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NITROGEN FERTILIZER APPLICATION TIME EFFECT ON THE SENESCENCE AND GRAIN YIELD OF DURUM WHEAT (*TRITICUM DURUM DESF*.)

Z. ALHABBAR[1,2*], S.I. ALOBAIDI[1], A.N.A.N. AL-DEEN[3], O.A. ABDULQADER[1], M.A. ABDULATEEF[1], and M.A. HAJI[1]

[1]Department of Field Crops, College of Agriculture and Forestry, University of Mosul, Mosul, Iraq
[2]Australia China Centre for Wheat Improvement, School of Veterinary and Life Sciences, Murdoch University, Murdoch, Western Australia, Australia
[3]Iraqi Ministry of Education, General Directorate of Education of Nineveh, Iraq
*****Corresponding author's email: zaid.alhabbar@uomosul.edu.iq
Email addresses of co-authors: saddam.alobaidi@uomosul.edu.iq, anharnsr418@gmail.com, edu3ab@uomosul.edu.iq, mohammed.akram1985@uomosul.edu.iq, msc.mohammed.ameen@uomosul.edu.iq

SUMMARY

The experiment progressed to evaluate the effects of time nitrogen fertilizer application on the senescence of durum wheat and its association with grain yield and its components. The experimental treatments, set up in a split-plot design, used five durum wheat cultivars (Adnham, Grecale, Duma, Sardar, Svevo) as the main plots, and the time nitrogen fertilizer application (100% tillering, 100% stem elongation, and 50% tillering + 50% stem elongation stages) under two locations in Northern Iraqi conditions. The results of this study indicated that late senescence cultivars (Grecale and Svevo) produced the highest flowering date (123.89 and 123.61 days), number of spikes/m^2 (267.2 and 265.6 spikes), number of grains/10 spikes (330.5 and 323.6 grains), grain yield g/m^2 (532.2 and 511.6 g), and harvest index% (38.72% and 39.86%). Moreover, the time of nitrogen fertilizer application at the tillering stage (early application) influenced most of the studied traits. Finally, extending photosynthesis capacity during the grain-filling phases by delaying the rate of senescence could be a better approach to improving the grain yield of wheat cultivars.

Keywords: Durum wheat (*Triticum durum desf*.), cultivars, senescence rate (SR), time nitrogen fertilizer application (TNFA), grain yield (GY)

Key findings: Leaf senescence is a developmental process controlled by genetic and environmental factors, such as the dose and time of nitrogen fertilizer application. Together, stay-green and early-senescence traits can be good strategies to improve the wheat grain yield. The stay-green trait can maintain the uptake and remobilize the nutrients, while the early senescence can avoid the stress conditions during grain-filling stages in wheat crops.

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INTRODUCTION

Proper usage of fertilizer, particularly nitrogen (N), is critical to minimize input costs and harmful environmental effects. Wheat is the third most important crop behind maize and rice; however, the environment substantially impacts wheat yield and quality. It can help to explain why different regions of the world cultivated many different kinds of wheat (Lobell and Gourdji, 2012). In the Mediterranean climate, wheat is grown in an area characterized by most temperature and rainfall favorable at the early-growing stage, followed by heat stress and water shortage at the developmental stages (Lo´pez-Bellido *et al.,* 2005). In wheat, the grain yield (GY) and quality have effects mainly from the quantity of N remobilized at the filling stages from accumulated N in vegetative parts at the early stage (Kichey *et al*., 2007). Generally, the GY and its components often incur influences from genotype and environmental conditions, such as the application of N rate and time, water deficit, and heat stress, especially at the developmental stages (Abedi *et al.,* 2010; Farooq *et al.,* 2011; Altaf *et al.,* 2021). According to these studies, selecting the proper application of nitrogen rate and time is critical to maximize crop production and minimize N losses by leaching. In cereal crops, the N recovery at sowing is lower than the recovery at late applications between the tillering stage (TS) and stem elongation stage (STES) (López-Bellido *et al.,* 2005; Abedi *et al.,* 2011). The low recovery of N application at the early stage can be due to the high nitrate leaching and denitrification. An explanation for this could be the low N demand of the small plants (root and shoot) during the early stages. Inversely, many studies mentioned that early N application between early TS and the end of STES stages could achieve the maximum GY in wheat. It may refer to the highest N demand generally occurring during TS when the biomass accumulates quickly (Malhi *et al.,* 2006). Nitrogen availability, light intensity, temperature, and water deficit significantly influenced the grain-filling duration in wheat. All these conditions lead to an increased grain-filling period, which results in well-filled grain (Garrido-Lestache *et al.,* 2004); whereas, under stress conditions, drought, and heat, as in the Mediterranean climate, where the grain-filling period is shorter, leads to lower GY.

