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**Optimizing Wheat Productivity Under Low Nitrogen input: Unveiling Key Characters and Inheritance of Nitrogen Use Efficiency**

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**Abstract**

Six parents *i.e.* 1) Line 1, 2) Line 2, 3) Line 3, 4) Sakha 93, 5) Giza 168 and 6) Gemmeiza 10 were crossed in a half diallel to demonstrate performance, important criteria, gene action and heritability for NUE components. Available soil nitrogen 40.5 kg N/fad. was expressed as low N-level (L<sub>1</sub>), then 35 Kg N/fad was added to give 75.5 kg N/fad which represents the normal N-level (L<sub>2</sub>) and 70 kg N/fad was added to give 110.5 Kg N/fad as high N-level (L<sub>3</sub>), were implemented in three adjacent experiments, separately in a RCBD. Parents P<sub>1</sub>, P<sub>2</sub>, P<sub>6</sub> and F<sub>1</sub> crosses P<sub>2</sub> x P<sub>5</sub> and P<sub>3</sub> x P<sub>4</sub>, P<sub>4</sub> x P<sub>6</sub> made more efficient use of each amount of nitrogen uptake for grain and straw yield. N-stress tolerance indices showed that P<sub>1</sub>, P<sub>5</sub>, P<sub>1</sub> x P<sub>4</sub>, P<sub>1</sub> x P<sub>5</sub>, P<sub>3</sub> x P<sub>4</sub>, P<sub>3</sub> x P<sub>5</sub> and P<sub>5</sub> x P<sub>6</sub> were more tolerant to N-deficiency stress. Factor analysis shows that DH, DM, SGD, PH and yield components of first factor had high loading coefficients with highest participation in NUE. Also, SYP, BYP, SRI and NUtE of second factor were important. Hierarchical cluster analysis heatmap illustrated that P<sub>2</sub> and P<sub>6</sub> were the best for NUE, NUtE, DH, DM and SGD and cross P<sub>3</sub> x P<sub>6</sub> for NUtE and NUE. Both additive and dominance genetic components were significant for NUE, NUtE, SRI and SSI with greeter role for dominance. Narrow sense heritability was moderate for NUE, NUtE and SRI, but low for SSI.

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**Keywords:** Bread Wheat, N-use Efficiency, Gene Action, Heritability, Correlation, Path and factor Analyses, Heatmap.

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## تحسين إنتاجية القمح تحت المدخلات المنخفضة من النيتروجين: الكشف عن السمات الرئيسية ووراثة كفاءة استخدام النيتروجين

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## الملخص العربي:

تم تهجين ستة آباء من قمح الخبز هي (1 سلالة 1، 2 سلالة 2، 3 سلالة 3، 4 سلالة 4، 5 سلالة 5، 6 سلالة 6) مميزة 10 بنظام الدباليل لدراسة متوسط الأداء، المعايير الانتخابية، الفعل الجيني وكفاءة التورث لمقاييس كفاءة استخدام النيتروجين NUE. قُدر النيتروجين الميسر بالتربة 40.5 كجم/ن/ف وأعتبر انه المستوى الأقل، ثم تم إضافة 35 كجم/ن/ف لتعطي 75.5 كجم/ن/ف ليُمثل المستوي الثاني (الموصى به)، ثم تم إضافة 70 كجم/ن/ف لتعطي 110.5 كجم/ن/ف ليُمثل المستوى الثالث (المستوي العالي)، نفذت في ثلاث تجارب منفصلة لمستويات النيتروجين الثلاثة، علي الترتيب في تصميم القطاعات الكاملة العشوائية. كانت الآباء P<sub>1</sub> و P<sub>2</sub> و P<sub>6</sub> وهجنها P<sub>2</sub> x P<sub>5</sub>، P<sub>4</sub> x P<sub>6</sub>، P<sub>3</sub> x P<sub>4</sub> الاعلي في NUE. وأشارت دلائل تحمل إجهاد نقص النيتروجين ان P<sub>5</sub> يليه P<sub>3</sub> x P<sub>5</sub>، P<sub>1</sub> x P<sub>4</sub>، P<sub>1</sub> x P<sub>4</sub>، P<sub>3</sub> x P<sub>4</sub>، P<sub>1</sub> x P<sub>5</sub>، P<sub>5</sub> x P<sub>6</sub> أكثر تحملاً لنقص النيتروجين. وظهرت صفات العامل الاول " طرد السنابل والنضج، البقاء اخضر، ارتفاع النبات، طول السنبله ومكونات المحصول " وصفات العامل الثاني "المحصول البيولوجي، محصول القش، دليل مقاومة الإجهاد SRI وكفاءة الاستفاده من النيتروجين NUE أعلى معامل تحميل ومساهمة في NUE. وأظهر التحليل العنقودي الهرمي والخريطة الحرارية تفوق الآباء P<sub>2</sub> و P<sub>6</sub> في NUE، NUE، الطرد والنضج و البقاء اخضر، وتفوق الهجين P<sub>3</sub> x P<sub>6</sub> في NUE، NUE. كان المكون المضيف والسيادي معنوياً في وراثة NUE، NUE، SRI and SSI، مع دور اكبر للمكون السيادي. وكانت تقديرات كفاءة التورث بالمعنى الضيق متوسطة لـ NUE، NUE and SRI ومنخفضة لـ SSI.

**الكلمات المفتاحية:** قمح الخبز، كفاءة استخدام النيتروجين، الفعل الجيني، كفاءة التورث، معامل الارتباط، معامل المرور، الخريطة الحرارية

**INTRODUCTION:**

Wheat is typically grown at high N-input levels, and NUE have been estimated to be between 35 to 42% (Yan *et al.*, 2022). A major challenge is optimizing N-use (*i.e.*, selecting cultivars that make the best use of applied N; termed as N-efficient germplasm) while maintaining yield in order to minimize negative environmental impacts and production input costs (Kant *et al.*, 2011). Nitrogen (N) fertilizer represents a significant cost for the grower and may also have environmental impacts through nitrate leaching and N<sub>2</sub>O as greenhouse gas emissions associated with denitrification (Nehe *et al.*, 2018). Further improvement in NUE might require extending the genetic diversity and simultaneous assessment under low and high N levels (Tahir *et al.*, 2020). A recent study showed that modern cultivars have improved yield performance along with enhanced NUE (Voss-Fels *et al.*, 2019), demonstrating that high NUE is likely to be indirectly increased through selection for high yield.

Plant adaptation to less favorable nutrition regimes is complex with various endue- and exogenous mechanisms involved (Gastal and Lemaire 2002; Gorny *et al.*, 2011; Al-Naggar *et al.*, 2017). Different morpho-physiological characteristics, associated with N-uptake capacity and nitrogen NUtE appear to be critical components of NUE (Khalilzadeh *et al.*, 2011; Nehe *et al.*, 2018). Numerous reports on genotypic variation and types of gene action in NUE components already suggest potential application of this genetic knowledge for wheat improvements. A relatively fewer attempts have been made to breed wheat for these components (Brancourt-Hulmel *et al.*, 2005; Laschenberger *et al.*, 2008; Wolf *et al.*, 2008; Gorny *et al.*,

2011; Al-Naggar *et al.*, 2017; Tahir *et al.*, 2020). Tahir *et al.* (2020) registered significant genotypic variation between 12 bread wheat genotypes for NUtE and NUE and associated characters. Addition of N increased biomass and grain yield of all genotypes, however, the magnitude of the response varied among the genotypes. NUtE was highest in Debeira (47.4 kg grain/kg N taken up) followed by Wadi Elneel, Khalifa, and Elnielain. Whereas NUE was highest in Elnielain 90 (43.8 kg grain/kg N) followed by Khalifa 04 and Wadi Elneel 87. Furthermore, stress tolerance indices have been used by many investigators of them, Aga *et al.* (2021) and Ivić *et al.* (2021b) to differentiate between wheat genotypes to determine which are tolerant or susceptible.

It is considered important to understand the genetic basis of tolerance to nitrogen deficiency stress. In this regard, both additive and non-additive genetic effects were crucial for agronomic N-use efficiency components in F<sub>1</sub> hybrids between modern French cultivars (Le Gouis *et al.*, 2002). The additive gene action was only important for NUE components in F<sub>2</sub> progenies of wheat of Turkish origin (Yildirim *et al.*, 2007). The additive gene action was almost exclusively significant for the N-uptake, NUtE and NUE and GYP only under high N-fertilization. The role of dominance distinctly increased under low N-fertilization. Narrow sense heritability for N-efficiency components varied from low to moderate (Gorny *et al.*, 2011; Al-Naggar *et al.*, 2017).

Correlation, path, factor, hierarchical cluster and heatmap analyses of morpho-physiological and yield characters has been computed to determine the relationship between NUE components and

other indicators. **Kichey *et al.* (2007)** found that grain yield was moderately correlated with chlorophyll contents ( $r = 0.64$  and  $0.73$ ). A high correlation ( $r = 0.85$ ) was obtained between grain-N content and N-uptake after anthesis. The correlation between grain N content and leaf senescence was negative ( $r = -0.83$ ). The correlation between grain weight and any of the other agronomical, physiological or NUE characters was insignificant. **Khalilzadeh *et al.* (2017)** showed that nitrogen uptake efficiency contributed to about 85% of variation at both N-levels. While, contribution of total NUE and Harvest Index (HI) were only (2-15%). **Xu *et al.* (2020)** registered significant positive correlations between nitrogen uptake efficiency and each of nitrogen fertilizer productivity, NUtE and nitrogen harvest index as well as between NUtE and nitrogen harvest index. Whereas, wheat grain yield was negatively and highly significantly associated with all nitrogen measurements. Hierarchical cluster analysis heatmap was exploited for dividing bread wheat genotypes into

different clusters based on the importance of measurements and the recorded characters as previously utilized by **Lupini *et al.* (2021)** and **Sarker *et al.* (2023)**.

