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Integrated nutrient supply and varietal difference influence grain yield and yield related physio-morphological traits of durum wheat (*Triticum turgidum* L.) varieties under drought condition

Anteneh Agezew Melash* – Éva Babett Ábrahám

University of Debrecen, Institute of Crop Science, Department of Crop Production and Applied Ecology, 4032, Debrecen, Hungary
Correspondence: antenehagezew2008@gmail.com

SUMMARY

The ever-growing world population entails an improvement in durum wheat grain yield to ensure an adequate food supply, which often gets impaired by several biotic and abiotic factors. Integrated nutrient management, such as nitrogen rate × foliar zinc × sulphur fertilization combined with durum wheat varieties were investigated in order to examine the dynamics of yield and yield related physio-morphological traits under drought conditions. The four durum wheat varieties, three-level of nutrient supply (i.e. control, sulphur, and zinc), and two nitrogen regimes (i.e. zero and 60 kg ha^{-1}*) were arranged in split-split plot design with three replications. Zinc and sulphur were applied as foliar fertilisation during the flag leaf stage, both at a rate of 3 and 4 liters ha*$^{-1}$*, respectively. Results showed existence of genetic variability for grain yield, plant height, NDVI, SPAD and spike density. Foliar based application of zinc and sulphur at the latter stage improved the plant height. Nitrogen fertilized varieties with lower spike numbers showed to better yield formation. Co-fertilization of nitrogen and zinc improved grain yield of responsive varieties like Duragold by about 21.3%. Spikes per m*^{2} *were statistically insignificant for grain yield improvement. It could be inferred that the observed positive effect of sulphur, nitrogen and zinc application on physio-morphology and yield formation substantiates the need to include these essential nutrients in the cultivation system of durum wheat.*

Keywords: *Leaf area index; grain yield; spikes per m*^{2}*; chlorophyll content; NDVI*

INTRODUCTION

There is no doubt that, as the world population increases, wheat grain yield needs to be enhanced concomitantly which often gets hindered by several biotic and abiotic stresses (Fischer et al., 2014; Khobra et al., 2019). This proliferation enforces the agricultural sector to improve wheat production by about 40% in the coming two decades (Iqbal et al., 2021). Indeed, harvesting high grain yield could come about via more biomass accumulation and resource partitioning (Weina et al., 2022; Leilei et al., 2019). Improved partitioning of assimilates into the developing spike and grain has the single greatest impact on enhancing wheat grain yield under both potential and marginal environments (John et al., 2011). High biomass production has been correlated with the capacity of the canopy to intercept the incoming PAR as a function of LAI and translate the intercepted radiation into new biomass (Thomas and Russell, 1999). A leaf area index in combination with measurements such as chlorophyll content has been reported as a critical proxy for vegetation productivity and prevailing stress in vegetation (Gitelson et al., 2006; Sanglard et al., 2014).

Although to date enhancement in wheat grain yield potential has been based chiefly on yield per se, there is substantial evidence that understanding traits at the physiological and morphological level will help to forecast synergies between agronomy and breeding, and between biomass and partitioning traits as well (Reynolds et al., 2009; John et al., 2011). A number of reliable indicators, such as NDVI and SPAD, have been developed to determine crop nitrogen status, forecast the grain yield, and assess phenotypic variability, particularly under the current climate change scenarios (Qi et al., 2020; Kizilgeci et al., 2021). NDVI has been putatively associated with grain yield and plant height as well (Milan et al., 2018). Every 0.1unit improvement in the NDVI value enhances the grain yield by about 1.1 to 2.6 t ha^{-1} (Ewa and Dariusz, 2020). Importantly, leaf chlorophyll concentration has been responsible for 8.8 to 10.9% of grain yield variation (Roy et al., 2021). This effect could have varied with the change in developmental plasticity, where low chlorophyll content has been more advantageous at the grain filling stage (Araus et al., 2002). Although its robustness, higher LAI could negatively influence the overall varietal performance (≈8%) and grain yield (≈10%) (Srinivasan et al., 2017). However, whereas improved in photosynthesis and increased grain partitioning both ostensibly offer to enhance water and nutrient productivity (John et al., 2011), higher leaf area index reduces the photosynthetic efficiency of crops by creating a shading effect (Srinivasan et al., 2017).

The modern crop varieties would produce more leaves than is optimal for grain yield and thus reducing leaf area would give higher grain yield (Srinivasan et al., 2017). The yield regulatory effect of some physio-morphological traits and reliance on grain yield on NDVI, chlorophyll content, LAI, plant height, and tiller production has been confirmed in numerous studies (Roy et al., 2021; Tshikunde et al., 2019; Melash et al., 2019). Hence, consideration of this dependence could contribute to promoting specific responses that culminate in higher harvestable yield and stable physio-morphological traits, whether attempts at improvement be genetic or agronomic in approach. However, in many environments in which soil nitrogen and sulphur are inadequate, yield, NDVI, and SPAD values are substantially reduced due to lesser leaf expansion and assimilation rate (Ilze and Antons, 2017; Fernando and Daniel, 2008). This shows an absolute requirement for nitrogen and sulphur fertilization for wheat growth, and grain yield depend on substantial inputs.