In wheat, cultivars with a high capacity to uptake nitrogen from the soil to the plant and utilize nitrogen from the tissues to sink could store the highest GY under different growth conditions (Alhabbar *et al.,* 2018b). Many studies found that cultivars with late leaf senescence might have longer grain-filling duration by maintaining the nitrogen uptake and translocation in wheat (Hitz *et al.*, 2017). Therefore, selecting cultivars with a late-leaf senescence rate could benefit by providing extra carbon and nitrogen, leading to high grain yield and components, especially under favorable situations.

Senescence is the last developmental process when the plant tissues turn yellow and have genetic control and environmental conditions largely influencing it. Generally, it begins about 10–24 days from the beginning of anthesis, which allows more refilling of nutrients from leaf tissue into the sink (Gregersen *et al.,* 2013; Chapman *et al.,* 2021). All environmental factors influencing plant senescence could consist of two-factor classification: abiotic, such as water stress and nutritional deficiency, and biotic, such as pathogen infection. Nitrogen availability in the plant strongly influences the leaf's senescence rate (SR). For example, the low N condition could accelerate the onset of SR; however, the high availability of the N condition can lead to delays in the onset of SR in wheat (Havé *et al.,* 2017; Chen *et al.,* 2020). Early leaf senescence correlates to high grain protein content and slightly low GY; however, delayed leaf senescence interrelates with high GY and relatively low grain protein content in wheat. Early and late leaf senescence mainly has control from the senescence-regulation-associated genes and environmental conditions (Alhabbar *et al.,* 2018a; Sultana *et al.,* 2021). The current study aims to determine a correlation between the senescence rate and grain-filling duration. It could improve grain yield by extending the CO_2 assimilation duration and photosynthesis in durum wheat, especially under favorable situations.

MATERIALS AND METHODS

Site and crop management

Field experiments conducted in the Mosul region (North Iraq) had two locations for the winter agricultural season 2021–2022. The first experiment commenced at Nimrud (Balawat village), which lies 25 km southeast of the city of Mosul (longitude 36°13′35.1 North, latitude 43°25′10.4 East), and the second at Bashiqa, 12 km northeast of the city of Mosul (longitude 36°27′41.7 North, latitude 43°19′34.5 East). Both locations are in the Mediterranean region, with information about monthly rainfall and minimum and maximum temperatures shown in Table 1.

The experiment setup was a randomized complete block design with a split plot and three replications. The main parcels included the time nitrogen fertilizer application (TNFA) (100% tillering stage [TS], 100% stem elongation stage [STES], and 50% TS + 50% STES). The subplots comprised five durum wheat cultivars (Adnham, Grecale, Duma, Sardar, and Svevo). A subplot consists of five lines with a length of 1 m, and the distance between one line and another was 18 cm with a 30 cm gap across an experimental unit. The sowing date started on 1 December 2021, the recommended date for wheat crops in the northern Iraqi farming districts.

The TNFA gained synchronization with the Zadoks scale (Z) of wheat growth stages (Zadoks *et al.,* 1974). Hence, these were T1 = 80 kg N/ha (topdressing) supplied at early TS (Z21–Z23); T2 = 80 kg N/ha (topdressing) given at early STES (Z31–Z32), and T3 = 40 kg N/ha supplied at TS, and 40 kg N/ha applied at early STES. Urea fertilizer (46% N) was the chief source of nitrogen. In addition, the recommended dose (160 kg/ha) of Diammonium phosphate application proceeded by furrow placement before sowing. The soil sample collected was from 0 to 30 cm depth before wheat sowing. Soil analysis for nitrate content appears in Table 2. In the two locations, the rainfall season was only about 160–270 mm (Table 1), thus requiring only three times separate irrigation applications (50 mm every time) supplied in February and March.

