Original Research Article

Study the Effect of Nitrogen Fertilization Rates on Grain Yield and Some Physiological Characteristics for Wheat Varieties

ABSTRACT

The study goals were to assess the impact of various nitrogen (N) application rates on the grain yield and some physiological traits of four wheat cultivars as well as determine the relationship between these traits across the experimental factors. Four Egyptian bread wheat varieties (Sakh 93, Gemmiza 12, Seds 12, and Sakh 94) with four nitrogen rates were applied: control (N0), 75 kg N fad^{-1} (N1), 100 kg N fad^{-1} (N2), and 125 kg N fad^{-1} (N3) were used in two experimental fields during the 2020/2021 and 2021/2022 growing years at Sakha, Kafr El-Sheikh Governorate, Egypt. Grain yield (kg ha-1), relative water content (RWC) proline content, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) are the traits under study. The grain yield and the majority of the physiological parameters assessed were significantly influenced by the years, nitrogen treatments, and varieties as well as their first and second-order interactions ($p \leq 0.05$ or 0.01), according to Three-way ANOVA. Grain yield increased with N treatments increased, and N3 treatment was higher than N0, N1 and N3 treatments with values of 31.1%, 22.9%, and 10.6%, respectively. With increased N application there is a tendency for MDA and SOD to decrease and then increase, while RWC, proline content, CAT, and ABX to increase and then decrease. The highest values were recorded for grain yield, proline and CAT by Sakh 94 variety and for MDA, SOD, CAT and ABX by Seds 12 variety. According to the interactions among experimental factors, the highest wheat yield and the majority of physiological measures were observed by Sakha 94 variety with N3 rate under the second growing year. The Sakh 94 variety shows a positive correlation with grain production and the majority of physiological parameters under applying N3 treatment during the second growing year based on all variables examined by principal component analysis. Finally, our results recommended using the Sakha 94 variety with 125 kg N fad^{-1} to increase and improve wheat productivity.

Keywords: Years, Nitrogen Treatments, Varieties, Grain yield, Physiological traits, PCA, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a flowering and herbaceous plant and is a member of the Poaceae family. It is the second most extensively cultivated crop and one of the most significant food cereal crops in the world (Wang et al. 2021). In January 2023, the area, yield, and production of barley were 220.01 million ha, 3.55 metric tons ha^{-1}, and 781.31 million metric tons in the world, respectively, with increased production from the previous year of 0.26% (USDA 2023).

A sufficient supply of nitrogen (N) is essential to ensuring good crop yields because it is a crucial nutrient and a major limiting factor for crop growth and development, where wheat grain yield and quality depend on substantial inputs of nitrogen (Wang et al. 2021 and 2022). Nitrogen takes a prominent position in the metabolism of plants, where all biological processes and functions in plants are related to protein, of which nitrogen is an essential component (Alam 2014). It is taken in the form of chemical fertilizer predominantly as nitrate and ammonium, with nitrate being the predominant form in most agricultural soils (Crawford and Forde, 2002; Ivic´ et al. 2021).

Reduced N level in crops may lead to the overall decline in the physiological traits of and thus decresed crop yield (Qadeer et al. 2019). Wheat has evolved numerous morphological and physiological modifications as adaptive strategies and coping mechanisms to deal with N deficit in field conditions (Lv et al., 2020; Wang et al. 2021). According to Hitz et al. (2017), genotypes with high N use efficiency must be selected at low N levels. Therefore, testing and selection at low input levels should be a part of breeding projects that aim to create N-efficient cultivars for low-input conditions in order to maximize selection gains for grain production (Brancourt-Hulmel et al. 2005; Cormier et al. 2013). The interaction of N application with grain yield and the physiological traits of wheat must be taken into account in order to improve grain yield, which is the most frequently used indicator (Qadeer et al. 2019).

Relative water content (RWC) determines the water case in plant tissues, in which an increased RWC in tissue implies that a plant's performance is maintained under stress conditions (Rady et al. 2021). Plant tissues require a water content of 70–90% of their fresh weight in order for physiological and biochemical activities to be successful and antioxidants to function adequately (Gardner and Gardner 1983; Eid et al. 2022). Superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX), among others, are the primary enzyme antioxidants in plants that prevent adversity stress from damaging plant cell membranes (Ahmad et al. 2022; Wang et al. 2022). Positive effects on proline contents were observed by N addition (Qadeer et al. 2019). According to Liu et al. (2020), malondialdehyde (MDA) concentrations significantly increased in the wheat genotypes when they were exposed to N-deficient stress. Wang et al. (2022) reported an increased SOD level under an adequate N supply range of 0–240 kg ha^{-1}, while the activity of antioxidant enzymes decreased when the nitrogen application rate was increased to 300 kg ha^{-1}, demonstrating that an excessive nitrogen input led to diminishing grain yield of wheat. On the other hand, the drought-stressed plants showed a drop in MDA content and an increase in SOD and APX activity with increased N treatment rates (Abid et al. 2016). Qadeer et al. (2019) reported that N addition enhances proline and antioxidant enzymes in wheat plants.

Principle competent analysis (PCA) can be used to assess grain yield and physiological characteristics in connection to responses to N levels. Liu et al. (2020) in wheat and Eid et al. 2022 in cotton used PCA to assess the relationships between grain yield and physiological traits of wheat under various nitrogen applications. The current study was carried out to examine the effects of various nitrogen application rates on the performance of four wheat cultivars during the two growing seasons based on grain yield and some physiological traits of wheat, and to investigate the association between these traits across experimental factors.

