wheat in 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 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 Ca²⁺ (5.30), K⁺ (0.70), Na⁺ (0.31), Mg²⁺ (2.60) and the soluble anions in meq l⁻¹ are CO₃²⁻ (0.00), HCO₃⁻ (2.10), Cl⁻ (5.30) and SO₄²⁻ (1.51).

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 (100 GW) 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. [18]. 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: $GPC\% = N_g \times 5.70$ according to AACCC [19], 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. [4].

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 ® [20]. Moreover, each environment (HN and LN) was analyzed separately as lattice design for the purpose of determining genetic parameters using Genstat10th addition windows software. Least significant differences (LSD) values were calculated to test the significance of differences between means according to Steel et al. [21]. Simple correlation coefficients were calculated between pairs of studied traits under stress and non-stress conditions in parents, F₁ and F₂ generations according to Singh and Narayanan [22]. The genetic parameters and ratios were calculated according to methods developed by Jinks and Hayman [23], Jinks [24] and Hayman [25,26] and described by Sharma [27]. Narrow-

sense heritability (h^2_n) in F₁'s was estimated using the following equation: $h^2_n = [1/4D / (1/4D + 1/4H_1 - 1/4F + \hat{E})]$. Narrow-sense heritability (h^2_n) in F₂ was estimated using the following equation: $h^2_n = [1/4D / (1/4D + 1/16H_1 - 1/8F + \hat{E})]$, where \hat{E} = expected environmental component of variance. D = variance due to additive effects of the genes. F = mean of the covariance of additive and dominance effects across all arrays. H₁ = variance component due to dominance deviation. The expected genetic advance (GA) from direct selection as a percentage of the mean (\bar{x}) was calculated according to Singh and Narayanan [22] based on 1% selection intensity as follows: $GA = 100[(k \cdot h^2_n \cdot \delta_{ph}) / \bar{x}]$ Where: k = 2.64 (selection differential for 1% selection intensity), and δ_{ph} = square root of the dominator of the narrow sense heritability.

3. RESULTS AND DISCUSSION

3.1 Analysis of Variance

Combined analysis of variance across years (Y) of the split plot design in lattice (6x6) arrangement across 2008/2009 and 2009/2010 seasons for the studied 36 wheat genotypes (6 parents, 15 F₁'s and 15 F₂'s) under two levels of nitrogen was performed (data not presented). Mean squares due to years were highly significant for nine studied traits, namely days to heading (DTH), plant height (PH), spikes/plant (SPP), grains/ spike (GPS), 100 grain weight (100 GW), grain yield/ plant (GYPP), biological yield/ plant (BYPP), nitrogen utilization efficiency (NUTE) and nitrogen harvest index (NHI), indicating significant effect of climatic conditions on most studied traits,

Results also exhibit that mean squares due to nitrogen levels (N) were highly significant for all studied traits, indicating that the N level has an obvious effect on all studied traits of studied wheat genotypes. Mean squares due to genotypes (G) were highly significant for all studied traits, indicating that wheat genotypes used in this study were significantly ($P \leq 0.01$) different for all studied traits. Moreover, mean squares due to genotypes x nitrogen levels, i.e. G x N were significant ($P \leq 0.01$ or 0.05) for all studied traits, indicating that genotypes ranks differently from one nitrogen level to another and that selection can be done under a specific soil nitrogen environment as proposed by Al-Naggar et al. [28-37]. The significant GxN interaction for NUE was also a good evidence for varying responses of these wheat genotypes at various

N levels [38,39]. The interactions G x Y and G x Y x N were also significant ($P \leq 0.01$ or 0.05) for all studied traits, indicating that genotypes ranks differ from one combination of Y x N to another.