Hence, the objectives of the current study highlights performance of NUE- components, N-tolerance indices and determines the relevant morho-physiological traits associated with low N-input tolerance as well as focusing on the types of gene action and heritability to improve more efficient wheat genotypes to low N-input.

#### MATERIALS AND METHODS:

The present study was conducted at the Experimental Farm of Kafer El-Hamam Agriculture Research Station, Agriculture Research Center, Egypt ( $30^{\circ} 02' N$  latitude and  $31^{\circ} 13' E$  longitude with an altitude of 22.50 meters above sea level), during the two successive seasons of 2009/2010 and 2010/2011. Six bread wheat (*Triticum aestivum* L.) genotypes were chosen for their differences in tolerance to low N-input as well as earliness, morho-physiological and yield characters (Table 1).

**Table (1): Name, pedigree and origin of the six parental bread wheat genotypes involved in the present study**

Name	Pedigree	Origin
1- Line 1	SAKHA 93/SIDS 6 CGZ (16) -3GM-2GM-OGM	Egypt
2- Line 2	GIZA 168/SIDS 7 CGZ (7)-4GM-2GM. OGM	Egypt
3- Line 3	GIZA 168//CHIL/SLMI 75 CGZ(9)-2GM-2GM OGM	Egypt
4- Sakha 93	SAKHA 92/TR 810 328 S 8871-1S-2S-1S-0S	Egypt
5- Giza 168	MRL/BUC//SERI CM93046-8M-0Y-0M-2Y-0B-0GZ	Egypt
6-Gemmeiza 10	MAYA74"s"/ON//1160-147/3/BB /GLL/4/CHAT"S"151 CROW "S" CGM 5820-3GM-1GM-2GM-OGM	Egypt

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

#### Crossing technique and experimental layout:

In 2009 /2010 season, a half diallel of crosses involving the six parents was done to obtain the  $F_1$

seeds of 15 crosses. In the second season of 2010/2011, the obtained 15  $F_1$  cross seeds together with their 6 parental genotypes were sown on the

3<sup>rd</sup> week of November. Before N application, random samples were collected from each replication and composited to determine soil N-content. The amount of available soil nitrogen in Kg/fad. was assessed and found to be 40.5 kg N/fad. which expressed as the low nitrogen level ( $L_1$ ). Then 35 Kg N/fad. was added before the first irrigation to give 75.5 kg N/fad. which represents the normal level ( $L_2$ ) and 70 kg N/fad. was added to give 110.5 Kg N/fad. which considered as high N-level ( $L_3$ ) which applied in two equal doses before the first and second irrigations, respectively. Three adjacent experiments were conducted for the three nitrogen levels, separately. In each experiment, a randomized complete block design with three replicates was used. Nitrogen was added in the form of Ammonium nitrate (33.5% N). The amount of available nitrogen (including soil and added N) was calculated as g/plant. Each parent or  $F_1$  was sown in two rows; each row was 2.5 meters long; spaces between rows were 20 cm and 10 cm between plants; the plot size was 1.0 m<sup>2</sup>. All other agricultural practices were done according to the recommendation of Ministry of Agriculture for growing wheat in the experimental site. Physical and chemical properties of the experimental site are listed in Table (2).

**Table (2): Some physical and chemical properties of the experimental site (Kafer El Hamam).**

Properties	Value
<b>Physical analysis</b>	
Sand %	33.43
Silt%	31.50
Clay%	45.07
<b>Soil texture</b>	
Clay	
<b>Chemical analysis</b>	
Available (N) PPM	40.50
Available (P) PPM	21.23
Available (K) PPM	329.00

**Source:** Central Lab for Soil Analysis, Water and Environment Research Institute, ARC, Giza, Egypt.

The following characteristics were measured on a random sample of 10 labeled plants of each parent and  $F_1$  in each replicate to collect data on earliness characters *i.e.* DH, DM, as well as SGD which the latest measured as number of days from sowing to time when the flag leaf of the main spike turned yellow, also, morpho-physiological characters *i.e.* PH (cm), FLA (cm) and FLCC (SPAD - 502 apparatus Castelli *et al.*, 1996). Spike characteristics *i.e.* SL, NFSS and NSSS were measured. GYP (g), SYP (g) and BYP (g) were assessed. 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 and samples were ground in powder. The micro Kjeldahl method was used to determine nitrogen of grains and straw and multiplied by 5.75 to obtain the percentage of crude protein according to AACC (2000).

**Total N-uptake and NUE components:** Grain and straw N-content were determined, and the following measurements based on g/plant were calculated according to Fageria *et al.* (1997):

1. Grain N-uptake (GNU) in g N/plant = grain N content x grain yield (g N/plant).
2. Straw N-uptake (SNU) in g N/plant = straw N content x straw yield (g N/plant).
3. Total N-uptake (TNU) in g N/plant = grain N uptake + straw N uptake (g N/plant).

**Nitrogen use efficiency components:**

1. **Agronomic efficiency (AE)**, expresses the efficiency of a unit of applied N in producing grain yield.

$$AE = \text{Error! (g g}^{-1}\text{)}$$

Gf: Is the grain yield of the fertilized crop (g), Gu: Is the grain yield of the unfertilized crop (g), Na: Is the quantity of nutrient applied (g).

**2. Physiological efficiency (PE)**, expresses the efficiency of a unit (g) of N-uptake from applied N- fertilizer in producing the total biomass yield (g).

$$PE = \text{Error!}(g\ g^{-1})$$

Yf: Is the total dry matter yield of the fertilized crop (g), Yu: Is the total dry matter yield of the unfertilized crop (g), Nf: Is the nutrient uptake of the fertilized crop, Nu: Is the nutrient uptake of the unfertilized crop.

**3. Apparent recovery efficiency (ARE)**, expresses the efficiency of wheat plants in recovering N from added N fertilizer in percentage.

$$ARE = \text{Error!} \times 100 (\%)$$

**4. Agro physiological efficiency (APE)**, expresses the efficiency of a unit of N-uptake from applied N fertilizer in building up grain yield.

$$APE = \text{Error!}$$

Ntf: Is the nutrient uptake by straw and grains in the fertilized plants (g).

Ntu: Is the nutrient uptake by straw and grains in the unfertilized plants (g).

**5. Nitrogen Utilization efficiency (NUE)**, is the product of PE and ARE and hence expresses the efficiency of a unit of added N in building up the total biomass.

$$UE = PE \times ARE$$

**6. Nitrogen use efficiency NUE**, is considered as the ratio of

grain yield to available N from the soil and applied fertilizer ( $g\ g^{-1}$ )

$NUE = \text{Grain yield (g)} / \text{available N from soil and fertilizer (g)}$  (Ladha et al. 2005).

#### N-stress tolerance indices

Tolerance indices were used to determine the tolerance or sensitivity of wheat genotypes to N-deficiency stress and are listed in Table 3.

**Table (3): N-tolerance indices, formula and reference**

No.	Index name	Formula	Reference
The low values of these indices indicate to N-stress tolerance			
1	Stress resilience index (SRI)	$(Y_n - Y_s) / [1 - (\bar{Y}_n - \bar{Y}_s)]$ t = 1- Effect/SE of effect	Awaad et al. (2024) Current Research
2	Tolerance index (TOL)	$Y_n - Y_s$	Rosielle and Hamblin (1981)
3	Stress sensitivity index (SSI)	$[1 - (Y_s / Y_n)] / [1 - (\bar{Y}_s / \bar{Y}_n)]$	Fischer and Maurer (1978)
4	Reduction % (R%)	$(Y_n - Y_s) / (Y_n) \times 100$	Rybiński et al. (2003)
The high values of these indices indicate to N-stress tolerance			
5	Mean productivity (MP)	$(Y_n + Y_s) / 2$	Rosielle and Hamblin (1981)
6	Harmonic mean (HM)	$\{2 * (Y_n * Y_s)\} / (Y_n + Y_s)$	Jafari et al. (2009)
7	Nitrogen tolerance (NT)	$Y_s / Y_n$	Fereres et al. (1986) and Saulescu et al. (1995)
8	Relative performance (RP)	$(Y_s / Y_n) / (\bar{Y}_s / \bar{Y}_p)$	Abo-Elwafa and Bakheit (1999)

-  $Y_n$  and  $Y_s$  indicate to average grain yield of each genotype under normal and stress conditions, respectively.

-  $\bar{Y}_n$  and  $\bar{Y}_s$  indicate to average grain yield overall genotypes under normal and stress conditions, respectively.

**Statistical procedures:** Data were statistically analyzed and pooled analysis was performed to detect the interaction effect using the computer statistical program MSTAT-C and L.S.D. was used to compare between different means (Steel *et al.*, 1997). Diallel analysis was computed as outlined by Hayman (1954a and b). Simple correlation coefficient was estimated according to Snedecor and Cochran (1967) to be used to calculate path coefficient analysis (Singh and Chaudhary 1985) and factor analysis (Cattell 1965 and Walton 1972). Hierarchical Cluster Analysis Heatmap was applied using R statistical software (version 4.1.1).