MATERIALS AND METHODS

1. Climatic data of experimental region

Two experiments were carried out at a private farm in Sakha, Kafr El-Sheikh Governorate, Egypt, during the wheat growing seasons of 2020/2021 and 2021/2022. The average weather data in the region studied across the two growing seasons for wheat is given in Fig. 1. The highest and lowest values of average temperature (ºC) were recorded in May and January months in both growing season, respectively. The greatest average relative humidity (%) and precipitation (mm) in January month in both growing season were found. The lowest values of average precipitation (mm) were observed in May month in both season. While May and March months recorded the lowest values of Average relative humidity (%) during 2020/2021 and 2021/2022 growing season, respectively.

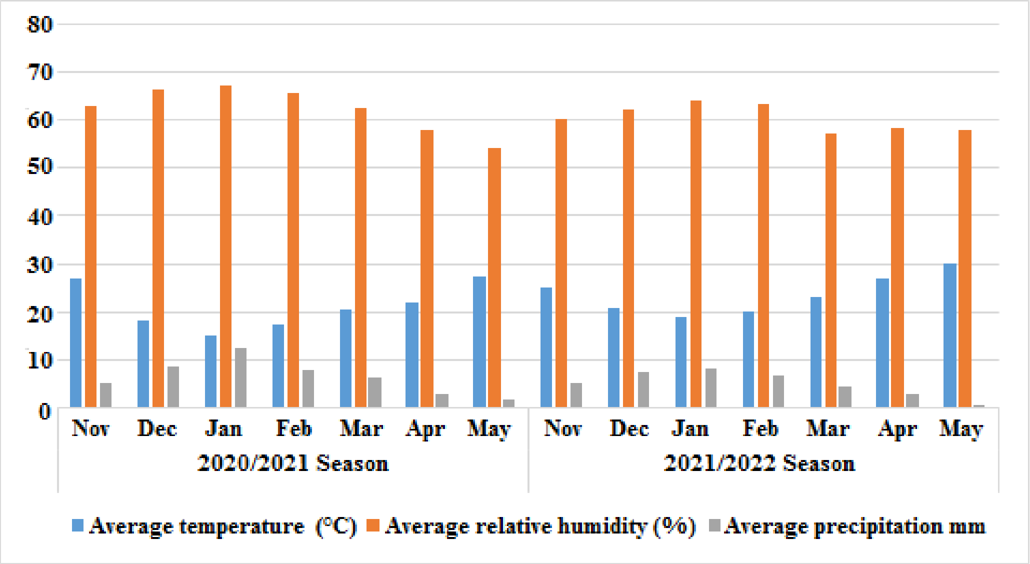

Fig. 1. The average of climatic data at Sakha, Kafr El-Sheikh Governorate, Egypt during the wheat growing season of 2020/2021 and 2021/2022.

2. Soil properties of experimental region

Before the trials began, soil samples totaling 0.5 kg were taken from the research area at depths ranging from 0-40 cm. To measure the physical and chemical properties of soil, each sample was divided into three replicates and sent to the lab in sealed tins in both the 2020/2021 and 2021/2022 growing seasons. The soil samples were air-dried, sieved, and utilized for determined the granulometric composition of the soil (sand%, silt%, clay %) according to Jackson (1967) and Rowell (1994). The physical analysis' findings are presented as a percentage of the original weight. The soil generally had the highest clay % followed by Silt % in the soil depth, indicating a clayey texture in the soil at the study location. The electrical conductivity (EC; ds/m) and pH values for each sample were measured using the soil-water paste (Jackson 1962). The soil chemical properties under study were determined Using a saturation paste devised by Tuzuner (1990). Table 1 lists the physicochemical characteristics of the soil in the research area at a depth of 0 to 40 cm. The soil and water lab at the Desert Research Center in Cairo, Egypt, evaluated the soil samples from the field region under study.

Table 1. Physical and chemical properties of the soil in the study region at a depth of 0–40 cm before sowing.

Soil Properties	Study Region		
	2020/2021	2021/2022	Mean
Physical Properties			
Clay %	57.03	56.9	56.97
Silt %	31.35	31.58	31.47
Sand %	11.62	11.52	11.57

Chemical Properties			
Organic Matter %	1.38	1.39	1.39
pH	8.19	8.19	8.19
Ec (ds m^{-1})	3.31	3.32	3.32
Total N (ppm)	518.47	520.98	519.73
Available P (ppm)	15.62	16.8	16.21
K^{+} (ppm)	15.51	16.24	15.88

3. Factors studied and Experimental design

Four broad wheat varieties including Sakh93, Gemmiza 12, Seds12 and Sakh94 were used in this study. Healthy seeds of wheat genotypes were obtained from the central administration of seeds production of the Egyptian ministry of Agriculture and land Reclamation. A split-split plot design based on a randomized complete block design with three replications was used to conduct the trials during the two growing seasons. The plot size was 3.5 x 4 m and included 13 rows of 3.5 m along, and 30 cm apart. The main plots included four levels of ammonium nitrate (33.5% N), as follows: the control (N0, no fertilizer applied), 75 kg N fad^{-1} (N1), 100 kg N fad^{-1} (N2), and 125 kg N fad^{-1} (N3). While the four types of wheat have occurred in the subplots. As prescribed, nitrogen fertilizer was given in three equal installments: one dose at after complete germination and two doses after 45 and 60 days respectively following first dose.

4. Agricultural management practices

On November 24th in the two growing seasons, grains of the four examined varieties were sown in rows at a rate of 50 kg fad-1 after one chisel plow. The field was completely prepared by plowing and harrowing then clearing up the various forms of plant waste. Basic fertilization of 150 kg P_2O_5 fad^{-1} (calcium super phosphate 15.5% P_2O_5) added during the soil preparation, while 50 kg K_2O fad-^{1} (potassium sulfate 48.5% K_2O) in one dose before first irrigation was applied. According to the prescribed agricultural procedures for the research region across the two growing seasons, all agronomic practices were carried out, and the crop was sown in a single day under uniform field conditions to reduce environmental changes to the maximum feasible extent.

