Evaluation of gene expression and enzymatic and biochemical activities of leaves in native and foreign genotypes of bread wheat before and after anthesis

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Abstract

Wheat flag leaf plays a valuable role in the production of dry matter, and the transfer of photosynthetic material to the grain and related physiological properties such as chlorophyll content and photosynthetic efficiency are important factors in increasing yield. This experiment was carried out in Golestan province, in a randomized complete block design (RCBD) with four replicates. Wheat genotypes included N-91-9, N-91-17, Tiregan, Nogal, Euclide, Agorazado, Lucullus, and Antonius. Sampling from flag leaf and other leaves was done at 10 stages included 7 DBA (day before anthesis), 3 DBA, anthesis, 3 DAA (day after anthesis), 7 DAA, 11 DAA, 15 DAA, 19 DAA, 23 DAA, and 27 DAA, and the samples were carried to laboratory in liquid nitrogen and stored at -80 ℃. Part of the leaf samples was carried with a paper bag and incubated in an oven at 80 ℃ for 48 hours. Biochemical traits such as chlorophyll content, net photosynthetic rate, sucrose content, sucrose synthase (SS), sucrose phosphate synthase (SPS) content, grain yield, and photosynthetic genes expression including *RbcL*, *RbcS*, and *Rca* were measured in all stages. Results showed that flag leaf in the post-anthesis stages has a significant role in photosynthesis and sucrose production in the plant and cultivars with higher chlorophyll content also had higher photosynthesis. Also, in genotypes with higher SS enzyme activity, more sucrose was synthesized and due to the role of SPS, increasing this parameter was involved in the transfer of sucrose from leaves to other organs. The *RbcL*, *RbcS*, and *Rca* genes had higher expression in cultivars with higher photosynthesis than the control (Tiregan). Among the studied genotypes, Nogal cultivar and line N-91-17 was superior in terms of grain yield and other measured traits and can be recommended for use in future research.

Keyword: chlorophyll content, gene expression, photosynthetic, sucrose, wheat

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Introduction

Flag leaf plays a valuable role in the production of dry matter, and the transfer of photosynthetic material to the grain and related physiological properties such as chlorophyll content and photosynthetic efficiency are important factors in increasing yield. Photosynthetic efficiency, continuity, and dependent regulatory mechanisms are all controlled by gene expression pattern (Navabpour, 2011). Evaluation of the expression of genes involved in photosynthesis and leaf

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ageing processes makes it possible to justify many physiological interactions that lead to changes in important agronomic traits at both cellular and molecular levels (Zhao et al., 2018).

Chlorophyll is a key chemical compound in plants that is essential for photosynthesis. The amount of photosynthetic pigment chlorophyll in living plants is one of the important factors in maintaining their photosynthetic capacity (Roca et al., 2016). Numerous indicators have been presented to evaluate the stability of physiological processes of the plant, and one of the most important physiological criteria is chlorophyll stability (Fahad et al., 2017). Chlorophyll content is low in the early stages of plant growth, and as it gets closer to the physiological maturation of the seed, its amount increases, which is probably due to the plant's severe need for grain reserves. Finally, with the onset of ageing and yellowing of the leaves, the amount of chlorophyll has decreased significantly. As the phenological stages progresses, the chlorophyll content of the cultivars decreases, which is related to the yellowing of the plant at the end of the plant growth stages (Shi et al., 2016).

Current photosynthesis, as one of the most important sources of carbon for grain filling, depends on the effective absorption of light by the plant's green surface after the pollination stage (Golabadi et al., 2015). This source is generally limited by the natural ageing of the leaves and the occurrence of various stresses. However, the demand for photosynthetic materials during the grain development and the demand for respiration of plant biomass retention also increase during this period (Ezat Ahmadi et al., 2012). The role of current photosynthesis in grain yield can be considered as a selective mechanism because the remobilization process in both the accumulation and remobilization stages requires energy. In other words, when the material from the current photosynthesis is sufficient to fill the grain, the flow and retransfer of the photosynthetic material is limited (Millard et al., 2015). The capacity of source tissues in the production of photoassimilates from flowering to full maturity, during which grain formation occurs, plays an important role in the grain filling process. Leaf photoassimilates in this period are mainly in the form of sucrose and high photosynthesis in leaves produces enough sucrose which is useful in improving yield (Lemoine et al., 2013). In sugar metabolic pathways, two enzymes, namely sucrose synthase and sucrose phosphate synthase, are involved in the production of sucrose, and these enzymes have been reported to increase photosynthesis, as well as help maintain a high rate of photosynthesis (Stein and Granot, 2019). Results of other studies have shown that the genotypes with a higher photosynthetic capacity in the pre- and post-pollination stages are more successful in the grain filling process than other genotypes, and they will have a higher yield.