Measurements

The flowering date recording ensued when around 50% of the spikes in each plot showed visible stamens (Z61–65). Total above-ground biomass or AGB (grain and straw) collection transpired on 3 June 2022, when all plants, upon inspection, reached full maturity. The number of grains/10 spikes measurement comprised counting the number of grains of 10 spikes/plot using a grain counter machine. The number of spikes estimation consisted of totaling the spikes in three middle rows/plots. The Harvest index (HI) was calculated by the grain ratio to AGB at harvest (Richards et al. 1987).

Stay-green cultivars (late of senescence)

The Chlorophyll Concentration Meter, IC-CCM-200 (Apogee Instruments), has helped estimate the amount of chlorophyll in the flag leaf. The IC-CCM-200 measures the Chlorophyll Content Index by dividing the transmission of radiation (931 nm) by the transmission of radiation (653 nm) (Padilla *et al.,* 2019). The values of five plants were taken per plot at the middle position on the flag leaf at every stage. The CCI measurements recording occurred at seven developmental growth stages (Ear emergence from boot [Z55-Z59], anthesis [Z61-Z65], seven, 14, 21, 28, and 35 days after anthesis [DAA]) based on the Zadok scale. The SR of the flag leaf estimation resulted when the chlorophyll content decreased rapidly after the anthesis stage (Ommen *et al.,* 1999; Gelang *et al.,* 2000). The maximum chlorophyll content index appeared seven days after anthesis (7DAA) in all five durum wheat cultivars. Therefore, the calculation of SR was (MaxCCI – XCCI) / MaxCCI × 100, where MaxCCI is the plant's maximum CCI value before it declines, and XCCI is the CCI value at the specific stage used to calculate flag leaf SR reduction (Alhabbar *et al.,* 2018b).

Table 1. Average of monthly rainfall during growing season.

Locations	December	January	February	March	April	May
Bashiqa	76	117	16.5	24	13	21.5
Nimrud	51	43	13	10	20	21

The weather data came from the General Authority for Meteorology and Seismic Monitoring at Rashidiyah City.

Table 2. Chemical analysis of the experimental soil.

Locations	Clay %	Silt %	Sand %	Textural	N (ppm)	P (ppm)	K (ppm)	Organic matter	PH	EC (us/cm)
Bashiqa	37.7	37.55	27.75	Silty-clay	0.023	10.58	28	1.857	8.8	1400
Nimrud	23.95	33.37	42.67	Mix	0.012	13.83	9.143	1.724	7.5	1080

The Central Laboratory, College of Agriculture and Forestry, University of Mosul.

Statistical analysis

Analysis of variance ANOVA employed the GenStat software to investigate the effects of location, cultivar, and TNFA when F-values were significant, and the least significant difference (LSD) test and standard deviation served to compare treatment differences in means. In all cases, the differences seemed noteworthy if $P < 0.05$. The correlation analysis advanced to examine the relationship between the SR, GY, and its components. Rate bar graphs construction materialized for the significant effect of cultivars, growth stages, TNFA, and the interaction between five durum wheat cultivars and different growth stages on the senescence, applying error bars with standard deviation.

RESULTS

The climate data showed acceptable rain amounts in April and May, which is unexpected in the study region (Mediterranean climate) (Table 1). The stay-green cultivars possess a longer grain-filling phase, allowing additional CO_2 assimilation and photosynthesis. These advantages for stay-green cultivars could lead to high GY, especially under favorable conditions during grain-filling.