Integrated use of macronutrients and mineral fertilizers for tackling soil fertility depletion and improving yield sustainably by optimizing the physiological efficiency of wheat has paramount importance. However, most physio-morphological traits that drove the remarkable yield improvement and their association to harvested product of durum wheat in a range of nutrient supply appear to have a little remaining potential for further improvement. A circumstantial evidence from agronomic efforts suggests that sulphur containing fertilizers enhances the leaf area index, which allowed a higher radiation interception, radiation use efficiency and consequently the biomass and grain yield (Fernando and Daniel, 2008). Moreover, the combined application of sulphur and nitrogen has been more effective for improving chlorophyll content and grain yield (Piotr et al., 2012). The application of zinc fertilizers has been suggested to enhance nutrient use efficiency and absorption (Xiaolong et al., 2021). Hence, the positive effect of sulphur, nitrogen and zinc application on physiology, morphology and yield formation in durum wheat substantiates the need to include these essential nutrients in the cultivation system of this species. With the above-mentioned issues in mind the present study was aimed **i**) to scrutinize the genotypic variability in response to the applied inputs and **ii**) evaluate dynamics in yield and yield related physio-morphological traits of durum wheat varieties under a range of nutrient supply.

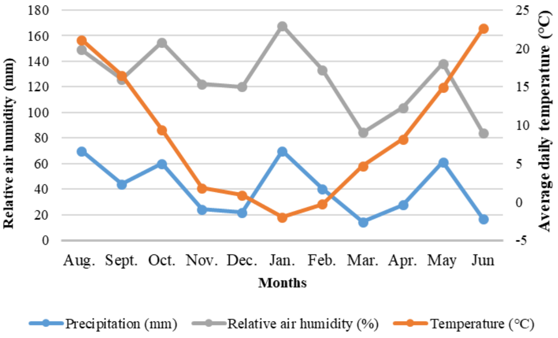
MATERIALS AND METHODS

The field experiment was conducted at Látókép field research station of the University of Debrecen, Hungary, during 2021 spring season. It is laid at 47˚33042″ N; 21˚ 27002″ E, and 15 km away from Debrecen (Csajbók et al., 2020). The chernozem soil of the long-term experiment contains 2.7–2.8% humus, and total depth of the humus enriched horizon was about 0.8 m. This calcareous chernozem soil is characterized by a specific plasticity index (KA) of 40 and nearly neutral pH (pH KCl = 6.46). During the experimental period, the soil contained 0.94 mg kg^{-1} of zinc, 57 mg kg^{-1} AL-soluble P and 199 mg kg^{-1} AL-soluble K. The soil has favourable water management characteristics. The soil sample of the experimental site was obtained from a 0 to 20 cm cultivated soil layer prior to application of the treatments. The daily average temperature, relative air humidity and seasonal precipitation during the test period (August 2020–June 2021) are shown in *Figure 1*.

Experimental design and treatments

The treatments consisting of two levels of nitrogen application regime (i.e. control and 60 kg ha^{-1}), four durum wheat varieties (i.e. *Colliodur*, *Durablank*, *Duragold*, *Tamadur*), and three-levels of foliar based fertilizers (i.e. control, zinc and sulphur) were arranged in a split-split plot design with three replications. The two levels of nitrogen application rates were assigned into the main plots, the four durum wheat varieties into the subplots, and the three-levels of foliar based fertilizers were assigned into the sub-sub plots. The sub plot size was 9 m in length and 1.5 m in width, with 15 cm row spacing. A liquid solution of sulphur and zinc in a form of fertilizers was supplied foliarly at flag leaf stage, both at a rate of 4 liters ha^{-1} and 3 liters ha^{-1}, respectively.

Figure 1: **Average daily precipitation, relative air humidity and temperature during 2020/21 cropping season, Látókép, Hungary**



Data collection

The relative leaf chlorophyll concentration (SPAD) was measured from 10 randomly selected leaves using a portable SPAD meter (Model SPAD-502, Minolta crop, Ramsey, NJ) at 65, 77, 92, and 128 days after sowing (DAS). The instrument records red light transmission at 650 nm and of infrared light transmission at 940 nm, at which chlorophyll absorbs light, and no absorption occurs, respectively. Hence, considering the two values of transmission, the portable SPAD meter, calculates a SPAD unit values that is well associated with relative leaf chlorophyll concentration (Markwell et al., 1995). The plant height was measured from the soil surface to the top of the spike, as the average height of the main tiller of five randomly selected plants. The leaf area index (LAI) was determined nondestructively by using a SunScan Canopy Analysis System (SSI) portable leaf area measuring instruments in four repetitions with five measurements per repetition. The final grain yield was determined after harvest in a kg ha^{-1} base. The normalized difference vegetation index (NDVI) was measured using a portable N tech "Trimble Greenseeker" NDVI meter at different developmental stages.

Statistical Data analysis

The mean data of five-ten randomly selected plants of each durum wheat variety was used to determine a range of physio-morphological traits, and it has been tested for normality and homoscedasticity. All the collected data such as spikes per square meter, SPAD, NDVI, LAI, plant height and grain yield were subjected to statistical analysis using GenStat (18th ed.) statistical software package (Payne et al., 2011). When the ANOVA revealed significant difference, the mean treatment values were compared using the least significance difference (LSD) at a 5% significance level. Duncan's multiple range test (DMRT) was employed for comparison of the various interaction means presented in various graphs and tables presented. The mean values were used to construct the graphs using excel graphing features. Additionally, paired Spearman's correlation analysis was performed using the same statistical software package, to see the strength of the relationship between pair of traits under the applied treatments.