5. Grain yield and physiological traits

In both seasons, bread wheat cultivars were harvested manually at full maturity on the May 10th (after 160 days from sowing). By harvesting a one-m^{2} area on each plot, grain yield was estimated and translated to kilograms per hectare (GY kg ha^{-1}). The relative water content (RWC; %) in wheat leaves samples was obtained following the equation described by Flexas et al. (2006). Proline content (mg g^{-1} FW) were determined in fresh leaves from plants at the flag leaf stage based on the procedures by Bates et al. (1973). Malondialdehyde (MDA; µmol/g FW), superoxide dismutase (SOD; mg^{-1} protein), catalase (CAT; mg^{-1} protein), and ascorbate peroxidase (APX; mg^{-1} protein) were determined following the procedures of Guo et al. (2015).

6. Statistical Approaches:

A three-way ANOVA test was performed on the measured data to identify the significant variations for the effects of the years, nitrogen levels, varieties, and their interactions. The obtained data was presented as mean ± standard error (SE). The least significant difference test (L.S.D.) at the 0.05 level of significance was used to determine mean differences between the experimental factors under study. When $p < 0.05$, differences were statistically significant. For a better understanding of the association between wheat grain yield and physiological traits across experimental factors under study, principal component analysis (PCA) was used. All statistical analyses were performed using Origin Pro 2021 version b 9.5.0.193 software.

RESULTS AND DISCUSSION

1. Three-way ANOVA

Table 2 shows the results of p-values from three-way ANOVA for the impact of two years, four nitrogen treatments, and four varieties on bread wheat grain yield and some physiological traits. The main effects of years, nitrogen treatments and varieties were all significant ($p \leq 0.05$ or 0.01) for grain yield and physiological traits, except years for MDA as well as nitrogen treatments for proline content and CAT. The first and second-order interactions of these three factors displayed statistically significant differences ($p \leq 0.05$ or 0.01) on grain yield and physiological traits under study. These findings indicate that there were sufficient variations and desirable in the four wheat varieties responses with N treatments during the two growing seasons, which may be used to increase wheat grain production. Our findings regarding grain yield and physiological traits of whaet are consistent with those of several earlier research, such as those by Abid et al. (2016), Qadeer et al. (2019), Liu et al. (2020), and Wang et al (2022). Weather often played a role in the variations between years (Delfine et al. 2005). We can therefore presume that the large variations in grain yield and physiological features of bread wheat were caused by meteorological factors and genotype variability (El-Hashash et al. 2022) as well as N treatments. Differences in genotypes can be crucial in assisting wheat breeders in creating bread wheat that is more resistant to climate change (Shew et al. 2020). Significant variations in the first- and second-order interactions for wheat grain production have been confirmed by Pačuta et al. (2021).

Table 2. Three-way ANOVA (p-values) for the impact of two years (Y), four nitrogen treatments (N) and four varieties (V) on bread wheat grain yield and some physiological traits.

Factors	GY ($kg\ ha^{-1}$)	RWC (%)	Proline ($mg\ g^{-1}$ FW)	MDA (μmol/g FW)	SOD	CAT (mg^{-1} protein)	ABX
Y	0.00^{**}	0.00^{**}	0.02^{*}	0.46^{ns}	0.00^{**}	0.00^{**}	0.00^{**}
N	0.00^{**}	0.00^{**}	0.59^{ns}	0.00^{**}	0.00^{**}	0.14^{ns}	0.00^{**}
V	0.00^{**}	0.00^{**}	0.03^{*}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}
Y x N	0.00^{**}	0.00^{**}	0.01^{*}	0.00^{**}	0.00^{**}	0.02^{*}	0.00^{**}
Y x V	0.00^{**}	0.00^{**}	0.01^{*}	0.00^{**}	0.00^{**}	0.01^{*}	0.00^{**}
V x V	0.00^{**}	0.00^{**}	0.01^{*}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}
Y x N x V	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}	0.00^{**}

GY: grain yield; RWC: relative water content; MDA: Malondialdehyde; SOD: superoxide dismutase; CAT: catalase; APX: ascorbate peroxidase. * and ** indicate statistically significant differences at $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively; ns: indicate the non-significant difference.

2. Effects of main factors

The main effects of years, nitrogen treatments and varieties on grain yield and physiological traits are presented in Table 3. Regarding the two years, 2021/2022 growing season significantly increased proline, SOD, CAT, and ABX contents, while other examined traits were higher in 2020/2021 growing season. Year-to-year variations significantly affected wheat production (El-Hashash et al. 2022). Variability in rainfall during the wheat-growing season may be the cause of the lower grain productivity (Pačuta et al. 2021). Wheat plants respond to stress conditions by changing a number of metabolic and physiological processes, according to Chandrasekar et al. (2000).

Table 3. Main effects of years, nitrogen treatments and varieties on bread wheat grain yield and some physiological traits (means ± standard error).