Identification of early-senescence and stay-green cultivars

The identification of early-senescence and stay-green cultivars was according to the Chlorophyll Concentration Meter after seven DAA (Maximum CCI). The SR showed a wide-ranging difference among the five cultivars during the developing stages (Figure 1a). Among growing periods, the SR significantly increased during phases shown in Figure 1b. Averaged over the TNFA, location, and stages, the SR significantly differed between the five cultivars. Grecale and Svevo cultivars (stay-green) have a lower senescence rate than the other cultivars (early-senescence). TNFA was insignificant; however, the late application (T2 and T3) achieved less SR than the early application (Figure 1c). The interaction of cultivars and growth stages significantly impacted the SR ($P < 0.001$). At the 14 DAA, all five cultivars have the same SR. However, at later stages, 21, 28, and 35 DAA, both cultivars Grecale and Svevo (stay-green) have lesser SR than the Sardar, Duma, and Adnham (early-senescence), as shown in Figure 1d. For the rest of the interactions, most had no significant difference in influence on the rate of senescence.

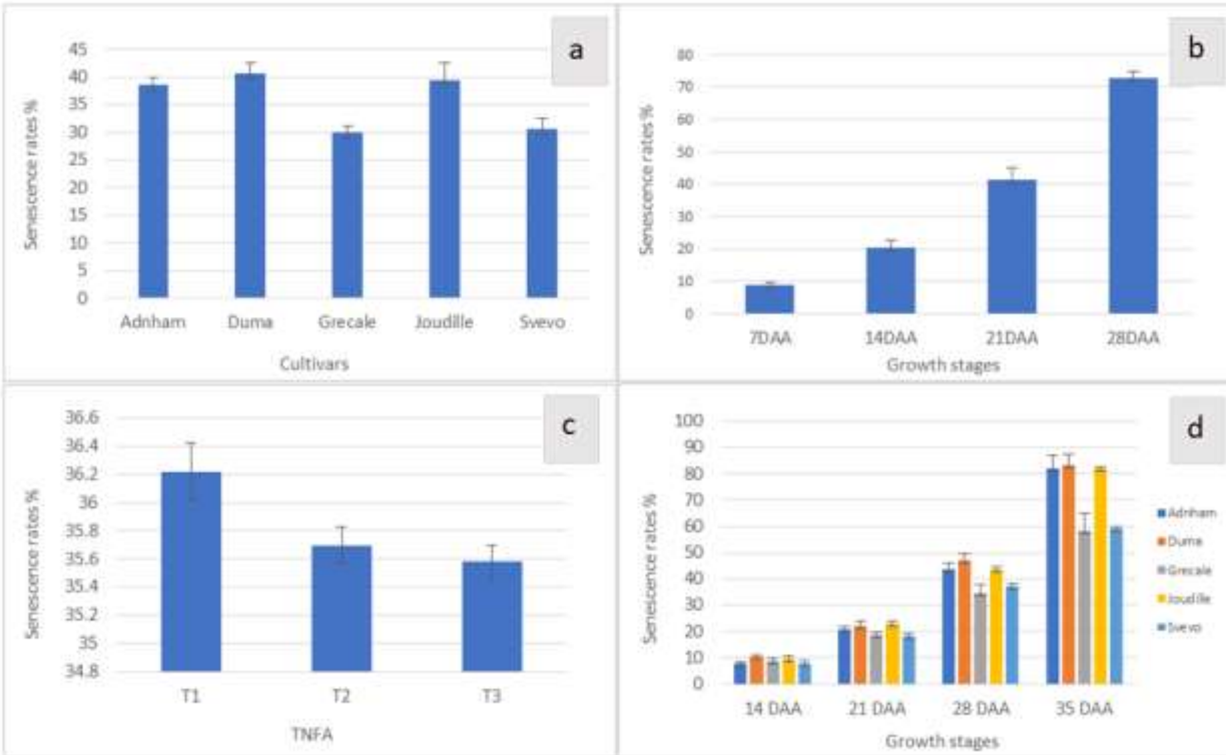

Figure 1. Effect of five durum wheat cultivars (a), growth stages (b), time nitrogen fertilizer application (TNFA) (c), and the interaction between the durum wheat cultivars and wheat growth stages (d) on the senescence rate%. The standard deviation is shown with error bars above each bar.

Effect of five cultivars, TNFA, and locations on the agronomic traits

Most vegetative traits had significant differences in locations, cultivars, and TNFA. The mean plant height in Nimrud (74.3 cm) was higher than that in Bashiqa (68.19 cm). The Duma cultivar had the tallest plant height compared with Sardar (Table 3). On the average of TNFA, the application at T1 gave the maximum plant height, whereas applying the N at T3 produced the lowest plant height.