Combined analysis of variance of a lattice design for all studied traits under each environment (high N and low N) across two seasons was also performed (data not presented). Mean squares due to genotypes, parents, F_1 's and F_2 's under the two levels of nitrogen were highly significant for all studied traits. Significant differences among parents of diallel crosses in all studied traits are pre-requisite for performing the diallel analysis for estimating the inheritance of studied traits under different N- application rates. Mean squares due to parents vs. F_1 's and F_1 's vs F_2 's were highly significant for all studied traits under the two levels of nitrogen, except for nitrogen uptake efficiency (NUPE) which were not significant for F_1 's vs F_2 's, indicating the presence of significant heterosis for all studied traits and the presence of inbreeding effects for most studied traits. Mean squares due to the interaction G x Y were highly significant for all studied traits under the two levels of nitrogen, except for NUPE and GPC under low N which were not significant. Mean squares due to the interaction P x Y under high level of nitrogen were significant or highly significant for 10 studied traits and non significant for DTH, BYPP, NUE and GPC. Mean squares due to the interactions F_1 's x Y and F_2 's x Y under high-N were significant or highly significant for all studied traits, except NUPE for F_1 's x Y and 100GW for F_2 's x Y, which were not significant. Mean squares due to the interactions F_1 's x Y and F_2 's x Y were significant or highly significant for all studied traits under low N, except for 100GW, GYPP, NUE, NUPE, NUTE and GPC for F_1 's x Y and NUE and NUPE for F_2 's x Y. Mean squares due to the interactions P's vs F_1 's x Y and F_1 's vs F_2 's x Y under the two levels of nitrogen were significant and highly significant for all studied traits, except GPC and NHI for F_1 's vs. F_2 's x Y and NHI for P's vs F_1 's x Y. The significance of the interactions P's vs F_1 's x Y and F_1 's vs F_2 's x Y indicates that heterosis and inbreeding effects differ from season to season in most studied traits.

3.2 Mean Performance

A comparative summary of means of all studied traits across all 36 genotypes (6 parents, 15 F_1 's and 15 F_2 's) subjected to two levels of nitrogen conditions and across two years is presented in

Table (1). In general, low N caused a significant reduction in 7 out of 8 studied traits, namely GYPP, SPP, 100 GW, GPS and HI. Mean grain yield/plant (GYPP) was significantly decreased due to low-N by an average of 18.96, 21.17, and 15.40% for parents, F_1 's and F_2 's, respectively. Reduction in grain yield of wheat due to low soil nitrogen was reported by several investigators. A positive relationship between N application levels and the grain yield has already been shown in many studies [39,40]. Significant reduction in grain yield as a result of low-N was associated with significant reductions in all yield components traits, *i.e.* SPP, 100GW and GPS. These reductions were relatively high in magnitude for number of spikes/ plant (SPP) for parents (23.65%), F_1 's (23.99%) and F_2 's (43.52%). This indicates that SPP is the most determining component of grain yield / plant of wheat under low-N stress. The importance of this trait (number of spikes or fertile tillers per plant) in wheat for grain productivity under abiotic stress conditions was previously reported by several investigators; e.g. Al-Naggar et al. [41]. Al-Bakry et al. [42] observed that increasing nitrogen application increased the number of fertile tillers per unit area. Geleto et al. [43] reported that grain yield is closely related to the number of spikes per unit area. Fertilized plots produced more spikes than control. Such response can be attributed to the adequate nitrogen availability which might facilitate the tillering ability of plants, resulting in a greater spike population. Ayoub et al. [44] also reported that spike population increased with increase in nitrogen level.

Moreover, low nitrogen caused a significant reduction in biological yield / plant (BYPP) by 12.49, 12.27 and 11.24%, grain protein content (GPC) by 25.06, 29.18, and 23.31% and harvest index (HI) by 6.57, 8.97 and 3.69% for parents, F_1 's and F_2 's, respectively. It was observed that low- N caused slight but significant earliness of DTM by 3.13, 5.33 and 4.52% (4.17, 7.16 and 6.07 days) and DTH by 0.70, 4.50 and 5.55 days for parents, F_1 's and F_2 's, respectively.

On the contrary, low-N caused increases in the averages of nitrogen use efficiency (NUE) by 89.56, 97.60 and 84.28% for parents, F_1 's and F_2 's, respectively. Significant increase in NUE due to low- N stress was associated with significant increases in averages of NUPE and NUTE. In agreement with these results, Ortiz-Monasterio et al. [5] also reported significant influence of N application rate on NUTE in which the highest efficiencies were measured at the

lowest application rate. Sinebo et al. [45] also found similar results in which they reported NUTE values of 29.3- 43.9 and 31.8-48.3 kg kg⁻¹ N, respectively.