Factors	GY (kg ha^{-1})	RWC (%)	Proline (mg g^{-1} FW)	MDA (µmol/g FW)	SOD	CAT (mg^{-1} protein)	ABX
Seasons							
2020/2021	4227.60±147.57a	71.80±1.53a	1.57±0.04b	21.92±0.68a	19.92±0.35b	18.17±0.55b	15.73±0.31b
2021/2022	4203.15±177.19b	70.79±1.71b	1.67±0.05a	21.61±0.58a	21.10±0.54a	20.60±0.69a	16.51±0.42a
LSD at 0.05	1.66	0.21	0.08	NS	0.05	1.04	0.06
Nitrogen Treatments							
N0	2992.50±37.98d	67.58±2.54d	1.64±0.05a	23.27±0.72a	20.90±0.50a	18.68±0.89a	16.23±0.47b
N1	3572.70±56.03c	77.16±2.22a	1.62±0.05a	20.14±0.78c	20.32±0.61c	19.85±0.80a	15.87±0.46d
N2	4603.50±109.19b	68.15±1.78c	1.65±0.06a	21.95±1.03b	20.01±0.93d	20.13±1.07a	16.06±0.70c
N3	5692.80±128.09a	72.29±2.09b	1.57±0.04a	21.70±0.94b	20.82±0.49b	18.86±0.90a	16.33±0.45a
LSD at 0.05	2.35	0.29	NS	1.17	0.06	NS	0.08
Varieties							
Sakh 93	4016.10±233.01d	74.62±1.62a	1.68±0.05a	21.67±0.74b	20.79±0.44b	17.32±0.92d	15.14±0.41d
Gemmiza 12	4106.40±196.59c	74.35±2.46a	1.60±0.06ab	22.35±0.84ab	20.53±0.56c	18.20±0.88c	16.37±0.48b
Seds 12	4170.00±242.53b	63.17±1.99c	1.52±0.04b	23.09±1.22a	21.69±0.46a	21.77±0.64a	17.22±0.45a
Sakh 94	4569.00±240.44a	73.05±2.23b	1.67±0.05a	19.94±0.55c	19.05±0.93d	20.23±0.95b	15.75±0.66c
LSD at 0.05	2.35	0.29	0.12	1.17	0.06	1.47	0.08

N0: kg N fad-1; N1: 75 kg N fad-1; N2: 100 kg N fad-1; N3: 125 kg N fad-1. Factors averages followed by different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test. ns: indicate the non-significant difference. Table 2 lists the key names of the traits under study.

In comparison with the N treatments, grain yield and ABX by the N3 treatment ($p \leq 0.05$), RWC by N1 treatment ($p \leq 0.05$), MDA and SOD by N0 treatment ($p \leq 0.05$), as well as proline and CAT by N2 treatment ($p > 0.05$) were recorded the highest values. While, grain yield, RWC and CAT with N0 treatment, MDA, SOD and ABX with N1 treatment as well as Proline with N3 treatment were recorded the lowest values. One of the most important methods to control crop development traits under stress seems to be a significant increase in antioxidant activities caused by high nitrogen levels in plant leaves (Ahmad et al. 2021). Grain yield and proline content of Sakh 94 variety, RWC and Proline content of Sakh 93 variety, MDA, SOD, CAT and ABX of Seds 12 variety were significantly higher than that of other varieties studied. Alam (2014), Litke et al. (2018), Kubar et al. (2022), Staugaitis et al. (2022) and Wang et al. (2022) earlier achieved comparable results.

Generally, with the increase in the N treatments, grain yield increased in the order N3 > N2 > N1 > N0, in which N3 treatment was higher than N0, N1 and N3 treatments with values of 31.1%, 22.9% and 10.6%, respectively. Also, Sakh 94 significantly outperformed other varieties in grain yield in the order Sakh 94 > Seds 12 > Gemmiza 12 > Sakh 93 with values of 4.57%, 5.33% and 6.44%, respectively. While with increasing N application there are tendency of decreasing and then increasing for MDA and SOD, and increasing and then decreasing for RWC, proline content, CAT and ABX. According to Wang et al. (2022), with increased nitrogen application, antioxidant enzymes and grain production showed an increasing and subsequently decreasing tendency. El-Hashash et al. (2022) reported that Sakha 94 variety has a good genetic background, leading to improving grain yield under different treatments and conditions. According to Gagliardi et al. (2020), the genotypes and years each had a unique impact on the grain yield of durum wheat. The genotypes' physiological characteristics are known to reflect their strong antioxidant capacity and crop productivity; the physiological traits may be used to successfully screen the cultivar with the maximum amount of N rate across various conditions (Eid et al. 2022).

3. Effects of interactions among main factors

As demonstrated in Table 4, interactions of years x N treatments were discovered to be significant for grain yield and physiological measures under investigation. During the second year, N3 treatment for grain yield, MDA and SOD, N1 treatment for RWC and proline as well as N0 treatment for CAT and ABX were the best combined treatments, where recorded the highest values for these traits. N0 treatment for CAT and ABX, N1 treatment for proline, MDA and SOD as well as N2 treatment for RWC were recorded the lowest values for these traits under the first year. Only, N0 treatment with the second year had the lowest grain yield. Previous reports on this trend include those by Litke et al. (2018), Qadeer et al. (2019), and Boulelouah et al (2022). Generally, the grain yield increased in both years with the gradual increase in the N treatments (in the order N3 > N2 > N1 > N0), but the second year was higher than the first year. Also, the N3 treatment was higher than N0, N1 and N3 treatments with values of 27.4%, 17.6%, and 7.0% during the first year and 34.60%, 28.01% and 14.01% during the second year. Our findings are consistent with those of Ahmad et al. (2022), who found that various N rates had an impact on grain yield as well as SOD and CAT activities over the course of two growing seasons.

Table 4. Effects of years and nitrogen treatments interaction bread wheat grain yield and some physiological traits (means ± standard error).