The flowering date has significant influences from the locations, cultivars, and TNFA (Table 3). The plants of Bashiqa have an early flowering date (118.2 days) compared with the Nimrud location (128.51 days). On average, the Sardar cultivar reached the flowering date earlier than other cultivars. The AGB also had locations, cultivars, and TNFA affecting it. The plants of Bashiqa produced the maximum AGB (1365 g/m2) versus Nimrud (1269 g/m2). TNFA contributes to the AGB; the highest value reached was when adding N at the TS (1410 g/m2). Overall, there were no significant differences in most agronomic traits between interactions among locations, cultivars, and TNFA (Table 3).

Effects of locations, durum wheat cultivars, and TNFA on the GY and its components

The GY and its components significantly varied among locations, cultivars, and TNFA, as shown in Table 4. Across the cultivars and TNFA, the plants of Bashiqa gave a maximum thousand-grain weight than Nimrud. The five cultivars indicated that Duma has the highest thousand-grain weight (54.77 g), while Adnham has the lowest (44.85 g). Among the TNFA, the late application (STES) produced the highest thousand-grain weight (54.77 g) compared with TS (early application) (45.80

Table 3. Effects of locations, durum wheat cultivars, and TNFA on Plant Height, Flag Leaf Area, Flowering, AGB, and Chlorophyll Content Index.

Locations (L)	Plant Height (cm)	Flag Leaf Area (cm^2)	Flowering (Day)	AGB (g/m^2)	Chlorophyll Content Index (CCI)
Bashiqa	68.19	20.54	118.2	1365	44.46
Nimrud	74.3	30.99	128.51	1269	44.14
Test F	<.001***	<.001***	<.001***	0.022*	0.610
LSD	1.775	1.292	0.466	81.4	N.S
Cultivars (CV)					
Duma	75.06	23.96	123.28	1266	43.04
Grecale	71.61	26.07	123.89	1387	45.16
Adnham	68.11	26.72	123.56	1365	43.64
Sardar	66.89	25.04	122.44	1237	44.89
Svevo	74.57	27.03	123.61	1328	44.77
Test F	<.001***	0.021*	0.003**	0.110	0.164
LSD	2.806	2.042	0.736	NS	NS
Time of N applications (TNFA)					
T1	72.28	26.53	123.10	1410	44.67
T2	72.21	25.52	123.40	1269	44.01
T3	69.26	25.25	123.57	1271	44.22
Test F	0.008**	0.241	0.259	0.008**	0.682
LSD	2.174	NS	NS	99.7	NS
L x CV	0.003**	0.061 NS	<.001***	0.191 NS	0.052 NS
L x T	0.139 NS	0.666 NS	0.114 NS	0.009**	0.010*
CV x T	0.039*	0.162 NS	0.936 NS	0.191 NS	0.144 NS
L x CV x T	0.002**	0.577 NS	0.457 NS	0.023*	0.908 NS

- aboveground biomass (AGB); *, **, *** indicate significance at 0.05, 0.01, and 0.001, respectively; NS = nonsignificant.

Table 4. Effects of locations, durum wheat cultivars, and TNFA on the thousand-grain weight, number of spikes and grains, grain yield, and harvest index.