In the present study, magnitude of increase in NUPE (65.87, 83.08 and 78.70%) was much higher than that in NUTE (14.39, 3.93 and 20.21%) for parents, F₁'s and F₂'s, respectively. This indicates that NUPE is the most determinant component of NUE. Al-Naggar et al. [33-37] showed that N efficiency diminishes as N fertilizer rates increase. Gorny et al. [46] reported that NUPE, NUTE and NHI tended to increase under low-N. According to Le Gouis et al. [47], in wheat, Al-Naggar et al. [33,37] in maize and Al-

Naggar et al. [28,29] in grain sorghum, N uptake in biomass was the most important factor in NUE determination regardless of N level. Abeledo et al. [48] reported that both N conversion and N capture have played a role in the improvement of NUE. On the contrary, Gaju et al. [49] found that NUPE effect explained only a small amount of phenotypic variance in NUE amongst cultivars, but NUTE affected it up to 61% and 77% under high -N low-N, respectively. The differences in results might be attributed to the different genetic background of genotypes used in different studies. In the present study, it is also observed that low- N stress caused a significant (but slight) increase in plant height of F₁'s (6.80%) and F₂'s (4.80%).

Table 1. Means of studied wheat traits under low-N (0 Kg N/fed) and high-N (75 Kg N/fed) and relative reduction compared to high-N combined across parents, F₁'s and F₂'s across two seasons

Traits	Parameter	Parents		F ₁ crosses		F ₂ crosses	
		High-N	Low-N	High-N	Low-N	High-N	Low-N
DTH	Average	88.64	87.94	89.61	85.11	89.04	83.49
	Reduction%	---	0.78	---	4.95**	---	6.13**
DTM	Average	132.33	128.17	133.33	126.17	132.76	126.69
	Reduction%	---	3.13*	---	5.33**	---	4.52**
PH(cm)	Average	82.74	81.21	89.54	83.96	102.95	99.05
	Reduction%	---	1.74	---	6.22**	---	3.78**
GPS	Average	80.23	69.81	79.95	71.76	74.48	64.78
	Reduction%	---	13.47**	---	9.80**	---	12.47**
100 GW(g)	Average	4.66	4.05	4.33	3.84	3.37	2.61
	Reduction%	---	12.96**	---	10.51**	---	21.72**
SPP	Average	11.88	9.11	12.13	9.14	12.95	7.31
	Reduction%	---	18.96**	---	23.99**	---	43.52**
GYPP(g)	Average	27.53	22.41	29.12	22.83	25.65	21.54
	Reduction%	---	18.96**	---	21.17**	---	15.40**
BYPP(g)	Average	63.14	54.98	64.87	56.78	59.29	52.59
	Reduction%	---	12.94**	---	12.27**	---	11.24**
HI (%)	Average	43.67	40.73	45.11	40.51	43.50	41.37
	Reduction%	----	6.57**	---	8.97**	---	3.96
NUE(g/g)	Average	14.72	28.03	15.57	28.53	13.72	26.91
	Reduction%	----	-89.56**	----	-97.60**	----	-84.28**
NUPE (%)	Average	16.00	26.87	16.14	29.43	17.13	29.97
	Reduction%	-----	-65.87**	-----	-83.08**	---	-78.70**
NUTE (g/g)	Average	0.94	1.07	0.98	1.00	0.84	0.98
	Reduction%	----	-14.39**	----	-3.93	---	-20.21**
GPC (%)	Average	14.35	11.33	14.61	12.64	14.86	12.91
	Reduction%	-----	21.3**	-----	14.71**	-----	13.12**
NHI (%)	Average	56.10	55.94	56.21	58.08	56.75	57.73
	Reduction%	-----	0.28	-----	-3.33	-----	-1.73

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

3.3 Interrelationships

Estimates of simple correlation coefficients between each of grain yield /plant (GYPP), nitrogen uptake efficiency (NUPE) and nitrogen utilization efficiency (NUTE) and other studied traits across the two seasons under high-N and low-N were calculated across all parents, F₁'s and F₂'s and presented in Table (2). Grain yield/plant showed perfect positive association with NUE (r=1.00) under both high-N and low-N for all groups of genotypes, *i.e.*, parents, F₁'s and F₂'s, that is why the estimates of correlation coefficients between GYPP and other traits are very close to those between NUE and the same traits.

The strongest correlations ($r \leq 0.90$) was observed between grain protein content (GPC) which is an expression of grain nitrogen content (N_g) and each of NUPE and NUE (GYPP) for all genotypes under both environments, *i.e.* high-N and low-N.