## RESULTS AND DISCUSSION:

### Analysis of variance and mean performance :

Estimates of genetic variability for studied characters in six parental wheat genotypes and their  $F_1$ 's under low, normal and high-N levels represents stress, favorable and high N containing environments, respectively. Frey and Maldnado (1967) defined the stress environments as the one in which mean performance for a certain attribute is low. As presented in Table 4, significant mean squares were detected due to N- levels, genotypes, parents and  $F_1$ 's in all characters, except parents for NSP and SYP as well as GPC and SPC for N-levels. P.Vs. $F_1$  as indicated to average heterosis was significant in DM, FLA, FLCC, NSSS, NSP, NGS, 1000 GW, BYP, GPC and SPC. Mean squares of G x N were significant for DM, FLA, NSSS, NGS, GPC and SPC; Parents x N for DM, FLA, NSSS, NGS, 1000 GW, BYP, GPC and SPC;  $F_1$ 's x N for FLA, NGS, GPC and SPC as well as P.Vs. $F_1$  x N for SGD, FLA, GPC and SPC. Therefore, each of the genotype and N-level had a marked effect on the previous characters, and the orders of genotypes differ from nitrogen

level to another. The significant interaction was also good evidence for varying responses of wheat genotypes to various N-levels, and the studied materials behaved somewhat differently from environment to another (Earl and Ausubel 1983; Barraclough *et al.*, 2010; Cormier *et al.*, 2016).

### Earliness characters

DH, DM and SGD illustrated in Fig 1, were delayed across genotypes from 89.87, 140.30 and 138.29 under L1; 91.63, 145.76 and 144.26 under L2 to 92.26, 147.26 and 146.23 under L3, respectively. This may be due to increasing cell elongation prolonging the vegetative plant growth period. N application ensures good establishment of sink capacity (Mader *et al.*, 2007) by increasing nitrogen availability for cell elongation, growth, photosynthesis and metabolism (Haberle *et al.*, 2008). Sohail *et al.* (2018) showed an increase in grain weight and protein contents, with N application, as attributed to sufficient distribution of nitrogen to flag leaf which enhanced dry matter accumulation, healthier and increased stay green duration (Gaju *et al.*, 2014).

### Morpho-physiological characters

As clarified in Fig. 2, PH reached 100.04, 107.15 and 106.33 under  $L_1$ ,  $L_2$  and  $L_3$ , respectively. Also, FLA and FLCC were enhanced in ascending order from  $L_1$ ,  $L_2$  and  $L_3$ . The increase in FLCC with increasing nitrogen levels may be due to the effect of nitrogen as a key component of biomolecules, such as proteins, nucleic acids, chlorophyll synthesis, and various secondary metabolites. Similar results were obtained by Saleh (2006) and Prakash Raigar *et al.* (2022).

### Spike characteristics

As illustrated in Fig. 3, the increase in the N rates significantly increased both SL and NFSS from

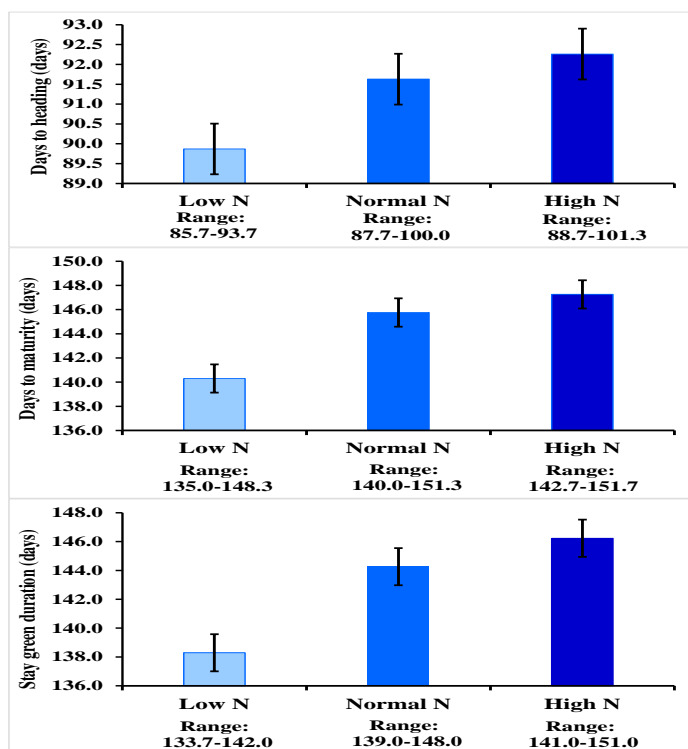
L<sub>1</sub>, L<sub>2</sub> and L<sub>3</sub>, respectively. Otherwise, NSSS took the opposite direction. This refers to the number of reproductive organs per spike either fertile florets at anthesis or grains at maturity can be increased by channeling resources to the spikes

more efficiently to produce more grains, this is strongly correlated with nitrogen supply. A similar interpretation was detected by Mónica Elía et al. (2016).

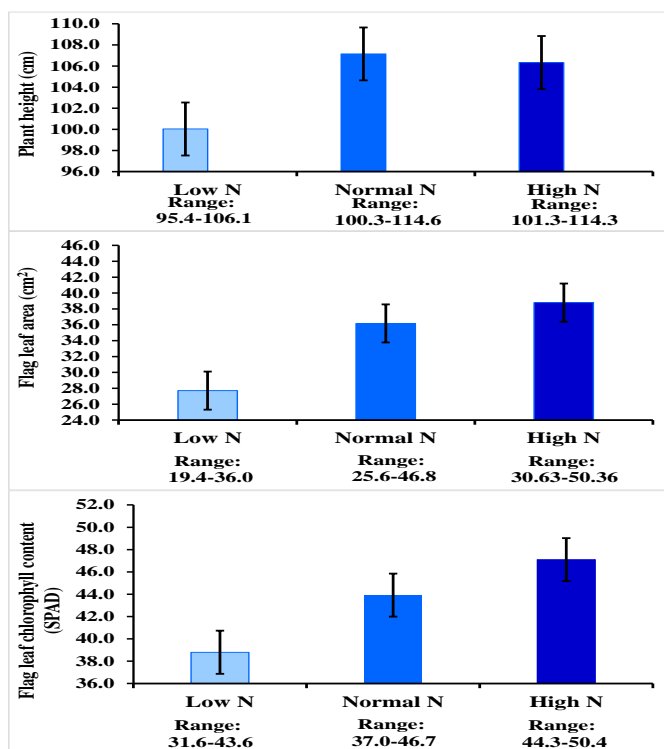
**Table (4): Observed mean squares due to parents and F<sub>1</sub>'s for the studied characters of wheat genotypes in each and across N-levels**

S.O.V	df	Earliness characters			Morpho-physiological characters			Spike characters			Yield and its component characters						Protein content		
		DH	DM	SGD	PH	FLA	FLCC	SL	NFSS	NSSS	NSP	NGS	1000 GW	GYP	SYP	BYP	GPC	SPC	
N-Levels	2	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	**	ns	ns
Reps/(N-Levels)	6	ns	ns	Ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Genotypes	20	**	**	**	**	**	**	**	ns	**	**	**	**	**	**	**	*	*	
Parents	5	**	**	**	**	**	**	**	**	**	**	**	**	**	ns	**	**	*	
F <sub>1</sub> 's	14	**	**	**	**	**	**	**	**	**	ns	**	**	**	**	**	**	**	
P. Vs. F <sub>1</sub>	1	ns	**	Ns	ns	**	ns	ns	ns	**	ns	**	ns	ns	ns	**	**	**	
G×N	40	ns	*	Ns	ns	**	ns	ns	ns	*	ns	**	**	ns	ns	ns	*	*	
Parents×N	10	**	*	Ns	ns	**	ns	ns	ns	*	ns	**	*	ns	ns	*	**	**	
F <sub>1</sub> 's×N	28	*	ns	Ns	ns	**	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	*	*	
P. Vs. F <sub>1</sub> ×N	2	ns	ns	**	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	**	
Error	120	1.17	1.81	2.02	45.5	16.90	6.01	0.38	0.39	0.17	0.63	5.94	5.68	5.37	18.12	18.12	0.07	0.06	

Ns,\* and \*\* indicate not significant, significant and highly significant at 0.05 and 0.01 probability levels, respectively. SOV, source of variance; df, degrees of freedom. DH, days to heading; DM, days to maturity; SGD, stay green duration; PH, plant height; FLA, flag leaf area; FLCC, flag leaf chlorophyll content; SL, spike length; NFSS, number of fertile spikelets/spike; NSSS, number of sterile spikelets/spike; NSP, number of spikes/plant; NGS, number of grains/spike; 1000 GW, 1000 grain weight; GYP, grain yield/plant; SYP, straw yield/plant; BYP, biological yield/plant; GPC, grain protein content; SPC, straw protein content.