RESULTS AND DISCUSSION

Response of grain yield to nutrient supply and genetic variation

The combined analysis of variance revealed that the grain yield was significantly ($p < 0.05$) influenced by varietal difference (*Table 2*). Considering genotypic mean, *Duragold* was relatively the highest yielder, which exceeded the grain yield of *Durablank* by about 2.18% (*Table 2*). The most preferred varieties, *Duragold* and *Durablank*, are the first and second highest yielders with an average grain yield of 7,000 kg ha^{-1} and 6,851 kg ha^{-1}, respectively. In comparison with *Tamadur* (4,953 kg ha^{-1}), both varieties gave an overall yield advantage of 41.3% and 38.3%, respectively (*Table 2*). This implies substantial genetic variability for grain yield within the durum wheat varieties, which will provide ample scope for selecting superior varieties under the current climate change scenario. Wider genetic variation for grain yield has been reported previously in durum wheat genotypes (Mengistu et al., 2019). These observations could reinforce the idea that screening large numbers of spring wheat varieties for grain yield and wider adaptability could significantly contribute to breeding programs.

The interaction effect of nitrogen and durum wheat varieties were significant for grain yield (*Table 3*). When the relationship between grain yield and the measured physio-morphological traits was examined, the varieties having better relative leaf chlorophyll content and NDVI profiles were achieved maximum grain yield under 60 kg N ha^{-1} than did in the control (*Table 2*). Variety *Duragold* and *Durablank* set a potentially higher grain yield of 7,469 kg ha^{-1} and 6,901 kg ha^{-1}, respectively. In contrast, variety *Colliodur* prevented achieving relatively high (6,702) grain yield (*Table 2*) despite its higher spike density per m^2 (*Table 3*). However, the lowest (4,014 kg ha^{-1}) grain yield was measured for variety *Tamadur* even under the same nutrient supply (*Table 3*). This variation could be generated in the allocation and conversion of the applied inputs into grain formation and the sensitive influence of pedoclimatic conditions. Indeed, wheat yield is a highly polygenic trait, with variation in grain yield often arising from differences in other phenotype features, each with different genetic background (Noah et al., 2020).

A higher correlation between grain yield and relative leaf chlorophyll content has been reported in wheat, suggesting the use of chlorophyll content to screen stress-tolerant genotypes (Yasir et al., 2019). However, our result confirmed that under adequate nitrogen fertilization, relatively higher chlorophyll producing varieties would not be established maximum grain yield (*Table 3*). It had been clearly observed in the first two high yielder varieties, variety *Duragold*, for instance, had been measured higher leaf chlorophyll content than *Durablank*, but the reverse has occurred for grain yield formation (*Table 2–3*). This means that both too high and low leaf relative chlorophyll concentration might affect grain yield formation, although to the extent that varies with varietal performance. Higher grain yield established under relatively low chlorophyll producing varieties could be due to less photochemical damage of leaves absorbing more light energy than required for maximum photosynthesis (Hamblin et al., 2014; Araus et al., 2002). The chloroplast is a nutrient-rich organ and less leaf chlorophyll content may spare nutrients essential for crop growth and development under marginal soil nutrient status (Hamblin et al., 2014).

Table 1: **Dynamics of grain yield (kg ha^{-1}) under co-fertilization of nitrogen, zinc and sulphur influences grain yield**

Nitrogen	Varieties	Foliarly applied nutrients		
		Control	Sulphur	Zinc
60 kg ha^{-1}	Colliodur	7166	6505	6434
	Durablank	7064	7035	6604
	Duragold	7356	7352	7696
	Tamadur	5785	6088	5806
Control	Colliodur	6698	6191	6637
	Durablank	6824	6747	6830
	Duragold	6751	6488	6354
	Tamadur	3819	4690	3532
LSD $_{0.05}$	893.8**			
CV (%)	5.5			

The grain yield was significantly determined by the level of applied nitrogen and foliar-based zinc fertilization. When nitrogen applied at a rate of 60 kg ha^{-1} combined with foliar zinc fertilization had been enhanced the grain yield of *Duragold* by about 14% than did in the control (*Table 1*), which corroborated to Piotr et al. (2012). Although the variety *Tamadur* attained minimum grain yield, its yield achievement was much higher than the yield depicted under the sole application of nitrogen fertilizer (*Table 1*). This variation might be attributed to a difference in root development, which might influence the ability of the varieties to exploit the applied inputs during the season. These results further suggested that the combined application of nitrogen and zinc in a form of fertilizers under drought episodes could improve the tolerance