Concerning the components of grain yield, significant and high-in-magnitude correlations were observed between GYPP and each of GPC (N_g) (0.92), HI (0.82), GPS (0.71), 100GW (0.63) and SPP (0.43) across parents, NUPE (0.80), HI (0.67) and NUTE (0.62) across F₁'s and HI (0.82) and SPP (0.32) across F₂'s under high-N and GPS (0.86), GPC (0.95) HI (0.55), SPP (0.57) and 100GW (0.36) across parents, GPS (0.60), BYPP (0.74), HI (0.71), GPC (0.44) and SPP (0.36) across F₁'s and SPP (0.29), GPS (0.24), NUPE (0.39), HI (0.55) and BYPP (0.38) across F₂'s under low-N conditions. These results indicate the importance of SPP, GPS, GPC and NUPE for getting high grain yield / plant under both high and low N conditions. Sinclair and Jamieson [50] reported that, it is logical that the number of grains produced is not only important for determining the yield, but is also related to the NUE. However, the results imply that breeding efforts improving yield by increasing the number of grains should also result in higher NUE as well according to Moll et al. [5].

The results indicate also that under low-N, it is possible to obtain wheat genotypes (parents, F₁'s and F₂'s) that have high grain yield of high grain protein contents. 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.

[51]. In other studies, Ayoub *et al.* [44] and Ortiz-Monasterio *et al.* [5] reported negative associations of grain yield with protein content. Sinebo *et al.* [45] found significant negative correlations of NUTE with grain protein content.

Concerning the components of NUE of parents, results (Table 2) showed that NUE had positive and significant correlations with NUPE (0.83 and 0.88) and negative and significant correlation coefficients with NUTE (-0.62 and -0.46) under low-N and high-N, respectively. So, parents data indicate that NUE is based mainly on NUPE, especially under low-N. Data of F₁'s showed that NUE is depending only on NUTE under high-N (r=0.61) and on NUPE under low-N (0.75), suggesting that NUE of F₁ hybrids depends more on NUPE under high-N and NUTE under low-N. Regarding F₂ populations, data showed that NUE had significant and positive, but low-in-magnitude correlations with NUPE under low-N (r=0.39) and high-N (r=0.24) and not correlated with NUTE. This indicates that F₂ hybrids express their NUE due to NUTE under both low and high N conditions, with higher degree under low-N.

In general, across all genotypes, NUE depends mainly on NUPE, except F₁'s under high-N which depends on NUTE. Thus under low-N, NUE increases by increase of NUPE for parents, F₁'s and F₂'s. The same conclusion was valid under high-N, for parents and F₂'s, but for F₁'s, NUE increases by increasing NUTE. Numerous studies indicated that NUPE accounts for more of the genetic variation in NUE at low N than at high N supply, *e.g.* amongst 10 spring wheat cultivars in Mexico [5] and 20 winter wheat cultivars in France [47]. Moreover, Dhugga and Waines [52] comparing 12 spring wheats in California, found that NUPE was the most important component of NUE at all N levels. Genetic gains in NUE with breeding under low N supply have been related mainly to improvements in NUPE in spring wheat in Mexico [5] and Finland [8] and to NUTE in winter wheat in France [9] and the UK [10].

In general, grain yield or nitrogen use efficiency (NUE) were significantly correlated with nitrogen uptake efficiency (NUPE) in most cases; this correlation was more pronounced in parents (0.88 and 0.83) than in F₁ crosses (0.80 and 0.75) and F₂ crosses (0.24 and 0.39) under high-N and low-N, respectively. Harvest index (HI) showed significant and positive correlation coefficients in most cases with GYPP and NUE for parents, F₁'s and F₂'s of medium to high magnitude under low-N and high-N.

Since NUPE and HI showed strong correlations with NUE or GYPP in this study and nitrogen translocation efficiency (NTRE) (grain N/total plant N) proved be strongly correlated with HI in other studies [33], grain yield or NUE of parents, F₁'s and F₂'s in the present study could therefore be considered of strong association with N-uptake and N-translocation efficiencies. Similar conclusion was reported by Al Naggar et al. [35-37] in maize and Gorny et al. [46] in wheat.