Years	Nitrogen	GY (kg ha^{-1})	RWC (%)	Proline (mg g^{-1} FW)	MDA (µmol/g FW)	SOD	CAT (mg^{-1} protein)	ABX
2020/2021	N0	3070.20±39.61g	72.08±4.05e	1.59±0.08bd	24.89±1.17a	20.46±0.65e	16.03±1.15d	15.01±0.52f
	N1	3771.60±64.69e	74.04±2.22c	1.47±0.06cd	18.51±0.87c	19.06±0.81h	19.08±0.99bc	15.75±0.66e
	N2	4682.40±210.65c	62.78±1.56g	1.70±0.08ab	25.02±1.24a	20.83±0.72d	19.17±0.79bc	16.05±0.71c
	N3	5386.20±201.37b	78.30±2.09b	1.52±0.05c	19.25±0.95c	19.35±0.57f	18.39±1.27c	16.13±0.60cd
2021/2022	N0	2914.80±58.00h	63.09±2.63g	1.69±0.05ab	21.64±0.55b	21.34±0.76c	21.33±0.82a	17.45±0.63a
	N1	3373.80±41.86f	80.28±3.73a	1.76±0.07a	21.77±1.15b	21.58±0.78b	20.62±1.27ab	15.99±0.67c
	N2	4524.60±65.98d	73.52±2.37d	1.59±0.10bd	18.88±1.07c	19.19±1.72g	21.09±1.99ab	16.07±1.25cd
	N3	5999.40±104.73a	66.29±2.72f	1.63±0.07bc	24.16±1.29a	22.30±0.54a	19.34±1.31abc	16.52±0.69b
LSD at 0.05		3.32	0.41	0.17	1.66	0.09	2.07	0.12

Factors averages followed by different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test. ns: indicate the non-significant difference. Tables 2 and 3 lists the key names of the traits and nitrogen treatments under study, respectively.

Interactions of years and varieties had significant effect on grain yield and physiological traits (Table 5). Sakh 94 variety produced the highest grain yield and RWC in the first and second years, respectively. While the highest proline and MDA contents were observed by Sakh 93 and Seds 12 varieties during the first year, respectively. SOD, CAT, and ABX activities of the Seds 12 variety under the second year were higher than that of other interactions of years and varieties. On the other hand, significant decreases were noticed in the first year with Seds 12 variety for grain yield and Proline, Sakh 94 variety for SOD, Gemmiza 12 variety for CAT and Sakh 93 for ABX. Also, the minimum values of RWC by Seds 12 variety and MDA by Sakh 94 variety were registered during the second year compared with their values in other interactions. Generally, Sakh 94 variety in both years showed more grain yield comparatively than other varieties, but the first year was greater than the second year. Also, the Sakh 94 variety was higher than Sakh 93, Gemmiza 12, Seds 12 varieties with values of 8.6%, 6.0%, 8.7% in the first year and 4.17%, 4.66%, and 0.46 in the second year, respectively. During the interaction between genotypes and years, the genotypes' behavior varied according to the growing seasons (Gagliardi et al. 2020). RWC is a physiological indicator for determining the genotype most suited for stressful conditions since it is associated with biological membranes in plant cells (Eid et al. 2022).

Table 5. Effects of years and varieties interaction bread wheat grain yield and some physiological traits (means ± standard error).

Years	Varieties	GY (kg ha^{-1})	RWC (%)	Proline (mg g^{-1} FW)	MDA (µmol/g FW)	SOD	CAT	ABX
						(mg^{-1} protein)		
2020/2021	Sakh 93	3985.20±303.67g	74.55±2.30b	1.76±0.07a	20.45±1.29c	20.61±0.59e	15.46±0.67c	14.59±0.49e
	Gemmiza 12	4205.40±258.74d	76.16±3.63a	1.49±0.08bc	23.27±1.38a	19.23±0.64g	16.07±1.08c	16.16±0.65c
	Seds 12	3981.00±242.00h	66.56±3.49e	1.43±0.05c	23.51±1.74a	21.26±0.64c	20.79±0.96ab	16.52±0.68b
	Sakh 94	4738.80±346.12a	69.94±2.00d	1.60±0.07a	20.44±0.76c	18.59±0.73h	20.35±0.68b	15.67±0.55e
2021/2022	Sakh 93	4047.00±366.97e	74.69±2.39b	1.61±0.08ab	22.88±0.62ab	20.96±0.66d	19.18±1.57b	15.70±0.65e
	Gemmiza 12	4007.40±304.77f	72.53±3.40c	1.71±0.06a	21.44±0.94bc	21.83±0.78b	20.34±1.10b	16.58±0.72b
	Seds 12	4359.00±425.34c	59.78±1.51f	1.61±0.08ab	22.68±1.77ab	22.11±0.65a	22.75±0.79a	17.92±0.53a
	Sakh 94	4399.20±341.62b	76.16±3.88a	1.74±0.09a	19.45±0.80c	19.51±1.75f	20.11±0.81b	15.83±0.99d
LSD at 0.05		3.32	0.41	0.17	1.66	0.09	2.07	0.12

Factors averages followed by different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test. ns: indicate the non-significant difference. Table 2 lists the key names of the traits under study.