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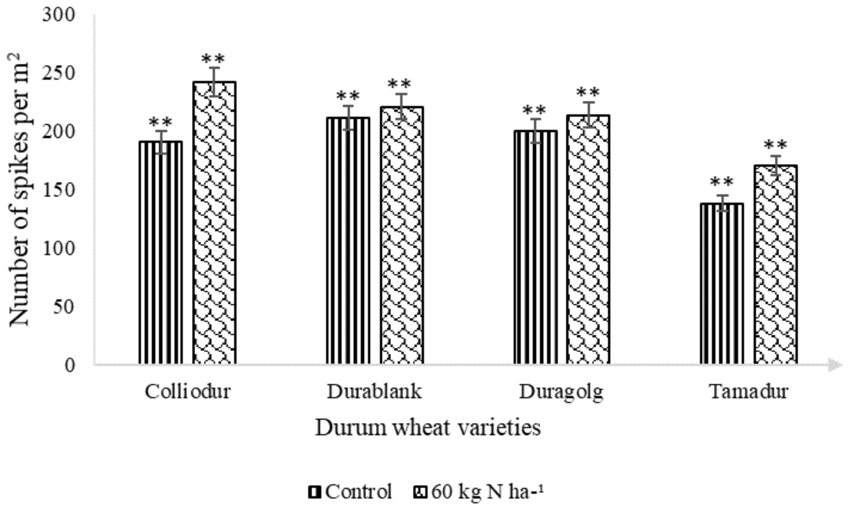
level of susceptible varieties, and thus maintain the grain yield. In line with this, Mosavian et al. (2021) reported that the combined application of zinc and nitrogen fertilizers could reduce the adverse effect of drought stress on the grain yield of wheat. Wheat grain yield loss due to terminal drought stress could be corrected by application of zinc through stimulating and producing antioxidant mechanism, proline, soluble proteins and thus regulate the wheat growth (Sattar et al., 2021).

Number of spikes per m^2 as affected by nutrient supply and genetic variation

The number of spikes per square meter was found significantly ($p < 0.05$) dependent on the plant nutrition and varietal difference (*Table 2–3*). A substantial phenotypic variation in spikes density per square meter was observed amongst the tested varieties, where higher (216) spike density was measured for *Durablank* and *Colliodur* (216) followed by *Duragold* (206) despite its high yielding potential. While the very minimum (154) spikes density per m^2 was measured for variety *Tamadur* (*Table 2*). This variation in spike density per m^2 could be associated with phenological plasticity and length of maturity period under drought condition. Würschum et al. (2018) verified that varieties having the capacity to stay green under prolonged drought episode often remain photosynthetically active, which in turn possess better spike fertility. Overall reduction in number of spikes under terminal drought condition was reported previously (Lin et al., 2020).

While the genetic variation in spike density was low, particularly in the first two varieties, relative differences varied substantially under the two nitrogen fertilization regimes (*Table 3, Figure 2*). Higher spikes density per m^2 was measured in the varieties *Colliodur* (242) and *Durablank* (221), while the minimum density was measured for *Tamadur* (170) under 60 kg N ha^{-1} than did in the control (*Table 3*). In line with this, Shahzad et al. (2010) observed higher number of spikes per m^2 under adequate nitrogen fertilization. This implies that adequate nitrogen application could enhance the number of spikes per m^2, may be at the expense of tillering potential of the varieties. Lesser spike density per m^2 measured for variety *Tamadur* could be associated with either its poor tillering capacity or non-responsiveness to the applied inputs. It has been previously reported that, establishment of higher number of spikes per m^2 had a result of significant genetic gain of fertile tillers (Boussakouran et al., 2021). Hence, as far as spike per m^2 concerned, it could be improved by enhancing the ability of durum wheat varieties to revert applied resources and form additional tillers (Subira et al., 2015).

Figure 2: **The interplay between nitrogen and varietal difference on the spike density per m^2. Bars indicated with double asterisks are statistically significant at 0.01% probability level**



Leaf chlorophyll content as influenced by nutrient supply and genetic regulation

The SPAD units as estimates of relative chlorophyll content was significantly ($p < 0.05$) influenced by developmental stages, nitrogen application, but not by foliar application of zinc and sulphur (2–4). A quadratic response in relative chlorophyll content was observed along with the shift in crop developmental plasticity (*Table 2–5*). Its value was increased rapidly at the first stages (65 DAS), followed by a relative stabilization in mid-season and then progressively decline in the final growth stages (128 DAS), even though zinc and sulphur fertilizers are applied foliarly at vegetative stages (*Table 4*). A decline in SPAD values at 128 DAS and onwards could express high green biomass reduction due to a progressive falling and discoloration of leaves.

This assumption has been reported in numerous studies that SPAD values increase with increase in age and decrease at maturity stage (Kandel et al., 2020). While the relative chlorophyll content could be declined at the later growth stage, it is often not agronomically and economically desirable to supply zinc and sulphur-containing fertilizers late in the season to improve this particular trait. We then suggested that zinc and sulphur fertilizers should be applied at early growth stages than late in the growing season, as far as chlorophyll content is considered.