**Fig. 1** Performance of earliness characters under three nitrogen levels across genotypes



**Fig. 2** Performance of morpho-physiological characters under three nitrogen levels across genotypes



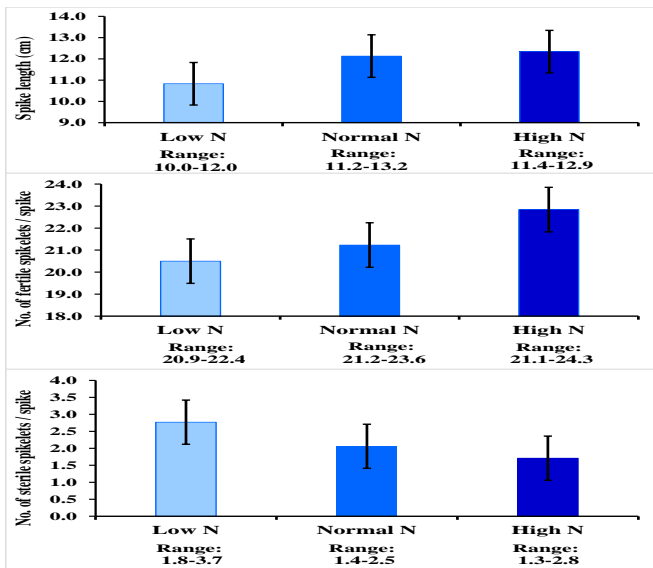


Fig. 3 Performance of spike characteristics under three nitrogen levels across genotypes

**Yields and its components:**

As illustrated in Fig. 4, GYP and 1000 GW tended to increase from (17.11 and 48.31g.) to (22.86 and 63.65g.) with increasing nitrogen levels from low

to normal and valued (22.16 and 54.08 g.) under high N-level, respectively. Moreover, SYP and BYP as well as other yield components tended to increase with increasing nitrogen levels from low, normal to high N-level. Such increase may be due to the important role of nitrogen in stimulating assimilation activities and hence vegetative growth which reflected in enhancing yield characters. **Yousaf et al. (2021)** showed that N affects all levels of plant function from metabolism to resource allocation, growth, and development. Numerous reports on genotypic variation of yield characters, already suggest potential application of this genetic knowledge for wheat improvements in bread wheat (**Brancourt-Hulmel et al. 2005; Laschenberger et al. 2008; Wolf et al., 2008; Gorny et al., 2011; Al-Naggar et al., 2017; Tahir et al., 2020**).

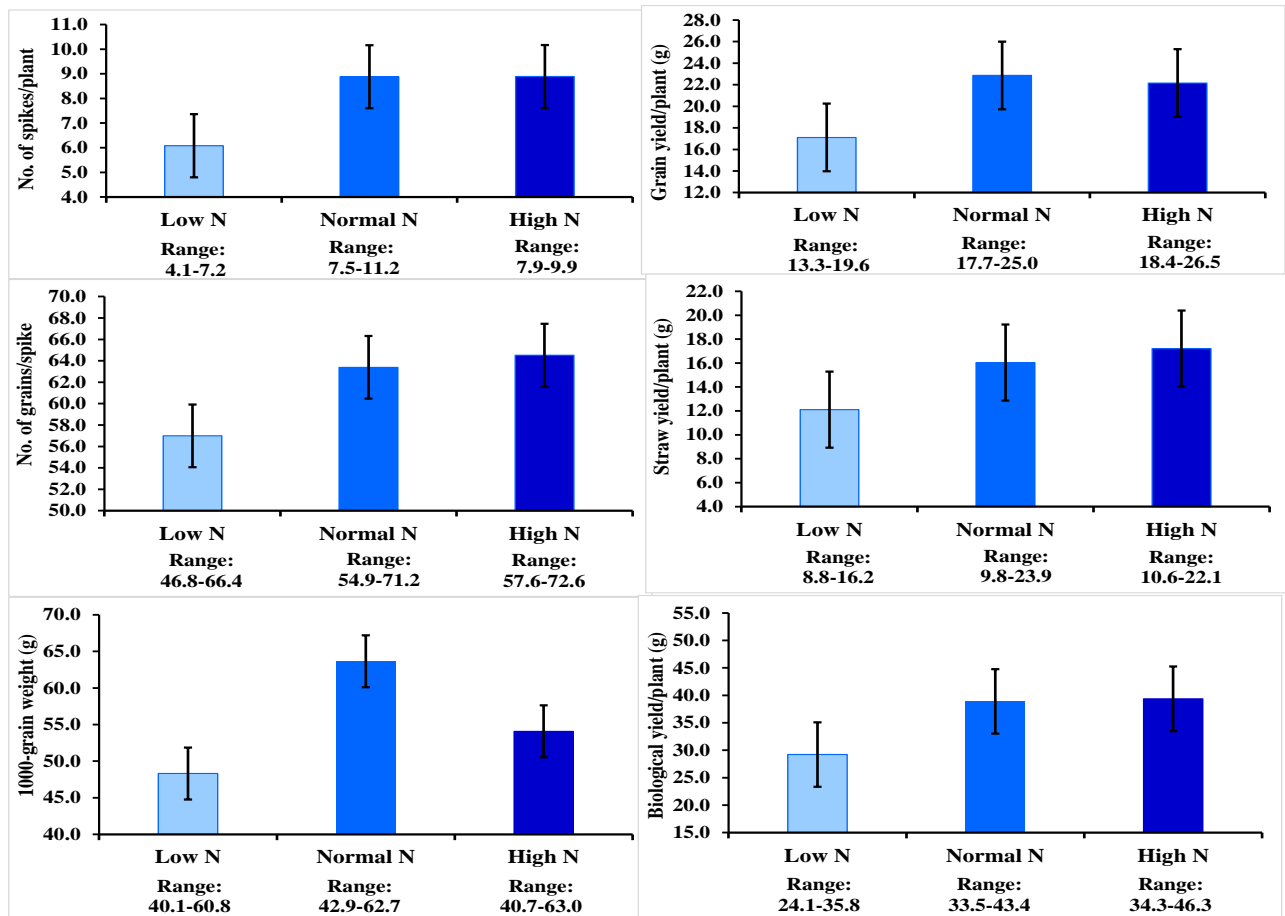
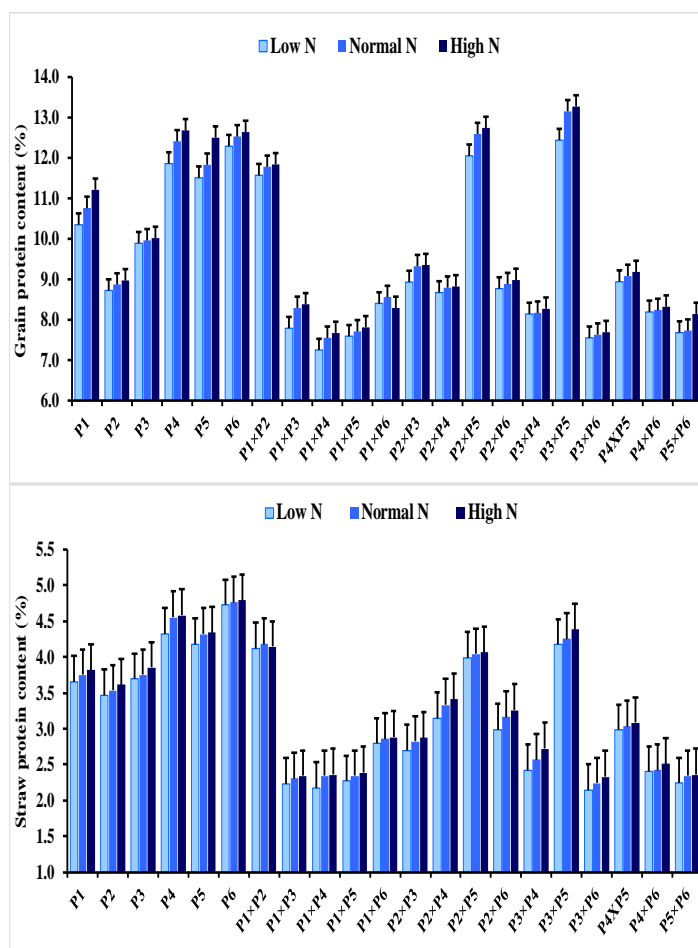


Fig. 4 Performance of yields and its components under three nitrogen levels across genotypes

### Protein content and N-uptake:

Results illustrated in Figs. 5 and 6 showed significant differences between parental wheat genotypes and their F<sub>1</sub> crosses for both grain and straw protein contents and N-uptake under different N-fertilization levels. However, insignificant effect was detected for nitrogen levels for both grain and straw protein contents. Whereas, significant interaction was observed between genotypes x N-levels (Table 4), hereby wheat genotypes differed in their response to N-levels. The parental cultivars P<sub>4</sub>, P<sub>5</sub> and P<sub>6</sub> and

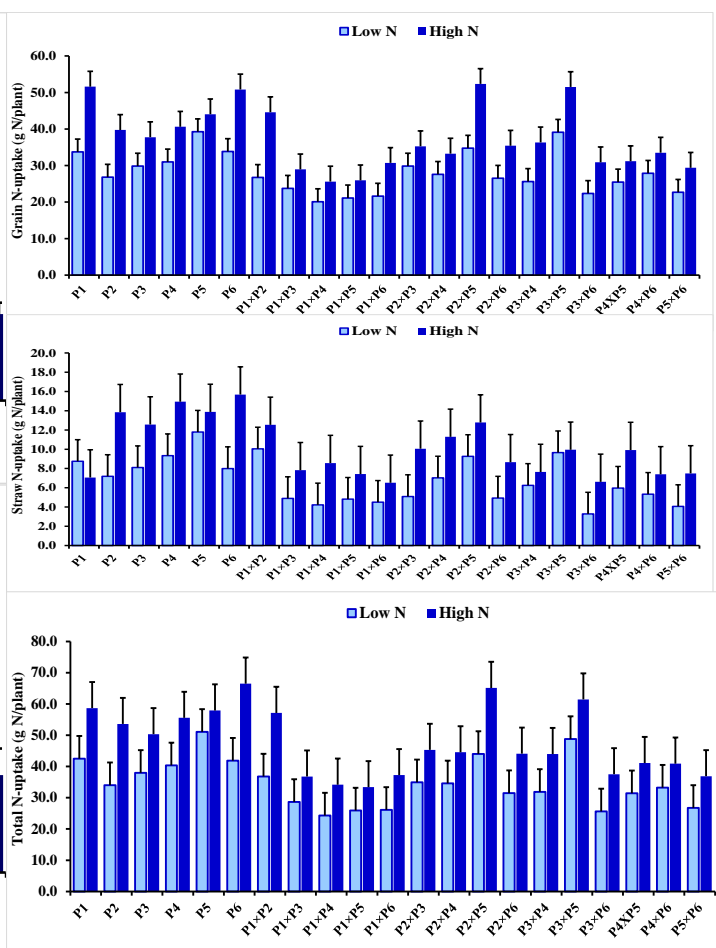
their respective crosses P<sub>2</sub> x P<sub>5</sub> and P<sub>3</sub> x P<sub>5</sub> produced the highest grain and straw protein contents under the three N-levels. Also, they gave the highest total N-uptake under low and high N-levels. Thus, abovementioned genotypes could be used for isolating new recombinations with high protein content and more efficient in N-uptake. **El-Moselhy, Omnya (2009)** recorded high degree of genetic variability among six bread wheat genotypes and their F<sub>1</sub> crosses in grain protein content, as well as by **Khalilzadeh *et al.* (2011)** for N-uptake efficiency.