As given in Table 6, the interaction between nitrogen treatments and varieties had a significant impact on grain yield and physiological parameters under study. Compared with the other varieties, Sakh 94 variety had recorded the highest grain yield with N3 treatment, RWC and CAT with N1 treatment and proline with N2 treatment. While maximum ABX and MDA were observed by Seds 12 variety under N2 and N3 treatments. Also, the highest SOD was noticed in Gemmiza 12 variety with N2 treatment. On the other hand, the effect of nitrogen treatments and varieties interaction showed that the lowest grain yield was observed in Sakh 93 variety with N0 treatment. Under N0 and N3 treatments, Seds 12 variety displayed the lowest levels of RWC and proline content, respectively. Sakh 94 variety produced the lowest MDA with N1 treatment as well as SOD, CAT and ABX with N2 treatment. Compared to low N levels, high N rates considerably boosted grain yield, SOD, and CAT activities in the cultivars that were investigated according to Ahmad et al. (2022). Generally, Sakh 94 performed better under N3 treatment with values ranged from 1.9% to 12.1% increase in grain yield compared to other nitrogen treatments and varieties interactions. The interaction effects of N treatments and varieties were evaluated by Borzouei et al. (2014 and 2020) and Yousuf et al. (2017). The antioxidant enzyme activities of nitrogen-efficient varieties were found to be higher than those of nitrogen-inefficient varieties with an increase in nitrogen, according to research by Yousuf et al. (2017). N rates effects on varieties studied led to increased grain yields of these varieties (Borzouei et al. 2020). Increased nitrogen treatment was crucial in helping wheat cultivars resist the effects of salinity on antioxidant enzymes and yield (Borzouei et al. 2014). Guo et al. (2011) and Liu et al. (2020) stated that some physiological measures, such as MDA, may serve as valuable markers to assess the sensitivity or tolerance of wheat genotypes to N deficiency, where significantly higher MDA contents in high N-deficiency sensitive cultivars than in low N-deficiency sensitive ones (Liu et al. 2020).

Table 6. Effects of nitrogen treatments and varieties interaction bread wheat grain yield and some physiological traits (means ± standard error).

Nitrogen	Varieties	GY (kg ha^{-1})	RWC (%)	Proline (mg g^{-1} FW)	MDA (µmol/g FW)	SOD	CAT (mg^{-1} protein)	ABX
N0	Sakh 93	2763.60±55.29p	72.07±2.26f	1.74±0.08ac	21.55±0.84cd	20.30±1.07f	16.32±1.65fh	15.02±0.79k
	Gemmiza 12	3064.80±46.18n	74.18±7.66d	1.48±0.11dg	25.75±2.14a	20.21±1.14g	17.08±2.45fg	15.51±0.91j
	Seds 12	2943.60±28.48o	54.83±0.95l	1.55±0.09cdg	22.87±1.29bc	22.36±0.98b	20.03±1.11cde	16.82±0.98e
	Sakh 94	3198.00±29.24m	69.25±2.85h	1.80±0.07ab	22.90±0.77bc	20.73±0.73e	21.30±1.06ade	17.57±0.94d
N1	Sakh 93	3512.40±68.17k	78.48±1.01b	1.68±0.08ad	19.59±0.99de	21.58±0.85c	16.00±0.97gh	14.92±0.73k
	Gemmiza 12	3534.00±36.86j	73.75±5.46de	1.62±0.15bcd	22.94±1.01bc	18.88±0.73i	19.14±1.09cdef	16.23±0.99g
	Seds 12	3404.40±43.49l	70.53±3.78g	1.69±0.12ad	19.98±2.39de	20.88±0.74e	20.62±1.65bcd	15.80±0.88i
	Sakh 94	3840.00±37.34i	85.88±4.36a	1.48±0.08dg	18.04±1.03e	19.95±2.05h	23.65±0.99a	16.53±0.95f
N2	Sakh93	4027.20±29.15h	74.72±3.52d	1.64±0.18adf	25.14±1.04ab	20.02±0.87h	22.01±2.21ac	16.26±0.98g
	Gemmiza 12	4306.80±26.08g	73.60±2.80de	1.64±0.08adf	20.00±1.41de	23.09±1.01a	20.68±1.67be	17.77±0.90c
	Seds 12	4945.20±78.90f	60.76±2.38k	1.44±0.07efg	22.58±3.49bd	22.24±0.86b	23.00±0.95ab	18.25±0.75a
	Sakh 94	5134.80±87.30e	63.52±1.66j	1.86±0.12a	20.09±0.74df	14.71±2.20j	14.83±2.07gh	11.97±0.99m
N3	Sakh 93	5761.20±59.59b	73.20±5.02e	1.68±0.10ad	20.39±1.96df	21.25±0.75d	14.96±0.98gh	14.38±0.74l
	Gemmiza 12	5520.00±41.46c	75.86±3.77c	1.67±0.09ade	20.74±1.15be	19.95±1.02h	15.92±1.19gh	15.98±0.78h
	Seds 12	5386.80±48.90d	66.57±4.88i	1.40±0.08g	26.95±1.63a	21.27±1.12d	23.44±1.01ab	18.02±079b
	Sakh 94	6103.20±31.46a	73.55±2.79e	1.55±0.07cdg	18.75±0.77ef	20.82±1.17e	21.13±1.22ace	16.95±0.73e
LSD at 0.05		4.69	0.59	0.23	2.35	0.13	2.93	0.16

Factors averages followed by different lowercase letters in the same column indicate statistically significant differences at $p \leq 0.05$ according to the LSD test. ns: indicate the non-significant difference. Table 2 and 3 lists the key names of the traits and nitrogen treatments under study, respectively.

4. Principal component analysis (PC)

Based on grain yield and physiological parameters under study, the triple influence of years, N treatments, and varieties was analyzed using PCA, and a total of seven principal components were found (Table 7). Out of these PCs, the PC1, PC2 and PC3 were extracted with eigenvalues ≥1 (3.42, 1.42 and 1.00, respectively) and explained 83.56% of the total variance in the variables under consideration. Liu et al. (2020) found a similar result in their study on responses of PCA in MDA and other traits to N-deficiency stress in some wheat genotypes. While 16.44% of the total variability was explained by the other PCs, which all had eigenvalues below one. According to studied traits, only roughly 48.93% of the overall variability among the years, N treatments, and varieties is described by PC1, which had higher contributions to the total variance than PC2 (20.31%) and PC3 (14.33%). Gioia et al. (2015) and Liu et al. (2020) had previously achieved findings that were similar.