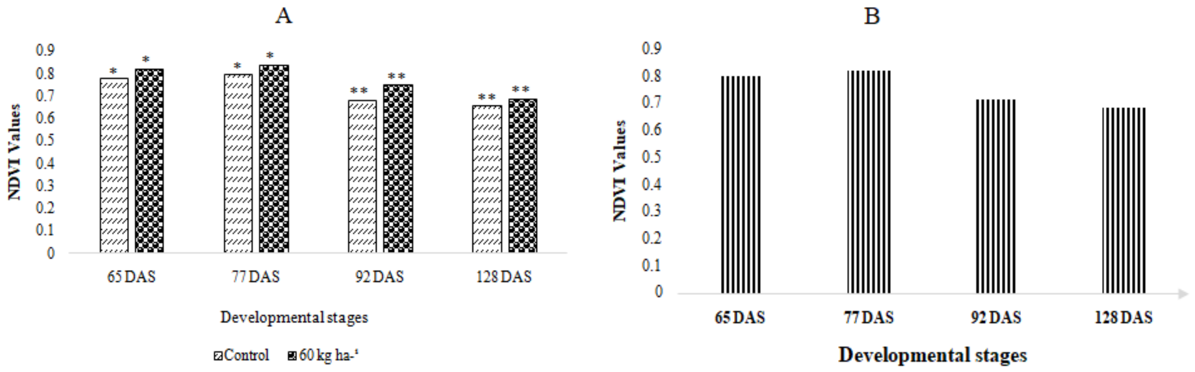
Variation in genetic landscape was statistically significant ($p < 0.05$) for relative leaf chlorophyll content (*Table 2*). Since the onset of greening and yellowing of leaves differs from varieties, the corresponding SPAD value ranged from 49.0 to 52.3 and 54.3 to 62.7 for *Colliodur* and *Durablank*, respectively (*Table 2*). This implies that the variety *Durablank* could maintain relatively higher relative leaf chlorophyll content than the other varieties under drought conditions, which might be its drought tolerance response. A substantial genetic variation has been frequently reported for leaf chlorophyll content (Hamblin et al., 2014; Kizilgeci et al., 2021). It seems that the use of leaf chlorophyll content as a trait may improve the grain yield, especially under stress conditions, by improving the light interception and its conversion effectiveness (Kapoor et al., 2020).

The interaction effect between nitrogen application dose and durum wheat varieties was statistically ($p < 0.05$) significant for chlorophyll content (*Table 3*). The interaction was most clearly observed in *Durablank*, which in the higher (60 kg ha^{-1}) nitrogen application rate had maximum (54.9–64.2) chlorophyll content, consistently throughout the growing cycle (*Table 3*). This showed that the relative leaf chlorophyll content is more responsive to nitrogen fertilizer application than variation in the genetic landscape. High leaf chlorophyll content could indicate that the tested durum wheat varieties received sufficient nitrogen fertilizer throughout the entire growth period. It is therefore more useful to study allometric relationships between dynamics in genetic response and nutrient supply to improve the stay-greenness of durum wheat varieties simultaneously with grain yield. The development of stay-green varieties has been frequently reported as an important tool to ameliorate the grain yield under stress conditions (Muhammad et al., 2021; Ramkumar et al., 2019). Higher grain yield under such conditions could be attributed to the production of high cytokinin content, low ethylene perception, and better water intake maintenance of the varieties (Monteoliva et al., 2021).

Shifting of LAI and NDVI in wheat supplied with zinc, sulphur and nitrogen under drought condition

The most important photosynthesis acceptor, the leaf area index (m^{2} m^{-2}) was regulated by the change in wheat developmental plasticity (*Table 2–5*). It had been confirmed that LAI and NDVI profiles exhibited the same tendency to increase and then fall as the growth stages progressed, where LAI (55.3) and NDVI (0.82) values reached maximum units at 77 DAS, respectively (*Figure 3B*). This means that the LAI and NDVI value during the leaf production, and leaf senescence stages may not be the same. It may be attributed to the fact at the early growing season, leaves are fully green while in the latter stages, the leaf colours became a mixture of green, yellow and dead leaves which deteriorates the numerical values of the traits. Improvement in LAI prior to heading stage has been reported due to the expansion of the leaf photosynthetic area, and the production of carbohydrates, which reflects later on leaf dry weight and overall biomass production (Din et al., 2017).

Figure 3: **The effect of nitrogen application rate (A) and crop developmental stages (B) on NDVI profile of durum wheat varieties. Bars indicated with single, and double asterisks are statistically significant at 0.05% and 0.01% probability level, respectively**



Important interaction was observed between nitrogen × varieties × foliarly applied nutrients on LAI and NDVI (*Table 5*). The result confirmed that foliar-based application of sulphur combined with 60 kg N ha^{-1} was increased the LAI (2.7 to 4.9) and NDVI (0.67 to 0.82) for *Colliodur* (*Table 5*). This implies that the

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LAI and NDVI profiles could be improved in a nitrogen and developmental stage dependent manner, to the extent that even the smallest changes in the nitrogen application rate could cause significant alterations in LAI and NDVI values. A number of studies reported similar results that nitrogen application in a form of fertilizers improves LAI and NDVI values in wheat (Yin et al., 2003; Kizilgeci et al., 2021). However, the unit measurements of LAI and NDVI profiles were varied with phenotypic response to developmental plasticity and the applied fertilizers (*Table 4*). In the absence of nitrogen fertilizer, variety *Durablank*, for instance, measured higher LAI and NDVI (0.75) values due to the sole effect of foliar-based zinc fertilization (*Table 4*). This means that different varieties of the same species could respond differently to the applied inputs, suggesting the necessity of appropriate varietal selection with a specific set of agronomic management practices. The main inference drawn is that relating the LAI and NDVI profile to the developmental stages of spring wheat varieties gives a better and universal relationship, which could be a potential yield improvement avenue. It has been verified that every 0.1 unit of increment in the NDVI value enhances the grain yield of cereals by about 1.1 to 2.6 t ha^{-1} (Ewa and Dariusz, 2020).