**Fig. 5** Performance of grain and straw protein contents under three N-levels for parental wheat genotypes and their F<sub>1</sub>

### Nitrogen use efficiency components

N-use efficiency components were determined for six parental wheat genotypes and their F<sub>1</sub> crosses based on low (available soil nitrogen, 40.5 Kg N fad) and high level (40.5 available + 70 Kg

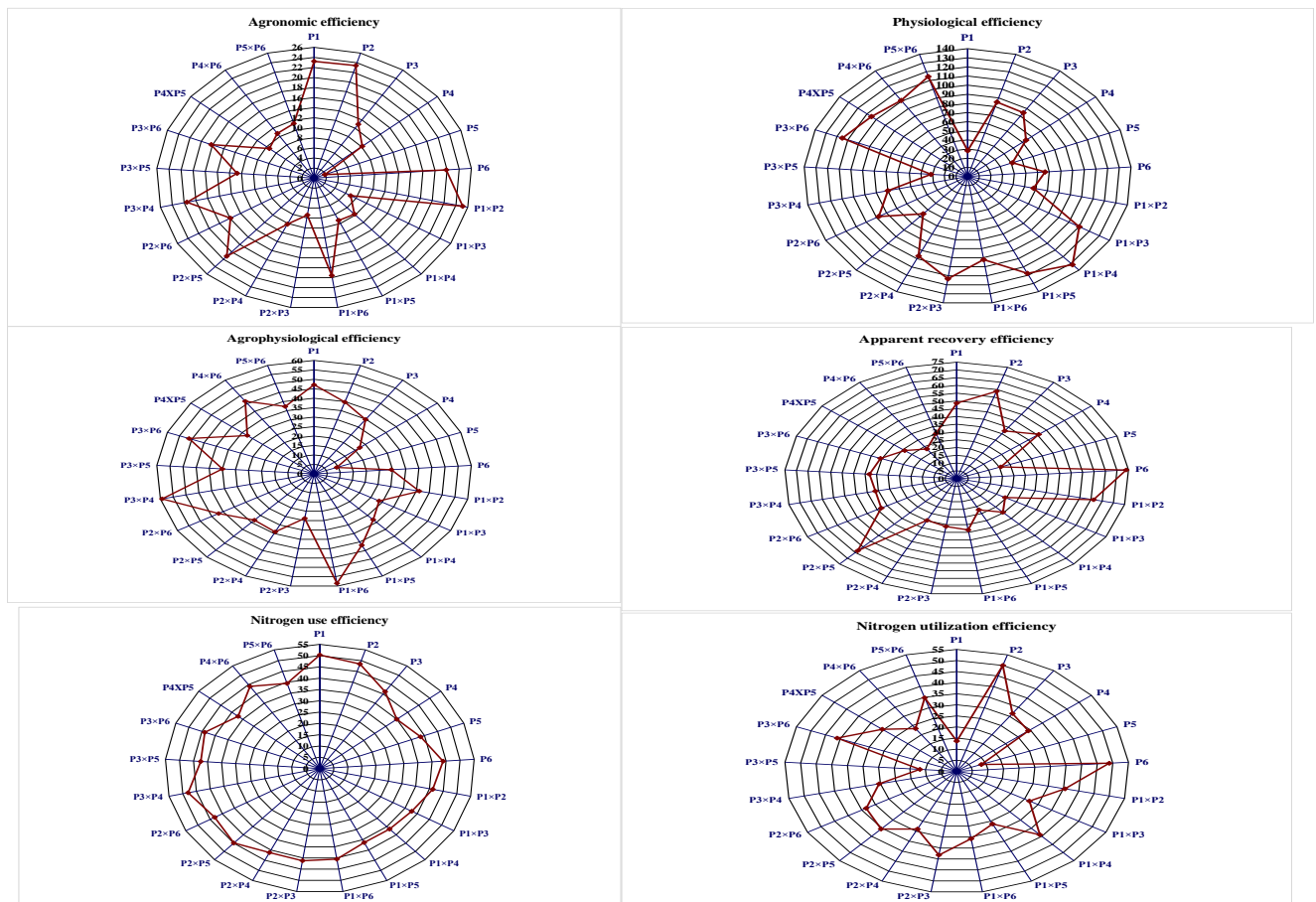


**Fig. 6** Performance of N-uptake under low and high N-levels for parental wheat genotypes and their F<sub>1</sub> crosses.

N/fad applied). As shown in Fig 7. Significant differences were detected between parents and their F<sub>1</sub>'s for nitrogen efficiency components. It is quite clear that the most excellent wheat

genotypes exhibited desired estimates of N-efficiency components were P<sub>2</sub>, P<sub>3</sub> and their F<sub>1</sub> crosses P<sub>1</sub> × P<sub>3</sub>, P<sub>1</sub> × P<sub>4</sub>, P<sub>1</sub> × P<sub>5</sub>, P<sub>1</sub> × P<sub>6</sub>, P<sub>2</sub> × P<sub>3</sub>, P<sub>2</sub> × P<sub>4</sub>, P<sub>2</sub> × P<sub>6</sub>, P<sub>3</sub> × P<sub>6</sub>, P<sub>4</sub> × P<sub>5</sub>, P<sub>4</sub> × P<sub>6</sub> and P<sub>5</sub> × P<sub>6</sub> for PE; P<sub>1</sub>, P<sub>2</sub>, P<sub>4</sub>, P<sub>6</sub> and their F<sub>1</sub> crosses P<sub>1</sub> × P<sub>2</sub> and P<sub>2</sub> × P<sub>5</sub> for ARE; P<sub>1</sub>, P<sub>2</sub> and their F<sub>1</sub> crosses P<sub>1</sub> × P<sub>2</sub>, P<sub>1</sub> × P<sub>5</sub>, P<sub>1</sub> × P<sub>6</sub>, P<sub>2</sub> × P<sub>6</sub>, P<sub>3</sub> × P<sub>4</sub>, P<sub>3</sub> × P<sub>6</sub> and P<sub>4</sub> × P<sub>6</sub> for APE; P<sub>1</sub>, P<sub>2</sub>, P<sub>6</sub> and their F<sub>1</sub> crosses P<sub>1</sub> × P<sub>2</sub>, P<sub>2</sub> × P<sub>5</sub> and P<sub>3</sub> × P<sub>4</sub> for AE; P<sub>2</sub>, P<sub>6</sub> and the F<sub>1</sub> crosses P<sub>1</sub> × P<sub>2</sub>, P<sub>1</sub> × P<sub>4</sub>, P<sub>2</sub> × P<sub>3</sub>, P<sub>2</sub> × P<sub>5</sub> and P<sub>3</sub> × P<sub>6</sub> for NUtE as well as wheat parents P<sub>1</sub>, P<sub>2</sub>, P<sub>6</sub> and the F<sub>1</sub> crosses P<sub>2</sub> × P<sub>5</sub>, P<sub>2</sub> × P<sub>6</sub>, P<sub>3</sub> × P<sub>4</sub>, P<sub>3</sub> × P<sub>6</sub> and P<sub>4</sub> × P<sub>6</sub> for NUE. Whereas, the remaining genotypes were less efficient. It should be illustrated that the quantity of changes due to low N-stress in N-use efficiency components and other studied characters were mainly genotype-dependent. These results clearly indicate that the parental wheat genotypes P<sub>1</sub>, P<sub>2</sub> and P<sub>6</sub> and their F<sub>1</sub> crosses

P<sub>2</sub> × P<sub>5</sub>, P<sub>3</sub> × P<sub>4</sub> and P<sub>4</sub> × P<sub>6</sub> made more efficient use of each observed amount of nitrogen uptake for grain and straw yield. Previous results are in agreement with the results beforehand reported in wheat by **Le Gouis et al. (2002)** and **Mansour et al. (2017)**. Thus, effective use of N helps to reduce the cost of N-fertilizer inputs and minimize nitrate contamination (**Yousaf et al., 2021**). **Ivić et al. (2021a)** recorded significant differences among 48 wheat genotypes under two comprised N-fertilization levels, 0 kg N ha<sup>-1</sup> (LN) and 100 kg N ha<sup>-1</sup> (HN) for PH, GY, GPC, Grain N-yield and harvest index and higher at HN compared to LN. Whereas NUE, NUtE and Biomass production efficiency were reduced at HN. The significant differences between the deliberated genotypes of a diallel crosses in all studied characters represent a prerequisite for identifying the most important criteria and inheritance of the studied characters.