Grain yield and physiological traits in seven PCs were mixed with positive and negative loadings under the variables studied (Table 7). CAT and ABX in the three first PCs, MDA, and SOD in PC1, grain yield in PC2 as well as proline content in PC3 were positive loading variables. While the other variables studied were negative loading factors during the first three PCs. The highest positive loading factors were APX, grain yield, and CAT in PC1, PC2, and PC3, respectively, which had loading factors ≥0.5. Liu et al. (2020) reported that PC1 was strongly correlated with MDA contents in wheat genotypes.

As shown in Table 7, the first three PCs were positively correlated with most factors evaluated including years, N treatments, and varieties. Based on the results of years, N treatments, and varieties variables, PC1 was strongly associated with Seds 12 variety, N0 treatment, and the second year. While PC2 had a high positive association with Sakh 94 variety, N3 treatment, and the second year. As for PC3, the highest positive correlation was observed with Sakh 94 variety, N0 treatment, and the second year. These results indicate these variables of the wheat grain yield and physiological traits contributed to the PC1 and PC2. It is evident that the first two PCs can be interpreted as responses of genotypes related to the N treatments under the two years for grain yield and physiological traits. Using PCA, it was discovered that the nitrogen treatments had a significant impact on all of the genotypes' phenotypic responses (Gioia et al. 2015). As a result, PC1 and PC2 were retained for the final analysis. According to Sharma (1996), the two PCs express greater variability and justify choosing the trait with a positive loading factor since they explain variance more thoroughly than a single characteristic.

Table 7. Results of seven PCs for the evaluated factors based on grain yield and some physiological traits of bread wheat.

PCs	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Traits loadings							
GY	-0.01	0.60	-0.58	-0.38	0.39	0.01	0.08
RWC	-0.46	-0.01	-0.20	0.61	0.18	0.36	0.47
Proline	-0.36	-0.16	0.50	-0.42	0.58	0.28	-0.03
MDA	0.41	-0.46	-0.20	-0.37	-0.06	0.27	0.61
SOD	0.40	-0.30	-0.10	0.35	0.69	-0.37	-0.09
CAT	0.29	0.52	0.56	0.11	0.04	-0.20	0.53
ABX	0.49	0.21	0.08	0.21	0.07	0.74	-0.33
Factors Scores							
2020/2021	-0.59	-0.28	-1.00	-0.11	-1.16	-0.22	0.01
2021/2022	0.58	0.27	1.03	0.09	1.17	0.23	-0.01
N0	1.03	-2.13	0.79	-0.40	-0.35	0.19	-0.11
N1	-1.51	0.29	0.73	1.62	-0.18	-0.20	0.04
N2	0.04	0.63	0.47	-1.19	-0.06	0.00	0.16
N3	0.42	1.20	-1.95	-0.06	0.63	0.03	-0.08
Sakh 93	-1.98	-1.59	-0.45	-0.24	0.79	-0.55	0.02
Gemmiza 12	-0.02	-0.65	-0.80	0.47	-0.16	0.80	0.07
Seds 12	4.23	0.56	0.25	0.30	-0.23	-0.39	0.01
Sakh 94	-2.20	1.70	0.94	-0.48	-0.45	0.10	-0.10
Eigenvalues	3.42	1.42	1.00	0.54	0.46	0.14	0.01
Variance %	48.93	20.31	14.33	7.68	6.60	2.05	0.10
Cumulative%	48.93	69.24	83.56	91.24	97.85	99.90	100.00

Table 2 and 3 lists the key names of the traits and nitrogen treatments under study, respectively.

PC1 and PC2 primarily distributed and discriminated the variables and examined traits in various groups based on all measurable data. Therefore, a biplot was drawn using the first two PCs (Fig. 2). The biplot diagram showed how years, N treatments, and varieties all contributed to the variability of all grain yield and physiological parameters assessed. Sharp and obtuse angles between variable vectors indicate positive and negative correlation, respectively (El-Hashash et al. 2022). Most of the analyzed traits showed a positive association, however, they varied in terms of degree and quantity consistency. Grain yield had a low positive correlation with RWC, CAT and ABX, while had negative correlations with proline content, MDA, and SOD. MDA was positively associated with SOD and ABX, while a positive correlation was observed between proline and RWC as well between SOD, CAT and ABX. According to Yehia and El-Hashash (2021), positive relationships for the evaluated traits showed that selection for one trait's higher value will boost the value of the other. These findings indicate that physiological measurements such as RWC, CAT, and ABX might be useful indices for the indirect selection of wheat yield potential for genotypes under N treatments. Wang et al. (2022) reported that grain yield was positively and negatively associated with SOD and MDA, respectively, indicating a strong synergistic effect between grain yield and physiological indicators such as SOD. The associations between crop physiological traits and grain yield demonstrated that these traits may be the primary factor affecting wheat grain productivity (Qadeer et al. 2019).

Based on PC1 and PC2, the effects of years, N treatments, and varieties on grain yield and physiological traits were divided into two groups. Liu et al. (2020) noticed similar trends when a comprehensive evaluation of the physiological traits in wheat under nitrogen stress using PCA. The first group (G1) was related to PC1 and includes MDA, SOD, CAT, and ABX, which are strongly associated with Seds 12 variety and N0, N1, and N3 treatments across the second year (the first and fourth quarters). During the second group (G2), grain yield, RWC and proline are highly associated with Sakh 94 variety and N1 treatment during the second year, which was related to PC2 (the second and third quarters). Generally, the Sakh 94 and Seds 12 varieties fertilized with N3 treatment during the second season were located with grain yield CAT and ABX in the first quarter (the highest PC1 and PC2) and second quarter (the highest PC2 and the lowest PC1). PC2 is considered very important to increase grain yield related to CAT and ABX in Sakh 94 variety under N3 treatment across the second year. While, PC2 seems to represent MDA, SOD, CAT, and ABX in Seds 12 variety with N0 and N3 treatments across the second year. Also, the best triple effect on grain yield and most physiological traits was observed by Sakh 94 variety with N3 treatment under the second year, which can effectively improve the physiological traits and enhance an increased grain yield of wheat. According to Borzouei et al. (2020), grain yield and a few physiological indicators showed a favorable association under different N levels in both years.