Table 2: **The effect of divergence in genetic landscape on selected physiological traits of durum wheat varieties measured at different growth stages**

Varieties	**65 DAS**			**77 DAS**			**92 DAS**			**128 DAS**			**PH**	**Grain Yield**	**Spikes**
	LAI	**NDVI**	**SPAD**	**LAI**	**NDVI**	**SPAD**	**LAI**	**NDVI**	**SPAD**	**LAI**	**NDVI**	**SPAD**	**(cm)**	**(kg ha^{-1})**	**(m^2)**
Colliodur	2.0	0.81	49.0	2.3	0.81	49.0	3.9	0.71	56.8	3.4	0.68	52.3	82.3	6605	216
Durablank	1.3	0.78	54.3	2.8	0.85	56.6	3.9	0.77	65.3	3.8	0.73	62.7	81.8	6851	216
Duragold	1.3	0.79	53.6	2.8	0.82	55.0	3.7	0.74	65.9	3.5	0.72	61.6	78.2	7000	206
Tamadur	1.4	0.79	51.8	2.1	0.79	51.8	2.7	0.64	58.8	2.3	0.58	53.5	71.9	4953	154
LSD$_{0.05}$	0.11**	0.015*	1.19**	0.13**	0.019	1.03**	0.16**	0.016	1.36**	0.17**	0.027	1.9**	1.7**	238.27	10.3**
CV (%)	10.9	1.50	3.4	8.0	1.9	2.9	6.5	1.8	3.3	7.8	3.2	5.0	3.1	3.0	7.7

Key to abbreviations: DAS: Days after sowing; **LAI:** Leaf area index; **SPAD:** relative chlorophyll content; **NDVI:** Normalized difference vegetative index; **PH (cm):** plant height; **LSD$_{0.05}$** least significant difference; **CV (%):** Coefficient of variation

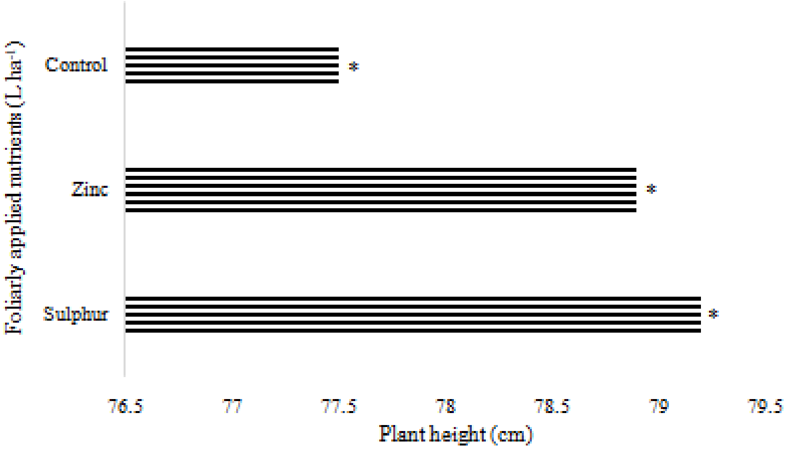
Table 3: **A combined effect of nitrogen and spring wheat varieties on selected physiological traits at different growth stage**

Nitrogen (kg ha^{-1})	**Varieties**	**65 DAS**			**77 DAS**			**92 DAS**			**128 DAS**					**Grain**
		LAI	**NDVI**	**SPAD**	**LAI**	**NDVI**	**SPAD**	**LAI**	**NDVI**	**SPAD**	**LAI**	**NDVI**	**SPAD**	**PH (cm)**	**Spikes (m^2)**	**Yield (kg ha^{-1})**
Control	Colliodur	1.6	0.79	47.2	2.1	0.78	43.0	3.1	0.67	54.9	3.1	0.66	48.6	78.3	190	6509
	Durablank	1.3	0.77	53.8	2.2	0.84	52.8	3.7	0.76	64.7	3.6	0.75	61.3	80.2	211	6800
	Duragold	1.3	0.78	53.3	2.1	0.81	51.7	3.5	0.73	64.9	3.4	0.72	59.8	74.6	200	6531
	Tamadur	1.3	0.76	49.8	1.5	0.76	47.3	2.3	0.56	56.0	1.9	0.52	50.1	66.0	138	4014
60	Colliodur	2.4	0.84	50.7	2.5	0.84	55.0	4.7	0.74	58.7	3.7	0.69	55.9	86.3	242	6702
	Durablank	1.3	0.80	54.9	3.5	0.86	60.4	4.1	0.78	65.8	4.0	0.71	64.2	83.4	221	6901
	Duragold	1.3	0.81	54.0	3.5	0.82	58.3	3.9	0.75	66.9	3.6	0.73	63.3	81.8	213	7468
	Tamadur	1.6	0.81	53.8	2.6	0.82	56.4	3.1	0.71	61.6	2.6	0.64	56.9	77.8	170	5893
LSD$_{0.05}$		0.31**	0.024*	2.34*	0.21**	0.025*	1.36**	0.44**	0.02**	3.69*	0.54*	0.042**	2.99	2.2**	15**	958
CV (%)		10.9	1.5	3.4	8.0	2.4	2.9	6.5	3.4	3.3	7.8	3.2	5.0	3.1	7.7	3.0