Locations (L)	Thousand-grain weight (g)	Number of spikes ($Spike/m^2$)	Number of grains (10 Spikes)	GY (g/m^2)	Harvest Index (%)
Bashiqa	52.32	247.9	213.8	464.9	34.43
Nimrud	49.68	231.1	399.4	478.8	37.74
Test F	0.010**	0.029*	<.001***	0.297	0.002**
LSD	1.991	14.98	11.82	N.S	2.004
Cultivars (CV)					
Duma	54.77	222.5	282.6	423.9	33.59
Grecale	49.60	267.2	330.5	532.2	38.72
Adnham	44.85	223.6	290.1	469.9	34.90
Sardar	54.27	218.6	306.1	421.6	33.36
Svevo	51.51	265.6	323.6	511.6	39.86
Test F	<.001***	<.001***	<.001***	<.001***	<.001***
LSD	3.148	23.69	18.68	41.55	3.168
Time of N applications (TNFA)					
T1	45.80	240.0	320.1	523.0	36.85
T2	54.45	231.2	300.5	417.9	33.25
T3	52.75	247.3	299.2	474.6	38.15
Test F	<.001***	0.219	0.008**	<.001***	<.001***
LSD	2.438	NS	14.47	32.18	2.454
L x CV	0.791 NS	0.511 NS	0.083 NS	0.188 NS	0.040*
L x T	0.333 NS	0.013*	0.016*	0.007**	0.743 NS
CV x T	0.072 NS	0.007**	<.001***	0.117 NS	0.305 NS
L x CV x T	0.166 NS	0.781 NS	<.001***	0.025*	0.868 NS

- Grain Yield (GY); *, **, *** indicate significance at 0.05, 0.01, and 0.001, respectively; NS = nonsignificant.

g). Regarding the mean of locations, the plants of Bashiqa produced more spike numbers (247.9 spikes/m2) than Nimrud (231.1 spikes/m2). The highest number of spicules were visible with Grecale, with an average of 267.2 spikes/m2. The mean of cultivars and TNFA indicated the superiority of the plants of the Nimrud location with the maximum number of grains (399.4 grains/10 spikes) over the plants of the Bashiqa location (213.8 grains/10 spikes). Regarding the mean values for the cultivars, Grecale produced the highest number of grains compared with Duma. Applying N fertilizer at TS achieved the maximum number of grains (320.1 grains/10 spikes). The GY differed among the five wheat cultivars and TNFA (Table 4). The Nimrud location provided the highest mean of GY (478.8 gm), but it was not significantly varied as that of the Bashiqa location (464.9 gm). The values of cultivars over sites and TNFA resulted in a 20.8% higher GY of Grecale than Sardar. As for the TNFA, the highest GY occurred when adding N fertilizer at TS (523.0 gm). Regarding HI, the plants of the Nimrud location produced higher HI (37.74%) than the Bashiqa location (34.43%). The Svevo cultivar has a better HI than the Sardar cultivar. Applied N 50% at TS + 50% STES has better HI than STES. Overall, there was a significant difference in most GY and its component traits over the interactions between locations, cultivars, and TNFA (Table 4).

Correlation between SR with agronomic traits and GY components

Results of the correlation analysis between SR, agronomic traits, and GY components of early cultivars (Duma, Adnham, and Sardar) and late cultivars (Grecale and Svevo) under different TNFA are available in Table 5. Overall, the results of correlation indicate a negative association among SR at seven, 14, 21, and 28 DAA and AGB (r = -0.06, -0.14, -0.12, and -0.36, respectively), harvest index (r = -0.50, -0.33, -0.34, and -0.31, respectively), and GY (r = -0.30, -0.07, -0.50, and -0.48, respectively) under early-maturity cultivars (Duma, Adnham, and Sardar). However, a positive association was evident among SR and AGB (r = 0.38, 0.56, 0.33, and 0.19, respectively), harvest index (r = 0.22, 0.30, 0.05, and 0.48, respectively), and GY (r = 0.51, 0.26, 0.54, and 0.58, respectively) under late-maturity cultivars (Grecale and Svevo).

DISCUSSION

Both stay-green and early-senescence traits could be valuable indicators for improving the GY of wheat in specific areas. Usually, under Mediterranean climates, such as Northern Iraq, the growing seasons of wheat ears for both temperature and rainfall are appropriate

Table 5. Correlation between SR during grain-filling stages with agronomic traits of early senescence and late senescence under locations and TNFA.