Table 2. Correlation coefficients among studied traits across parents, F₁'s and F₂'s under high and low-N

Genotype	High N			Low N		
	GYPP	NUPE	NUTE	GYPP	NUPE	NUTE
Parents						
DTH	0.71**	0.96**	-0.93**	0.51**	0.15	0.66**
DTM	0.14	0.10	-0.05	-0.21	-0.33*	0.12
PH	0.23	0.22	-0.25	-0.75**	-0.48**	-0.60**
SPP	0.43**	0.79**	-0.97**	0.57**	0.82**	-0.22
GPS	0.71**	0.85**	-0.73**	0.86**	0.91**	0.16
100GW	0.63**	0.84**	-0.79**	0.36*	0.28*	0.21
GYPP	---	0.88**	-0.45**	---	0.83**	0.54**
BYPP	0.07	0.85**	-0.73**	0.38*	0.40**	0.50**
HI%	0.82**	0.11	0.27	0.55**	0.58**	0.17
NUE	1.00**	0.88**	-0.46**	1.00**	0.83**	0.54**
NUPE	0.88**	---	-0.82**	0.83**	---	-0.02
NUTE	-0.45**	-0.82**	---	0.54**	-0.02	---
GPC	0.92**	0.99**	-0.76**	0.95**	0.99**	0.40*
NHI	0.32	-0.11	0.58**	-0.47**	-0.41*	0.55**
F₁ crosses						
DTH	0.12	-0.17	0.21*	0.70**	0.57**	0.50**
DTM	0.18	0.18	0.35**	0.69**	0.60**	0.48**
PH	0.05	0.16	-0.05	0.52**	0.45**	0.57**
SPP	0.35**	0.61**	-0.46**	0.36**	0.44**	-0.45**
GPS	0.30**	0.07	0.17	0.60**	0.28*	0.06
100GW	0.14	0.41**	-0.24*	-0.22*	-0.07	-0.64**
GYPP	--	0.08	0.62**	----	0.75**	0.06
BYPP	0.42**	0.76**	-0.30**	0.74**	0.69**	-0.11
HI%	0.67**	-0.53**	0.86**	0.71**	0.43**	0.51**
NUE	1.00**	0.08	0.61**	1.00**	0.75**	0.06
NUPE	0.80**	----	-0.73**	0.75**	----	-0.36**
NUTE	0.62**	-0.73**	---	0.36**	-0.36**	---
GPC	0.41*	0.37*	-0.77**	0.44**	0.84**	-0.56**
NHI	-0.37*	-0.61	0.23	-0.68**	-0.50**	0.10
F₂ crosses						
DTH	-0.33**	-0.55**	0.40**	0.20	0.23*	0.14
DTM	-0.11	-0.62**	0.56**	0.41**	0.49**	-0.20
PH	0.39**	0.29**	-0.08	-0.05	-0.15	0.14
SPP	0.32**	0.47**	-0.48**	0.29**	0.34**	-0.10
GPS	-0.04	0.56**	-0.54**	0.24*	0.43**	-0.07
100GW	0.15	0.43**	-0.39**	0.18	0.50**	-0.57**
GYPP	---	0.24*	0.11	----	0.39**	-0.08
BYPP	0.24*	0.59**	-0.57**	0.38**	0.39**	-0.19
HI%	0.82**	-0.12	0.41**	0.55**	-0.05	0.16
NUE	1.00**	0.24*	0.11	1.00**	0.39**	0.39**
NUPE	0.11	---	-0.91**	0.39**	---	-0.81**
NUTE	-0.91**	-0.91**	---	-0.08	-0.81**	----
GPC	0.27*	0.36*	-0.24	0.26*	0.89**	-0.69**
NHI	-0.11	-0.60**	0.61**	0.02	0.89**	0.31**

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

It is observed that a negative and significant correlation existed between NUPE and NUTE for parents under high-N (-0.82), F_1 's under high-N (-0.73) and low-N (-0.36) and F_2 's under high-N (-0.91) and low-N (-0.81). This observation was reported by many previous investigators (e.g. Gorny et al., [46]). The two processes (N-uptake and N-utilization) appear to be governed by different genetic factors [53-55]. For instance results of extensive molecular studies, on wheat and maize [51,56-58] revealed that different sets of genes (QTL regions) controlled various components of the two major measures of N efficiency, and that expression of the genes (loci) was considerably dependent upon soil N status. Hence, the appearance of the above mentioned negative relationship between NUPE and NUTE in the examined hybrids and their parents may be a genetic quandary. Most of modern parents of wheat were developed under enhanced N fertilization with strong selection for yielding capacity and grain quality. Thus, such a strategy may have favored high-yielding genotypes with increased NUTE, but not those with a high NUPE. This implies that the proposal of Martre et al. [59] to search for genotypes able to translocation of larger amounts of N grains without reducing plant biomass and photosynthetic capacity under N shortage has become particularly challenging for wheat breeders'.