**Fig. 7.** Nitrogen use efficiency components of six parental bread wheat genotypes and their F<sub>1</sub> crosses. P<sub>1</sub>: Line 1, P<sub>2</sub> Line 2, P<sub>3</sub>: Line 3, P<sub>4</sub> Gemmeiza 10, P<sub>5</sub>: Sakha 93, P<sub>6</sub>: Giza168, H1: P<sub>1</sub> × P<sub>2</sub>, H2: P<sub>1</sub> × P<sub>3</sub>, H3: P<sub>1</sub> × P<sub>4</sub>, H4: P<sub>1</sub> × P<sub>5</sub>, H5: P<sub>1</sub> × P<sub>6</sub>, H6: P<sub>2</sub> × P<sub>3</sub>, H7: P<sub>2</sub> × P<sub>4</sub>, H8: P<sub>2</sub> × P<sub>5</sub>, H9: P<sub>2</sub> × P<sub>6</sub>, H10: P<sub>3</sub> × P<sub>4</sub>, H11: P<sub>3</sub> × P<sub>5</sub>, H12: P<sub>3</sub> × P<sub>6</sub>, H13: P<sub>4</sub> × P<sub>5</sub>, H14: P<sub>4</sub> × P<sub>6</sub> and H15: P<sub>5</sub> × P<sub>6</sub>

**Nitrogen tolerance stress indices:**

A stress resilience index (SRI) formula has been devised in this search by the Author H.A. Awaad to classify wheat genotypes into three classes, the first class is "Tolerant", when the genotype displayed SRI value significantly less than unity (SRI <1.0), the second class is "Moderate tolerant", when the genotype value does not deviate significantly from the unity (SRI = or approached near 1.0). The third class is "Sensitive" when the genotype has an SRI value significantly greater than unity (SRI > 1.0). As shown in Table (5) the lowest values of SRI index deviated significantly from unity (SRI <1.0) were attained by P<sub>1</sub>, P<sub>5</sub>, P<sub>1</sub> x P<sub>4</sub>, P<sub>1</sub> x P<sub>5</sub>, P<sub>3</sub> x P<sub>4</sub>, P<sub>3</sub> x P<sub>5</sub> and P<sub>5</sub> x P<sub>6</sub>, hence, they are considered as tolerant to N-deficiency stress. The other indices took a similar trend with SRI, where the lowest values of

SSI, TOL and R% and the highest values of N-T, RP, MP, HM, indicate the tolerance of previous genotypes to N-deficiency stress. While, genotypes P<sub>3</sub>, P<sub>4</sub>, P<sub>6</sub>, P<sub>1</sub> x P<sub>3</sub>, P<sub>2</sub> x P<sub>3</sub>, P<sub>2</sub> x P<sub>4</sub>, P<sub>2</sub> x P<sub>6</sub>, P<sub>3</sub> x P<sub>6</sub> and P<sub>4</sub> x P<sub>6</sub> had values does not deviate significantly from unity (SRI = 1.0), and considered as moderate tolerant to N-deficiency stress, which agreed strongly in its denotations with the indices SSI, TOL, R%, NT-T, RP, MP and HM. Whereas, P<sub>2</sub>, P<sub>1</sub> x P<sub>2</sub>, P<sub>1</sub> x P<sub>6</sub>, P<sub>2</sub> x P<sub>5</sub> and P<sub>4</sub> x P<sub>5</sub> exceeded significantly the unity (SRI > 1.0) with highest values of SSI, TOL and R% and the lowest N-T, RP, MP and HM, emphasizes their sensitivity to N-deficiency stress. In this respect, **Aga et al. (2021 and Ivi'c et al. (2021b)** computed stress tolerance indices and recorded substantial differences among wheat genotypes in their tolerance to N-deficiency stress.

**Table (5) Nitrogen tolerance stress indices of six parental bread wheat genotypes and their F1 crosses under low and normal N-levels**

Index Genotype	SRI	SSI	TOL	R %	NT	RP	MP	HM
P1 (Line 1)	0.32**	0.29	1.53	7.55	0.92	1.25	19.51	19.47
P2 (Line 2)	1.38**	1.08	6.55	27.02	0.73	0.98	20.97	20.45
P3 (Line 3)	0.89ns	0.76	4.21	19.52	0.80	1.08	19.47	19.24
P4 (Gemmeiza 10)	0.86ns	0.83	4.10	21.43	0.79	1.06	17.08	16.83
P5 (Sakha 93)	-0.04**	-0.04	-0.21	-1.08	1.01	1.36	19.52	19.51
P6 (Gizal68)	0.86ns	7.00	4.09	20.52	0.79	1.07	0.27	17.65
P1XP2	1.37**	1.28	6.53	32.95	0.67	0.90	16.56	15.91
P1XP3	1.23	1.33	5.85	25.01	0.75	1.01	20.47	20.05
P1XP4	0.37**	0.38	1.74	9.84	0.90	1.22	16.81	16.76
P1XP5	0.75*	0.71	3.56	18.19	0.82	1.10	17.787	17.60
P1XP6	1.87**	1.46	8.90	37.57	0.62	0.84	19.24	18.21
P2XP3	1.07	0.83	5.06	20.84	0.79	1.07	21.75	21.46
P2XP4	0.91ns	0.76	4.30	19.03	0.81	1.09	20.45	20.22
P2XP5	1.60**	1.25	7.60	31.43	0.69	0.92	20.38	19.67
P2XP6	1.17ns	0.94	5.57	24.26	0.76	1.02	20.18	19.79
P3XP4	0.68**	0.59	3.21	15.06	0.85	1.14	19.71	19.57
P3XP5	0.08**	0.08	0.37	2.01	0.98	1.32	18.27	18.26
P3XP6	1.15	0.94	5.45	24.24	0.76	1.02	19.76	19.38
P4XP5	1.71**	1.32	8.13	33.16	0.67	0.90	20.46	19.65
P4XP6	1.14	0.86	5.42	21.68	0.78	1.06	22.29	21.96
P5XP6	0.79*	0.71	3.79	18.25	0.82	1.10	18.88	18.68
Mean	1.08	1.09	4.56	20.40	0.80	1.07	18.56	19.06

SRI, stress resilience index; SSI, stress sensitivity index; TOL, tolerance index; R%, reduction %; NT, nitrogen tolerance; RP, relative performance; MP, mean productivity; HM, harmonic mean.

**Correlation, path and factor analyses:****Correlation:**

Relationships among phenological, morpho-physiological, spike characteristics, protein, yield characters, NUtE, SRI and NUE, were computed under low N-input (Table 6). Earliness characters *i.e.* DH and DM showed positive and significantly correlation with each other, and with each of NFSS and NGS, but negatively and significantly correlated with each of PH, SL and 1000 GW. Meanwhile, FLA appeared to be negatively and significantly associated with DH and FLCC. A positive and significant correlation was recorded between SGD and NGS. Therefore, the delay in phonological characters led to an increase the associated characters and vice versa. Positive and significant association was found between grain yield and each of DH, FLCC and FLA (Awaad 2001).

Spike length as a visual selection criterion for wheat yield was positive and significant associated with each of PH, 1000 GW and NUE, but the direction of correlation was negative and significant with SGD. Meanwhile, FLCC was positively associated with NFSS and SPC. A positive and significant correlation was also verified between PH and each of SL, 1000 GW and NUtE, while negatively and significantly associated with NSP, pointing out that the short-statured varieties are distinguished by their high tillering capacity. There was a positive and significant relationship between NGS and DH, DM, SGD and NFSS and NSP. A positive and significant correlation was also proved between 1000 GW and each of PH and SL, while negatively and significantly associated with SGD, NSP, NFSS. BYP seemed to be significantly associated with each of GYP and SYP. While the

relationship was positive and significant between SPC and both FLCC and GPC. A positive and significant correlation was verified between NUE and SL. The significant positive correlation indicates the importance of SL in improving NUE. Negative and significant association was evidenced between SRI and each of GYP, SYP and BYP, indicates that the low SRI genotype is more tolerant to N-deficiency stress, represented by grain, straw and biological yields. Furthermore, significantly positive association was also observed between NUtE with each of PH, NSSS and SRI, but negative and significant with SYP and BYP. The significant positive correlation indicates the importance of correlated characters in improving NUE and vice versa. Meanwhile, Kichey *et al.* (2007) found that grain yield was moderately correlated with chlorophyll contents ( $r = 0.64$  and  $0.73$ ). High correlation ( $r = 0.85$ ) was recorded between grain-N content and N-uptake after anthesis. Between wheat grain yield and each of FLA, SL, NGS and GW, positive relationship was observed, while number of tillers/plant gave negative and significant association with grain yield by Iftikhar *et al.* (2012). Mandic *et al.* (2015) detected moderate positive correlations between PH with NGS; SL with NSS and WGS; NSS with NGS, 1000 GW, NS m<sup>2</sup> and GY; TGW with NS m<sup>2</sup> and N-agronomic efficiency NAE, as well as between NAE with NUE. Ivić *et al.* (2021a) recorded strong-to-very-strong negative correlations ( $r \geq -0.60$ ) between GY and each of PH, GPC, and NUE at both LN and HN levels. GY is intrinsically in positive linear relationship with NUE. A moderate positive relationship was observed between NUE and N-uptake efficiency under LN ( $r = 0.49$ ).