Traits	Early senescence				Late senescence			
	Sen. 7	Sen. 14	Sen. 21	Sen. 28	Sen. 7	Sen. 14	Sen. 21	Sen. 28
Plant height	-0.36	-0.38	-0.09	-0.08	0.06	-0.43	-0.27	0.17
Chlorophyll	-0.04	-0.03	0.26	0.19	-0.06	0.01	-0.19	-0.43
Flowering	-0.41	-0.54*	-0.35	-0.33	0.09	-0.22	-0.20	0.38
Flag Leaf Area	-0.38	-0.45	-0.36	-0.27	0.10	-0.28	-0.20	0.47
AGB	-0.06	-0.14	-0.12	-0.36	0.38	0.56*	0.33	0.19
Number of spikes	0.004	0.49*	0.26	0.30	0.04	0.40	-0.26	-0.25
Number of grains	-0.60**	-0.50*	-0.25	-0.25	0.01	-0.50*	-0.35	0.31
Thousand-grain weight	0.30	0.02	0.05	0.02	-0.55*	-0.20	0.11	-0.21
Harvest Index	-0.50*	-0.33	-0.34	-0.31	0.22	0.30	0.05	0.48
GY	-0.30	-0.07	-0.50*	-0.48*	0.51*	0.26	0.54*	0.58*

- *, ** indicate significance at 0.05 and 0.01, respectively. Senescence rate (SR) at 7DAA (Sen 7), senescence rate at 14DAA (Sen. 14), senescence rate at 21DAA (Sen. 21), senescence rate at 28DAA (Sen. 28).

through a vegetative stage (i.e., January, February, and March), whereas with regular heat, stress, and lack of rains, are ineffective through a grain-filling phase (April and May). However, in the presented study, there was an acceptable amount of rainfall during the maturity stage in both locations. The stay-green trait describes the plant's ability to maintain its leaves green for a longer duration, often, but not always exhibiting higher GY than early-senescence plants (Luche *et al.,* 2015; Alhabbar *et al.,* 2018b).

Many studies found that stay-green plants with a slow SR possess high N uptake and remobilization by extending grain-filling periods (Lopes and Reynolds*,* 2012; Hitz *et al.,* 2017; Alhabbar *et al.,* 2018b). Our results indicated that the SR completely differs for the five durum wheat cultivars (Figure 1). Duma, Adnham, and Sardar (early-senescence) cultivars own an early and fast SR; however, delayed SR occurred within the cultivars Grecale and Svevo (stay-green). Therefore, the two cultivars, Grecale and Svevo, have more chance to uptake and accumulate N during the post-anthesis growth stages. In general, this information could help select cultivars with an extending capacity of photosynthesis during the grain-filling phases by delaying the SR, increasing the GY potential (Tamrazov, 2022; Khanishova and Azizov, 2023; Turganbayev *et al*., 2023).

Optimum TNFA is a good approach for improving recovery of applied N up to 70%, thus raising the GY and quality of wheat. In the relevant study, the TNFA improved most GY and its component traits. The early applications (TS) increased the plant height, flag leaf area, AGB, number of grains per 10 spikes, GY, and HI; however, the late application (STES) improved the thousand-grain weight. A synchronization in N application between time supply and plant demand increases crop productivity and reduces N losses by leaching (Chen *et al.,* 2006; Gentile *et al.,* 2009). In wheat, the highest N demand ranged between TS and STES phases when plant targets produce high AGB and GY, and in an application at a later level, around the flowering stage, when plant targets high protein in grains.

Table 5 shows the correlations between the SR with studied traits among the early- and late-senescence cultivars. Overall, the results suggest a negative association between the SR at seven, 14, 21, and 28 DAA and AGB, HI, and GY under early cultivars, while a positive association with late cultivars. However, the thousand-grain weight correlated positively under early cultivars and negatively under late cultivars.

A possible explanation for this might be that early senescence could increase nutrient remobilization from tissue to grain, leading to an increase in a thousand-grain weight compared with the stay-green (Gregersen *et al.,* 2008; Alpuerto *et al.,* 2021; Sultana *et al.,* 2021). However, the stay-green maintains the capacity of N absorption and photosynthesis at the developmental stages, leading to a higher biomass accumulation (Borrell *et al.,* 2001).

CONCLUSIONS

The study concluded that the cultivars with late senescence own an extended grain-filling duration and generally produce better grain yield and enhance its components. Moreover, applying nitrogen fertilizer at the tillering stage (early application) could improve grain yield due to meeting the application time when the plant needed N fertilizer.

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