4. HERITABILITY

4.1 In F_1 Generation

Broad-sense heritability (h^2_b) in F_1 's for all studied traits (except GPC) in this experiment was of high magnitude and ranged from 61.7% (SPP) to 99.9% (GPS) under high-N and from 41.0% (NHI) to 100.0% (GPS) under low-N environments (Table 3), indicating that environment had a small effect on the phenotype of F_1 's for most studied traits. Grain protein content (GPC) trait showed very small h^2_b value under both high and low N environments (8.52 and 20.00%, respectively), indicating a large effect of environment on this trait.

Narrow-sense heritability (h^2_n) in F_1 's (Table 3) was generally of medium magnitude and ranged from 8.50% (NUTE) to 56.89% (GPS) under high-N and from 9.00% (NHI) to 73.55% (GPS) under low-N. It is observed that GPC trait recorded 0.0% h^2_n under both high- and low- N; the reason may be because its D value was negative; that is why we considered it zero.

The big difference between broad- and narrow-sense heritability estimated from F_1 '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 of the present study was generally higher in magnitude under low-N than under high-N for all studied traits, except for plant height. This increase in h^2_n under low-N compared to high-N was more pronounced in SPP, GPS, GYPP and NUE traits; *i.e.* the most important components of grain yield and consequently nitrogen use efficiency in wheat.

Expected genetic advance (GA) from selection (based on 1% selection intensity) across two years for F_1 's ranged from 0.12% for NHI to 23.9% for HI under high-N and from 1.02% for NHI to 23.26% for GPS under low-N. In general, the values of GA were higher under low-N than under high-N (for 11 out of 14 studied traits). The traits SPP, GPS, GYPP, BYPP, NUE and NUPE showed much higher estimates of GA (23.25, 23.26, 19.70, 10.44, 19.62 and 22.43%, respectively) under low-N than those under high-N (2.50, 15.87, 5.11, 5.44, 9.44 and 11.25%, respectively).

4.2 In F_2 Generation

Broad-sense heritability (h^2_b) in F_2 's for all studied traits (except GPC under both environments and NHI under low N) in this experiment was of high magnitude and ranged from 40.12% (SPP) to 100.00% (GPS) under high-N and from 63.71% (100 GW) to 100.00% (GPS) under low-N environments (Table 4), indicating that environment had a small effect on the phenotype of F_2 's for most studied traits. In F_2 generation, grain protein content (GPC) under both high and low N environments (13.00 and 20.00%, respectively) and NHI trait under low-N (14.30%) showed very small h^2_b value, indicating a large effect of environment on these traits.

Narrow-sense heritability (h^2_n) in F_2 's (Table 4) was generally of low to high magnitude and ranged from 2.11% (NHI) to 82.90% (GPS) under high-N and from 6.80% (NHI) to 93.00% (GPS) under low-N. It is observed that in F_2 's, GPC trait recorded 0.0% h^2_n under both high- and low- N; the reason may be because its D value was negative; that is why we considered it zero. The big difference between broad- and narrow- sense heritability estimated from F_2 's in this experiment could be attributed to the high estimates of dominance as compared to additive component.

Table 3. Estimates of heritability in broad (h^2_b) and narrow (h^2_n) sense and genetic advance from selection (GA) for studied traits under high and low-N in F_1 populations of 15 diallel crosses across two seasons