A combined effect of nutrient supply and varietal difference on plant height

The analysis of variance revealed that plant height was significantly influenced by genetic variation, nitrogen application, and by their interaction (*Table 2–5*). With reference to varietal difference, the tallest (82.3 cm) plant height was measured for variety *Colliodur*, while the shortest (71.9 cm) was measured for variety *Tamadur* (*Table 2*). This variation could be due to difference in genetic makeup. Variation in plant height among wheat varieties due to genetic material was reported by Mengistu et al. (2019). In the previous studies a strong association between plant height and wheat grain yield has been established, although grain yield is a polygenetic trait (Aisawi et al., 2015; Maeoka et al., 2020).

Figure 4: **The effect of foliar based zinc and sulphur application on plant height. Bars indicated with single asterisk is statistically significant at 0.05% probability level**



Interplay between nitrogen and varieties was statistically significant for plant height (*Table 3*). When nitrogen was applied at a rate of 60 kg ha^{-1}, the plant height of *Colliodur* was increased by about 10.22% than did in the control (*Table 3*). Increasing in plant height with augmenting nitrogen fertilizer was also reported in the previous studies (Hussain et al., 2006). Although their interaction with the imposed factors found statistically insignificant, the main effect of zinc and sulphur was significant for plant height (*Figure 4*). Where the maximum (79.2 cm) plant height was measured under sulphur fertilization than zinc (*Figure 4*). This implies that like any other essential nutrients, sulphur and zinc application in a form of fertilizers are very important for wheat growth and development as well.

Table 4: **The interaction effect of foliarly applied nutrients and durum wheat varieties on SPAD, NDVI, Spike density (m^2) and grain yield of durum wheat**

Nutrients	Varieties	65 DAS			77 DAS			92 DAS			128 DAS			Grain Yield (kg ha^{-1})	Spikes (m^2)
		LAI	NDVI	SPAD	LAI	NDVI	SPAD	LAI	NDVI	SPAD	LAI	NDVI	SPAD		
Control	Colliodur	1.9	0.81	49.8	2.4	0.82	49.4	3.8	0.71	57.4	3.4	0.67	52.6	6932	218
	Durablank	1.3	0.78	53.4	2.8	0.86	55.8	3.9	0.77	65.6	3.6	0.75	62.1	6944	214
	Duragold	1.3	0.80	53.3	2.7	0.81	54.1	3.7	0.75	66.8	3.6	0.73	62.3	7054	210
	Tamadur	1.3	0.78	51.0	2.1	0.78	52.8	2.4	0.64	56.9	2.0	0.58	52.7	4802	137
Sulphur	Colliodur	2.2	0.82	47.9	2.4	0.79	47.7	4.0	0.70	56.4	3.4	0.68	52.1	6348	208
	Durablank	1.4	0.78	54.3	2.9	0.84	56.4	4.0	0.77	64.3	3.8	0.70	62.5	6891	225
	Duragold	1.3	0.80	55.0	2.8	0.83	55.3	3.7	0.73	65.9	3.5	0.72	61.1	6920	211
	Tamadur	1.5	0.81	52.5	2.1	0.80	51.2	2.9	0.65	59.4	2.4	0.59	54.9	5389	163
Zinc	Colliodur	1.8	0.81	49.2	2.2	0.82	50.0	3.9	0.72	56.6	3.3	0.69	52.1	6536	223
	Durablank	1.3	0.79	55.3	2.9	0.84	57.5	3.8	0.78	66.0	3.9	0.75	63.6	6717	209
	Duragold	1.2	0.78	52.7	2.8	0.81	55.6	3.7	0.74	65.0	3.4	0.72	61.4	7025	199
	Tamadur	1.4	0.78	52.0	2.0	0.79	51.6	3.0	0.63	60.1	2.4	0.56	52.9	4669	163
LSD$_{0.05}$		0.07*	0.02*	2.10*	ns	0.026*	ns	0.26*	ns	2.48*	0.28*	0.035*	3.12*	397*	18*
CV (%)		10.9	1.9	3.4	8.0	2.4	2.9	6.5	3.4	3.3	7.8	3.6	5.0	5.5	7.7

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Table 5: **A summary of significance for variety, nitrogen rate, zinc and sulphur interaction on selected physiological traits of spring type durum wheat at different growth stages**