Table (6): Correlation coefficients between the studied characters under low N- input

L1	DH	DM	SGD	PH	FLA	FLCC	SL	NFSS	NSSS	NSP	NGS	1000 GW	GYP	SYP	BYP	GPC	SPC	SRI	NUE
<b>DH</b>	1																		
<b>DM</b>	0.82**	1																	
<b>SGD</b>	0.78**	0.80**	1																
<b>PH</b>	-0.70**	-0.53*	-0.37	1															
<b>FLA</b>	-0.53*	-0.41	-0.37	0.32	1														
<b>FLCC</b>	0.38	0.26	0.20	-0.10	-0.45*	1													
<b>SL</b>	-0.64**	-0.58**	-0.46*	0.73**	0.27	-0.03	1												
<b>NFSS</b>	0.47*	0.45*	0.36	-0.02	-0.28	0.47*	-0.34	1											
<b>NSSS</b>	-0.21	-0.32	-0.17	0.29	0.09	-0.04	0.16	-0.02	1										
<b>NSP</b>	0.40	0.43	0.40	-0.44*	-0.07	0.36	-0.26	0.35	-0.11	1									
<b>NGS</b>	0.69**	0.78**	0.70**	-0.35	-0.22	0.27	-0.32	0.60**	-0.40	0.49*	1								
<b>1000GW</b>	-0.64**	-0.69**	-0.48*	0.67**	0.17	-0.04	0.50*	-0.22	0.41	-0.55**	-0.75**	1							
<b>GYP</b>	-0.20	-0.13	-0.01	0.03	0.18	-0.16	0.11	0.23	-0.28	0.40	0.19	-0.03	1						
<b>SYP</b>	-0.13	-0.19	-0.24	-0.36	-0.05	0.20	-0.09	-0.17	-0.32	0.17	-0.03	0.05	0.31	1					
<b>BYP</b>	-0.20	-0.20	-0.17	-0.22	0.07	0.04	0.01	0.02	-0.37	0.34	-0.03	0.02	0.77**	0.84**	1				
<b>GPC</b>	0.03	0.17	0.07	-0.09	0.03	0.32	0.14	-0.04	-0.31	0.13	0.08	-0.40	-0.15	0.39	0.18	1			
<b>SPC</b>	0.05	0.11	0.02	-0.07	-0.01	0.44*	0.19	-0.01	-0.28	0.14	0.08	0.04	-0.14	0.34	0.13	0.96**	1		
<b>SRI</b>	-0.06	-0.12	-0.13	0.36	-0.18	-0.04	0.07	0.22	0.41	-0.53*	-0.24	0.33	-0.43*	-0.45*	-0.54*	-0.21	-0.18	1	
<b>NUE</b>	-0.25	-0.30	-0.22	0.38	-0.12	0.15	0.46*	-0.17	0.42	-0.29	-0.27	0.24	-0.38	-0.12	-0.30	0.21	0.26	0.13	1
<b>NUE</b>	-0.04	-0.08	-0.01	0.45*	-0.08	0.23	0.19	0.17	0.50*	-0.33	-0.021	0.30	-0.43	-0.65**	-0.68**	-0.21	-0.10	0.55**	0.07

DH, days to heading; DM, days to maturity; SGD, stay green duration; PH, plant height; FLA, flag leaf; FLCC, flag leaf chlorophyll content; SL, spike length; NFSS, number of fertile spikelets/spike; NSSS, number of sterile spikelets/spike; NSP, number of spikes/plant; NGS, number of grains/spike; 1000 GW, 1000 grain weight; GYP, grain yield/plant; SYP, straw yield/plant; BYP, biological yield/plant; GPC, grain protein content; SPC, straw protein content; SRI, stress resilience index; NUtE, N-utilization efficiency; NUE, nitrogen use efficiency.

**Path analysis:**

To identify the relative importance of different morpho-physiological and yield characters to NUE variation, path coefficient analysis was performed under low N-level (Table 7). Results indicated that the maximum positive direct effect on NUE was perceived by SPC (0.372) followed by DM and 1000 GW (0.246) and SRI (0.144). Desirable negative direct effect on NUE was observed by SGD (-0.029). Thus, under low N-input, direct selection for previous characters might be effective for arising NUE in wheat. The negative direct effect (-0.466) of GPC may be due to the inverse relationship and dilution effect between GPC and GYP, which included in NUE. The highest positive indirect effects on NUE were often observed through GPC via SPC (0.356) followed by SGD via DM (0.196); 1000 GW via GPC (0.186), FLCC via SPC (0.164). Hence, the simultaneous selection of the previous pairs of

characters is effective in improving NUE under low N-input. Whereas, negative indirect effects on NUE were observed through DM via each of 1000 GW and SL and with values of -0.170 and -0.142, in descending order. **Abo-Elwafa et al. (2023)** indicated that the direct effects of 1000 GW, biological yield and weight of spikes/plant on grain yield were different according to the rearrangement of the genetic make-up of genotypes. So, selection for SPC, 1000 GW with relatively moderate DM, SGD and SRI should be expressed as key characters for improving NUE. Estimates of heritability together with interrelationship between low input levels have been used for the prediction of relative efficiency of direct vs. indirect selection in wheat to give recommendations for further selection programs, aimed to producing cultivars acted well under low N-input (**Cormier et al., 2013; Šarčević et al., 2014; Khalilzadeh et al., 2017**).

**Table (7) Direct (diagonal) and indirect (off diagonal) effect of some wheat characters on NUE under low N- level**

Characters	DM	SGD	FLCC	SL	1000 GW	GPC	SPC	SRI
DM	<b>0.246</b>	-0.023	-0.038	-0.012	-0.170	-0.079	0.039	0.003
SGD	0.196	<b>-0.029</b>	-0.030	-0.009	-0.118	-0.034	0.007	0.003
FLCC	0.063	-0.006	<b>-0.149</b>	-0.001	-0.010	-0.147	0.164	0.001
SL	-0.142	0.013	0.004	<b>0.020</b>	0.122	-0.067	0.070	-0.001
1000 GW	-0.170	0.014	0.006	0.010	<b>0.246</b>	0.186	0.013	-0.007
GPC	0.042	-0.002	-0.047	0.001	-0.098	<b>-0.466</b>	0.356	0.004
SPC	0.026	-0.001	-0.066	0.004	0.009	-0.446	<b>0.372</b>	0.004
SRI	-0.030	0.004	0.005	0.001	0.082	0.098	-0.065	<b>0.144</b>

DM, days to maturity; SGD, stay green duration; FLCC, flag leaf chlorophyll content; SL, spike length; 1000 GW, 1000 grain weight; GPC, grain protein content; SPC, straw protein content; SRI, stress resilience index.

**Factor analysis:**

Factor analysis is used to identify the patterns of relationship among many dependent variables, with the goal of discovering something about the nature of the independent variables that affect them, even though those independent variables were not measured directly. The inferred independent variables are called factors. So,

factor analysis is a statistical method used because of its power to detect fundamental multivariate structures (**Walton 1972**).

Results of factor analysis for the nineteen deliberated characters under low N-level are presented in Table 8. Factor analysis divided the nineteen variables into five factors accounting for

80.830% of the total variability. The first factor included nine variables (DH, DM, SGD, PH, SL, NSP, NGS, 1000 GW and NSSS) and accounted for 30.809% of total variance. Factor two comprised four variables, namely SYP, BYP, SRI, and NUtE and accounted for 20.843% of total variability. Factor three contained three variables (FLCC, GPC and SPC) and accounted for 12.360% of the total variance. The fourth factor contained two variables *i.e.* (GYP and NFSS) and accounted for 9.431% of the variability in the dependence structure. The fifth factor contains

one variable *i.e.* FLA accounted for 7.388% of total variability in the dependence structure. These results agree with those obtained by **Siahbidi et al. (2012)**, **Ebrahimnejad and Rameeh (2016)** and **Charly Emmanuel et al. (2022)**. It is clear that DH, DM, SGD, NSP and NGS of the first factor together with GYP, SYP and BYP of second factor had high loading coefficients and participate much more in NUS under low N-input. Similarly, a considerable FLA, FLCC and NUtE were important to improve NUE.

**Table (8) Summary of factor loading for some important characters of wheat under low N-level on NUE**

Variables	Loading	Percentage of total	
<b>Factor 1</b>		30.809	
DH	0.865	13.130	
DM	0.876	13.160	
SGD	0.747	11.369	
PH	-0.745	11.308	
SL	-0.621	9.426	
NSP	0.608	9.988	
NGS	0.815	12.371	
1000 GW	-0.803	12.189	
NSSS	-0.465	7.058	
<b>Factor 2</b>		20.843	
SYP	0.826	26.314	
BYP	0.888	28.289	
SRI	-0.665	21.185	
NUtE	-0.760	24.212	
<b>Factor 3</b>		12.360	
FLCC	0.641	27.558	
GPC	0.810	34.824	
SPC	0.875	37.618	
<b>Factor 4</b>		9.431	
GYP	0.611	46.820	
NFSS	0.694	53.180	
<b>Factor 5</b>		7.388	
FLA	-0.607		
<b>Cumulative variance</b>		80.830	

DH, days to heading; DM, days to maturity; SGD, stay green duration; PH, plant height; SL, spike length; NSP, number of spikes/plant; NGS, number of grains/spike; 1000 GW, 1000 grain weight; NSSS, number of sterile spikelets/spike; SYP, straw yield/plant; BYP, biological yield/plant; SRI, stress resilience index; NUtE, N-utilization efficiency; FLCC, flag leaf chlorophyll content; GPC, grain protein content; SPC, straw protein content; GYP, grain yield/plant; NFSS, number of fertile spikelets/spike; FLA, flag leaf area.