Parameter	DTH	DTM	PH	SPP	GPS	100GW	GYPP
High N							
h^2_b %	91.15	85.88	94.50	61.70	99.90	81.00	91.27
h^2_n %	28.80	15.85	39.71	9.20	56.89	21.40	20.41
GA%	2.13	0.80	4.66	2.50	15.87	9.24	5.11
Low N							
h^2_b %	80.27	87.78	96.00	54.42	100.00	57.50	91.00
h^2_n %	42.52	21.80	33.11	66.32	73.55	27.43	67.14
GA%	2.27	1.30	4.38	23.25	23.26	9.90	19.70
Parameter	BYPP	HI	NUE	NUPE	NUTE	GPC	NHI
High N							
h^2_b %	96.32	87.30	91.20	96.00	96.60	8.52	63.85
h^2_n %	29.10	9.60	39.50	40.70	8.50	0.00	1.90
GA%	5.54	23.90	9.44	11.25	4.00	0.00	0.12
Low N							
h^2_b %	97.88	93.50	90.75	96.60	87.30	20.00	41.00
h^2_n %	40.20	15.87	66.90	46.36	11.11	0.00	9.00
GA%	10.44	5.42	19.62	22.43	5.63	-5.70	1.02

Table 4. Estimates of heritability in broad (h^2_b) and narrow (h^2_n) sense and genetic advance from selection (GA) for studied traits under high and low-N in F_2 populations of 15 diallel crosses across two seasons

Variance components	DTH	DTM	PH	SPP	GPS	100GW	GYPP
High-N							
h^2_b %	74.40	61.00	97.41	40.12	100.00	65.83	81.76
h^2_n %	56.00	46.75	16.45	16.10	82.90	30.60	42.70
GA%	3.00	1.40	2.60	3.20	20.40	12.80	7.70
Low-N							
h^2_b %	82.59	76.59	98.40	70.00	100.00	63.71	88.65
h^2_n %	79.10	37.80	17.72	43.76	93.00	25.56	85.10
GA%	5.38	1.70	3.20	22.00	28.12	12.31	23.14
Variance components	BYPP	HI	NUE	NUPE	NUTE	GPC	NHI
High-N							
h^2_b %	90.37	82.00	81.64	95.03	91.83	20.00	59.53
h^2_n %	65.40	19.30	42.64	33.37	20.41	0.00	2.11
GA%	7.00	3.60	7.70	9.52	6.80	0.00	0.12
Low-N							
h^2_b %	96.00	78.20	88.60	88.10	67.57	13.00	14.30
h^2_n %	55.44	33.38	85.00	72.80	27.00	0.00	6.80
GA%	13.00	7.50	23.10	27.43	6.80	0.00	6.70

It is observed that narrow-sense heritability (h^2_n) in F_2 's of the present study was generally higher in magnitude under low-N than under high-N for all studied traits, except for DTM, 100 GW and BYPP. This increase in h^2_n under low-N compared to high-N was more pronounced in SPP, GPS, GYPP, NUE and NUPE traits; *i.e.* the most important components of grain yield and nitrogen use efficiency in wheat.

Our results of heritability in F_1 's and F_2 's are in agreement with some researchers, e.g. Shabana et al. [60], Blum [61,62] and Al-Naggar et al. [28-37], who support the idea that heritability is higher under stressed than non-stressed environment. On the contrary, other investigators reported that heritability is higher under good (non-stressed) environment [63-65].

Expected genetic advance (GA) from selection (based on 1% selection intensity) across two years ranged from 0.12% for NHI to 20.40% for GPS under high-N and from 1.70% for DTM to 28.12% for GPS under low-N (Table 4). In general, the values of GA were higher under low-N than under high-N (for 12 out of 14 studied traits). The traits SPP, GPS, GYPP, BYPP, NUE, NUPE and NHI showed much higher estimates of GA (22.00, 28.12, 23.14, 13.00, 23.10, 27.43 and 6.70 %, respectively) under low-N than those under high-N (3.20, 20.40, 7.70, 7.00, 7.70, 9.52 and 0.12%, respectively).

These results in F_1 and F_2 generations indicated that to improve grain yield and nitrogen use efficiency traits in the present germplasm, it is better to practice selection for these traits under low-N conditions to obtain higher values of selection gain.

5. ALTERNATIVE SCREENING CRITERIA FOR NUE

The selection criterion for a given abiotic stress tolerance character, such as low-N tolerance, should be of strong association with tolerance to low-N, *i.e.* with nitrogen use efficiency and of high heritability in narrow-sense under low-N conditions. The results in Tables (2, 3 and 4) concluded that NUPE, GYPP, GPS, and SPP of parents, NUPE, GYPP, BYPP, DTH and GPS of F_1 's and NUPE, HI, BYPP, SPP and GPS of F_2 's had strong associations with high nitrogen use efficiency.