Nitrogen rate (kg ha^{-1})	**Varieties**	**Nutrients**	**65 DAS**			**77 DAS**			**92 DAS**			**128 DAS**			**PH (cm)**	**Spikes (m^2)**	**Grain Yield (kg ha^{-1})**
			LAI	NDVI	SPAD	LAI	NDVI	SPAD	LAI	NDVI	SPAD	LAI	NDVI	SPAD			
Control	Colliodur	Control	1.5	0.79	48.7	2.3	0.78	44.2	3.1	0.67	56.8	3.0	0.67	50.8	85.4	178.7	6698
		Sulphur	1.7	0.77	46.8	2.1	0.87	40.7	3.1	0.76	52.9	3.2	0.75	47.1	81.5	191.3	6824
		Zinc	1.4	0.78	46.1	2.0	0.80	44.2	3.2	0.74	55.1	3.0	0.72	48.1	79.9	201.0	6751
60		Control	2.4	0.75	51.0	2.4	0.74	54.5	4.4	0.55	58.1	3.8	0.51	54.4	75.6	257.3	3819
		Sulphur	2.7	0.83	48.9	2.7	0.85	54.7	4.9	0.74	59.9	3.7	0.67	57.1	79.2	224.3	7166
		Zinc	2.2	0.79	52.2	2.4	0.85	55.9	4.7	0.77	58.1	3.5	0.74	56.1	79.5	244.0	7064
Control	Durablank	Control	1.3	0.82	52.0	2.4	0.82	52.0	3.7	0.76	65.0	3.5	0.73	60.2	75.5	220.7	7356
		Sulphur	1.4	0.80	54.4	2.1	0.82	52.9	3.9	0.72	64.0	3.6	0.66	61.4	63.7	217.3	5785
		Zinc	1.2	0.79	55.0	2.2	0.76	53.4	3.5	0.65	65.2	3.7	0.65	62.2	86.7	195.7	6191
60		Control	1.2	0.76	54.8	3.2	0.82	59.6	4.0	0.76	66.1	3.7	0.74	64.1	84.1	207.7	6747
		Sulphur	1.3	0.79	54.2	3.7	0.81	60.0	4.2	0.72	64.5	4.1	0.71	63.7	83.5	232.3	6488
		Zinc	1.3	0.80	55.7	3.6	0.77	61.6	4.1	0.59	66.8	4.1	0.56	64.9	79.7	222.3	4699
Control	Duragold	Control	1.3	0.85	51.2	1.9	0.83	51.6	3.5	0.75	67.2	3.5	0.70	61.0	77.2	199.0	6505
		Sulphur	1.4	0.80	54.9	2.3	0.85	51.6	3.3	0.78	65.0	3.3	0.66	60.0	80.1	207.3	7035
		Zinc	1.1	0.82	53.7	2.0	0.84	51.9	3.7	0.75	62.4	3.3	0.73	58.4	74.7	194.3	7352
60		Control	1.3	0.83	55.3	3.4	0.82	56.7	3.9	0.70	66.3	3.7	0.62	63.5	67.3	221.3	6088
		Sulphur	1.3	0.79	55.0	3.4	0.80	59.1	4.0	0.69	66.8	3.6	0.66	62.2	86.9	214.0	6637
		Zinc	1.2	0.77	51.7	3.5	0.82	59.3	3.8	0.77	67.6	3.5	0.75	64.4	84.5	202.7	6830
Control	Tamadur	Control	1.1	0.77	48.4	1.6	0.81	47.8	2.1	0.72	53.1	1.7	0.72	48.2	81.9	126.3	6354
		Sulphur	1.4	0.74	51.4	1.5	0.75	47.6	2.4	0.55	56.8	2.2	0.48	52.2	78.2	148.0	3532
		Zinc	1.3	0.83	49.7	1.4	0.85	46.6	2.5	0.74	58.0	1.9	0.71	49.8	78.5	139.3	6434
60		Control	1.5	0.81	53.5	2.5	0.87	57.7	2.7	0.78	60.6	2.4	0.74	57.2	80.9	147.3	6604
		Sulphur	1.6	0.80	53.6	2.8	0.81	54.7	3.3	0.75	62.0	2.6	0.73	57.6	73.7	177.0	7696
		Zinc	1.6	0.82	54.2	2.6	0.82	56.7	3.5	0.70	62.1	2.9	0.64	55.9	66.9	186.7	5806
LSD$_{0.05}$			0.34	0.03	3.21	0.33	0.035	3.12	0.48	0.039	4.22	0.55	0.051	4.60	3.8	24.7	894
CV (%)			10.9	1.9	3.4	8.0	2.4	2.9	6.5	3.4	3.3	7.8	3.6	5.0	3.0	7.7	5.5

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CONCLUSIONS

In conclusion, it could be highlighted that increase in grain yield seems to be related not to spikes density per m^2 but relatively to leaf chlorophyll concentration and NDVI in spring season. The most promising strategy seems to rely on the co-fertilization of nitrogen and zinc, which could improve grain yield of responsive varieties like *Duragold* by about 21.3%. These observations could reinforce the idea that screening large numbers of spring wheat varieties for grain yield, nutrient use efficiency and their adaptability in a set of environmental conditions. Nitrogen fertilized varieties with low spikes density per m^2 could establish better grain yield, probably through minimizing the inter-plant competition of sparsely populated plants. Although robustness and reduction have been observed, the traits such as LAI, NDVI, and SPAD values could be improved in a nitrogen and developmental stage-dependent manner, even the smallest changes in the nitrogen application rate could cause significant alterations in these traits. Hence, the observed positive effect of sulphur, nitrogen and zinc application on physio-morphology and yield formation substantiates the need to include these essential nutrients in the cultivation system of spring wheat. However, before a wider use of this conclusion, field trails are required across year and under divergence agro-climatic conditions.

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