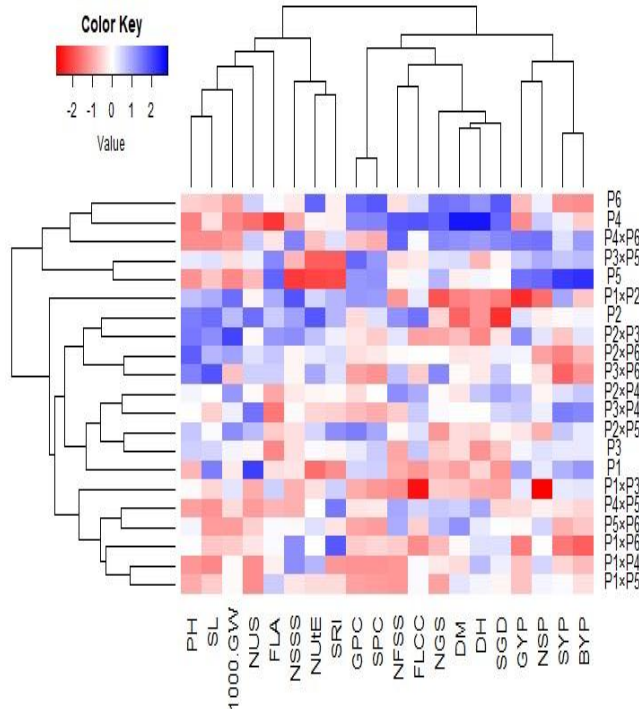
#### **Hierarchical cluster analysis heatmap:**

Hierarchical cluster analysis heatmap divided the evaluated 21 bread wheat genotypes into different

clusters based on NUE, NUtE and SRI as well as yield attributes (Fig. 8). Red and blue colors imply low and high values for the corresponding



parameters, respectively. The evaluated genotypes were allocated in four main clusters (Fig. 8).



**Fig. 8.** Hierarchical cluster analysis heatmap divide the evaluated six parental bread wheat genotypes and their F1 crosses into different clusters according to the studied characters. Red and blue colors imply low and high values for the corresponding parameters, respectively. PH, plant height; SL, spike length; 1000 GW, 1000 grain weight; NUE, N-use efficiency; FLA, flag leaf area; NSSS, number of sterile spikelets/spike; NUtE, N-utilization efficiency; SRI, stress resilience index; GPC, grain protein content; SPC, straw protein content; NFSS, number of fertile spikelets/spike; FLCC, flag leaf chlorophyll content; NGS, number of grains/spike; DM, days to maturity; DH, days to heading; SGD, stay green duration; GYP, grain yield/plant; NSP, number of spikes/plant; SYP, straw yield/plant; BYP, biological yield/plant.

The first cluster included P<sub>6</sub>, P<sub>4</sub>, P<sub>4</sub> × P<sub>6</sub>, P<sub>3</sub> × P<sub>5</sub> and P<sub>5</sub>. The second group contains P<sub>2</sub>, P<sub>2</sub> × P<sub>3</sub>, P<sub>2</sub> × P<sub>6</sub> and P<sub>3</sub> × P<sub>6</sub>. Whereas, the third group included P<sub>2</sub> × P<sub>4</sub>, P<sub>3</sub> × P<sub>4</sub>, P<sub>2</sub> × P<sub>5</sub>, P<sub>3</sub> and P<sub>1</sub>. However, the fourth group included P<sub>1</sub> × P<sub>3</sub>, P<sub>4</sub> × P<sub>5</sub>, P<sub>5</sub> × P<sub>6</sub>, P<sub>1</sub> × P<sub>6</sub>, P<sub>1</sub> × P<sub>4</sub> and P<sub>1</sub> × P<sub>5</sub>. Wheat genotype P<sub>2</sub> and P<sub>6</sub> were the best for NUE, NUtE, DH, DM, SGD, among them P<sub>2</sub> was superior for NFSS, FLCC, PH and SL, and P<sub>6</sub> for GPC and SPC. P<sub>1</sub> was the most distinguished genotype for NUE, DH, DM, SGD, GYP, SYP, BYP, SRI and

SL. The cross combinations P<sub>1</sub> × P<sub>4</sub> and P<sub>4</sub> × P<sub>6</sub> were the excellent for high NUtE, among them P<sub>1</sub> × P<sub>4</sub> was short PH and more tolerant to N-deficiency stress (SRI < 1.0), while P<sub>4</sub> × P<sub>6</sub> was high in GYP, BYP and NFSS and moderate tolerant to N-deficiency (SRI approached near 1.0). P<sub>3</sub> × P<sub>4</sub> was most distinguished in NUE, FLCC, GYP, SYP, BYP, moderate 1000 GW and tolerant to N-deficiency; P<sub>2</sub> × P<sub>5</sub> and P<sub>3</sub> × P<sub>6</sub> were moderately high for both NUE and NUtE. Wheat Line P<sub>3</sub> was moderate tolerant to N-deficiency and P<sub>5</sub> was more tolerant, and their F<sub>1</sub> cross P<sub>3</sub> × P<sub>5</sub> was more tolerant to N-deficiency, early DH, SGD, among them P<sub>3</sub> was higher in FLCC whereas, P<sub>3</sub> × P<sub>5</sub> was superior in FLA, GPC, SPC, GYP, SYP and BYP. Finally, the best tolerant genotypes to N-deficiency stress (SRI < 1.0) were P<sub>5</sub>, P<sub>3</sub> × P<sub>5</sub>, P<sub>1</sub>, P<sub>1</sub> × P<sub>4</sub>, P<sub>3</sub> × P<sub>4</sub>, P<sub>1</sub> × P<sub>5</sub> and P<sub>5</sub> × P<sub>6</sub>. It is interesting to note that parental wheat P<sub>5</sub> enjoys by the highest GYP, SYP, BYP, FLA, GPC, SPC, moderate early DH, DM, SGD, short PH with greatest NSP and tolerant to N-deficiency stress, but inefficient in NUE and NUtE. Hence, performing the hybridization between P<sub>5</sub> and one of the most efficient genotypes in NUE and NUtE, then selection in segregating generations for high yield and high N-efficiency segregants, leads to the development of new varieties that combine both advantages. Similar results were recorded by Lupini *et al.* (2021) and Sarker *et al.* (2023).

#### Assessment of genetic components and heritability:

Producing wheat genotypes with high NUE is a vital research target to reach better agricultural sustainability. NUE being complex characteristic relies on understanding of genetics, environment, management, and their interrelationships

(Prakash Raigar *et al.*, 2022) and involving various mechanisms (Gorny *et al.*, 2011; Al-Naggar *et al.*, 2017). Therefore, 6 × 6 diallel cross parents was used according to (Hayman 1954a and b) to test the types of gene action controlling NUE, NUtE, SRI and SSI.

Table (9) indicated that both additive (D) and dominance ( $H_1$  and  $H_2$ ) genetic components were highly significant for NUE, NUtE, SRI and SSI. The dominance genetic components were higher compared to the additive ones, resulting in average degree of dominance  $(H_1/D)^{0.5}$  was more than unity, confirming the importance role of over dominance gene effect in the genetic control of these parameters. In this joining, dominance genetic components were significant and greater than additive one in controlling NUtE under low N-level Gorny *et al.* (2011).

F value was positive and significant for NUE and NUtE. The overall dominance effects of heterozygous loci ( $h^2$ ) were positive and significant for NUE and NUtE, hereby dominance

was mainly attributed to heterozygous loci and seemed to be acting in the positive direction. Whereas, it was negative and insignificant for SRI and SSI. NUtE, SSI and SRI were influenced by the environmental conditions as revealed by significant E estimate. The proportion ( $H_2/4H_1$ ) was less than its maximum value (25%) for NUE, NUtE, RSI and SSI, indicating asymmetrical distribution of positive and negative alleles among parental populations.

Narrow sense heritability was moderate (30% - <50%) for NUE, NUtE and SRI, but low (8.89%) for SSI, hereby phenotypic 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 previous N-measurements. Low to moderate narrow sense heritability was detected for NUE, moderate for nitrogen uptake and utilization efficiency by Gorny *et al.* (2011) and reached 60% for NUtE by Ivić *et al.* (2021a).

**Table (9) Additive (D), dominance (H) genetic variances and their derived parameters for NUE, NUtE, SRI and SSI**

Characters Parameters	NUE	NUtE	SRI	SSI
D ± SE	248.26±40.25**	512.17±2.42**	0.248±0.12**	0.063±0.03*
$H_1$ ± SE	420.12±130.41**	3820.18±3.75**	0.319±0.16*	0.432 ±0.02**
$H_2$ ± SE	380.27±181.68**	2690.08±4.82**	0.224±0.11*	0.342±0.03**
F ± SE	216.42±139.37*	980.89±1.85**	0.058±0.21	0.181±0.24
$h^2$ + SE	165.62±82.04*	75.13±0.19**	-0.120±0.15	-0.045±0.04
E + SE	4.22±14.62	8.41±1.21**	0.268±0.13*	0.086±0.04*
Derived parameters				
$(H_1/D)^{0.5}$	1.30	2.73	1.134	3.674
$H_2/4H_1$	0.23	0.18	0.176	0.19
KD/KR	2.98	1.82	4.225	3.431
$h^2n\%$	26.53	32.69	36.76	8.89

\*, \*\* Significant at 0.05 and 0.01 levels of probability. NUE, nitrogen use efficiency; NUtE, N-utilization efficiency; SRI, stress resilience index; SSI, stress sensitivity index.

**Abbreviations:** DH, days to heading; DM, days to maturity; SGD, stay green duration; PH, plant height; FLA, flag leaf; FLCC, flag leaf chlorophyll content; SL, spike length; NFSS, number of fertile spikelets/spike; NSSS, number of sterile spikelets/spike; NSP, number of spikes/plant; NGS, number of grains/spike; 1000 GW, 1000 grain weight; GYP, grain yield/plant; SYP, straw yield/plant; BYP, biological yield/plant; GPC, grain protein content; SPC, straw protein content; AE, agronomic efficiency; PE, physiological efficiency; APE, Agro physiological efficiency; ARE, apparent recovery

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