As previously reported by Qadeer et al. (2019), Liu et al. (2020), Pauta et al. (2021), Ahmad et al. (2022), El-Hashash et al. (2022), grain yield and physiological traits have been increased by the combination of the experimental factors including growing seasons, varieties and different N levels, which recorded the highest values than those of every single factor. These results suggested that the experimental factors interactions showed that there was different behavior for examined genotypes traits, which was also reported previously by (El-Hashash et al. 2022). As a result, it might be used in the future to boost wheat grain productivity.

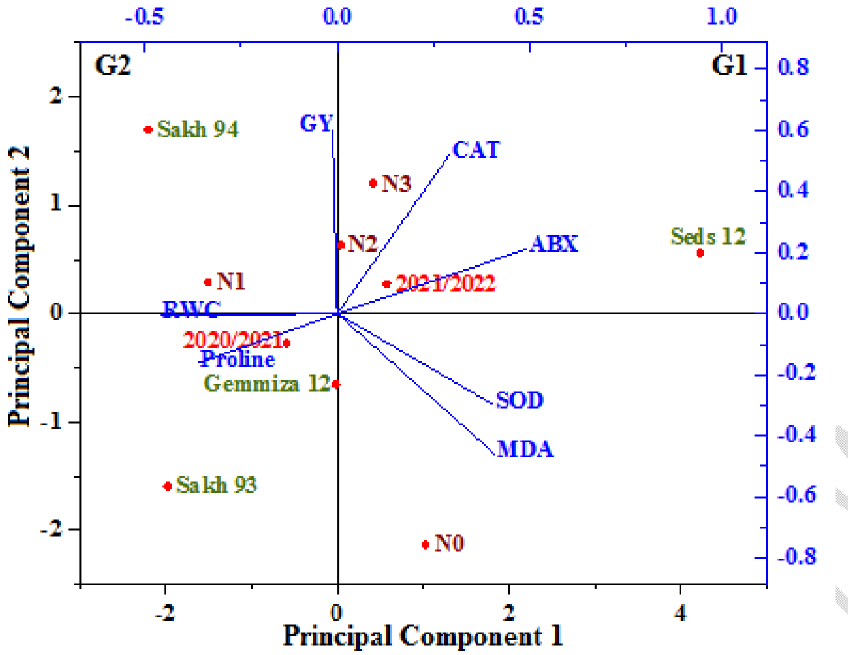

Fig 2. Biplot diagram shows similarities and dissimilarities in the correlation between grain yield and physiological traits during the three experimental factors based on PC1 and PC2. Table 2 lists the key names of the traits under study.

According to Wang et al. (2022), high nitrogen application caused excessive nutrient growth that decreased yield, whereas low nitrogen application could result in extreme plant growth, insufficient dry matter accumulation, and decreased wheat grain yield (Qadeer et al. 2019). On the other hand, the physiological and morphological traits, minimizing N losses, regulating the accumulation and transportation of assimilates as well as the increase in grain yield of wheat might all be significantly enhanced by the optimum application rate of N (Duan et al. 2018; Zhang et al. 2018; Qadeer et al. 2019; Wang et al. 2022). The activity of crops' defensive enzymes can be improved with the proper nitrogen fertilizer treatment, but the opposite is true (Iqbal et al. 2020).

Wheat exposed to N experienced improved root development and increased leaf water content (Qadeer et al. 2019). Since the quantity of nitrogen considerably impacts the activity of crop protection enzymes, nitrogen is a crucial component of plant protective enzymes (Wang et al. 2022), where increased N rates can affect wheat grain productivity and SOD, CAT, and APX (Borzouei et al. 2014). While decreasing grain yield resulted from an excessive nitrogen supply, the SOD activities increased as the nitrogen level increased (Wang et al. 2022). Abid et al. (2016) mentioned that maximum SOD and APX activities as well also minimum MDA content determined in given high N fertilization crops suggested their improved redox defense status to scavenge ROS damage. Also, Ahmad et al. (2022) reported that higher N rates, which indicate stress from ammonium toxicity, were the cause of the increase in antioxidant activity (Ahmad et al. 2022). The nutritional stress in plants increased ROS generation and antioxidant activity (Ahmad et al. 2019 and 2022). It has been discovered that adding N results in an increase in proline contents, which improves crop physiological processes, where proline is an essential stress protector (Srivastava et al. 2018; Qadeer et al. 2019). According to some physiological mechanisms, the increase in grain yield caused by nitrogen treatment may be attributable to its many functions, especially in the creation of N-containing molecules like proline and proteins (Borzouei et al. 2014; Borzouei et al. 2020).

CONCLUSIONS

Significant variations among years, N treatments, and wheat varieties as well as their interactions led to improve grain yield and most physiological traits and contributed to higher grain yield by Sakh 94 variety with 125 kg N fad^{-1} treatment. PCA results under study could be helpful and employed as an appropriate way for figuring out the best amount of nitrogen to apply in order to increase wheat varieties' physiological features and grain production. Finally, it is advised to apply 125 kg fad^{-1} of N along with the Sakha 94 variety to promote and increase wheat productivity.

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