The importance of number of spikes (fertile tillers per plant) in wheat for grain productivity under abiotic stress conditions was previously reported by several investigators [41]. CIMMYT breeders found that maize grain yield under low-N was closely related to some secondary traits such as improved N-uptake efficiency, high plant nitrate content, high-specific leaf-N content and late leaf senescence [3,65]. These results for NUPE and SPP are in consistency with those reported by Al-Naggar et al. [30,31,33-37].

Numerous studies indicated that NUPE accounts for more of the genetic variation in NUE at low N than at high N supply, *e.g.* amongst 10 spring wheat cultivars in Mexico [5] and 20 winter wheat cultivars in France [47]. Moreover Dhugga and Waines [52] comparing 12 spring wheats in California, found that NUPE was the most important component of NUE at all N levels. Genetic gains in NUE with breeding under low N

supply have been related mainly to improvements in NUPE in spring wheat in Mexico [5] and Finland [8], but to NUTE in winter wheat in France [9] and the UK [10]. Differences in results could be attributed to genotypes used in different studies; that might differ in genes controlling NUPE and genes controlling NUTE.

Harvest index (HI) and nitrogen harvest index (NHI) provide a means for determining the allocation of important nutrients within the plant tissues to grain during reproductive growth. Since NUPE and HI showed strong correlations with NUE or GYPP in this study and nitrogen translocation efficiency (NTRE) (grain N/total plant N) proved to be strongly correlated with HI in other studies [33], grain yield or NUE of parents, F_1 's and F_2 's in the present study could therefore be considered of strong association with N-uptake and N-translocation efficiencies. Similar conclusion was reported by Al-Naggar et al. [33] in maize and Gorny et al. [46] in wheat.

In the present study, a negative and significant correlation existed between NUPE and NUTE for parents under high-N (-0.82), F_1 's under high-N (-0.73) and low-N (-0.36) and F_2 's under high-N (-0.91) and low-N (-0.81). Both processes, N uptake and N-utilization, appear to be governed by different genetic factors [53-55]. For instance results of extensive molecular studies, on wheat and maize [51,56-58] revealed that different sets of genes (QTL regions) controlled various components of the two major measures of N efficiency, and that expression of the genes (loci) was considerably dependent upon soil N status. Hence, the appearance of the above mentioned negative relationship between NUPE and NUTE in the examined hybrids and their parents may be a genetic quandary. Most of modern parents of wheat were developed under enhanced N fertilization with strong selection for yielding capacity and grain quality. Thus, such a strategy may have favored high-yielding genotypes with increased NUTE, but not those with a high NUPE. This implies that the proposal of Martre et al. [59] to search for genotypes able to translocate of larger amounts of N grains without reducing plant biomass and photosynthetic capacity under N shortage has become particularly challenging for wheat breeders.

Selection based on improved performance under low N based on NUE alone has often been considered efficient, but the use of secondary traits can increase selection efficiency [33]. Plant

breeders have advocated the judicious incorporation of secondary traits within breeding programs [61,62]. Results of the present study suggest that to maximize the genetic gain from selection, for improving NUE in wheat and consequently, grain yield under low-N, future research should focus on the incorporation of secondary traits such as NUPE, GYPP, SPP and GPS in the selection programs along with NUE trait.

6. CONCLUSION

The results of this study indicated that GYPP, GPS, SPP and NUPE of parents, GYPP, NUPE, BYPP, DTH and GPS of F₁'s and HI, NUPE, BYPP, SPP and GPS of F₂'s are considered selection criteria for high nitrogen use efficiency (NUE). Nitrogen uptake efficiency (NUPE) is a common selection criterion for NUE in all studied genotypes, *i.e.* parents, F₁'s and F₂'s. Grains/spike (GPS) as a selection criterion for grain yield/plant (GYPP) is also selection criterion for NUE, since GYPP is perfectly correlated with NUE. Moreover, spikes/plant (SPP) is strongly correlated with both GYPP and NUE in parents and F₂'s. All these selection criteria (NUPE, GYPP, SPP and GPS) have high heritability in narrow sense and could be offered to wheat breeders for use in selection programs for getting high selection gain when improving nitrogen use efficiency. Results suggest that future research should focus on the incorporation of such secondary traits (NUPE, GYPP, SPP and GPS) in the selection programs along with NUE trait in order to maximize the genetic gain from selection for improving wheat NUE.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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