Rubisco is the most abundant leaf protein in C3 plants and as it catalyzes the first steps in photosynthesis and light respiration, it plays a key role in photosynthesis (Suzuki et al., 2009). Rubisco enzyme is made up of two subunits, small and large (Suzuki and Makino, 2013). Eight small subunits are encoded by a nuclear multi-gene family (*RbcS*) and eight large subunits are encoded by a single gene (*RbcL*) in the chloroplast genome. The coordination of the expression of these two genes plays an important role in photosynthesis due to the fact that each of them is expressed in a separate part of the cell (Vitlin Gruber and Feiz, 2018). Rubisco activase (*Rca*) regulates the activity of rubisco enzyme and plays a key role in photosynthesis. RCA removes inhibitory sugar phosphates from the active sites of rubisco in an ATP-dependent way and thus activates rubisco (Kumar et al., 2016). Accordingly, in this study, the traits and expression of photosynthetic genes, as well as the amount of grain yield, sucrose, and enzymes involved in the synthesis of sucrose in flag leaves and other leaves in domestic genotypes and foreign cultivars, were evaluated. The aim of this study was to investigate the role of photosynthesis and the amount of sucrose produced in this process during the formation of grain yield of different wheat genotypes.

Materials and Methods

Plant material

This experiment was carried out in Golestan, Kordkoy, and in the Research Farm of Georgian

Research Center (latitude and longitude 36.816067, 54.195777) arranged in a randomized complete block design (RCBD) with four replicates during 2018-2019. Wheat genotypes were grown using common agriculture practices and included N-91-9, N-91-17, Tiregan, Nogal, Euclide, Agorazado, Lucullus, and Antonius. N-91-9 and N-91-17 were Iranian advanced lines and Tirgan was one of the Iranian cultivars that commonly cultivated in the region. Nogal, Euclide and Agorazado were French cultivars, and Lucullus and Antonius were Austrian cultivars. Foreign cultivars are cultivated for the first time in Iran. For sampling with Zakoks scale, over 60 plants of all genotype were labeled, and sampling was carried out from flag leaf and other leaves at 10 stages including 7DBA, 3DBA, Anthesis, 3DAA, 7DAA, 11DAA, 15DAA, 19DAA, 23DAA, and 27DAA which were carried to the laboratory in liquid nitrogen and stored in a freezer at - 80 ℃. Part of the leaf samples was carried with a paper bag and incubated in an oven at 80 °C for 48 hours. From an area of one square meter, plants were harvested at the mature stage, and after separating the grains, the grain yields were calculated. The analysis of morphophysiological data was conducted using SAS 9.2 with proc GLM, and the comparisons of means were performed using LSD at 5% probability level.

Total chlorophyll

Poura et al. (1989) method was used to measure total chlorophyll content, where 0.5 g of leaf sample (stored frozen at - 80 ℃) was completely crushed and mixed with 10 ml of 80% acetone at room temperature until the tissue was completely bleached. After centrifugation at 5,000 g for 10 min, the absorbance (A) was recorded by a spectrophotometer at 646.6 and 663.6. The amount of total chlorophyll was calculated based on the following formula described by Chen et al. (2015):

Total Chlorophyll Content = 20.21 A645 + 8.02 A663

Net photosynthetic rate and sucrose content

Net photosynthetic rates of leaves were measured in the field from 10:00 AM to 12:00 AM with a portable photosynthesis system (Ciras-1, System, UK). The flag leaf and also the other leaves were placed in a chamber at a photon flux density of 1,000 $\mu molm^{-2}s^{-1}$; the flow rate through the chamber was 500 $\mu mols^{-1}$, and leaf temperature was nearly 28 ℃. The ambient CO_2 concentration was nearly 400 µmol CO_2 mol^{-1} air, and vapor pressure deficit was maintained at approximately 2.0 kPa (Chen et al., 2015). Seven plants were selected from each cultivar according to the sampling stage for measurement. Dried samples were ground to a fine powder for sucrose analysis. The sample powder (approximately 0.5 g) was extracted using 6 mL of 80% (v/v) ethanol for 40 min in a water bath at 80 ℃; then, the supernatant was collected after centrifugation at 5,000 g for 10 min. The supernatants were diluted with 80% ethanol to 25 mL for the measurement of sucrose content. Sucrose content was measured using resorcinol and estimated based on the absorbance at a wavelength of 480 nm and a standard curve (Shi et al., 2016).

Measurement of sucrose synthase (SS) and sucrose phosphate synthase (SPS) activities

Frozen leaf samples (approximately 0.5 g) were placed in a Chinese pounder and homogenized to a powder in liquid nitrogen. Approximately 2 mL of HEPES/NaOH (pH 7.5) buffer was added for enzyme extraction in an ice bath and centrifuged at 11,000 g and at 4 ℃ for 10 min. The supernatant was transferred to a new tube and used for the measurement of SS and SPS activities (Douglas et al., 1988). SS and SPS activities were determined according to Miron and Schaffer (1991) method.

Table 1
Primers sequences used in the RT-qPCR analysis

	(3' → 5') Forward	Reverse (5' → 3')	Product Length (bp)	Tm (℃)
RbcL	GGTGGAGGAACTTTAGGACAT	TCGCCTTCCATACTTCACAA	187	60
RbcS	ACTGGACAATGTGGAAGC	ACTCCTTCTTGACCTCCTC	84	60
Rca	TACGACATCTCCGATGACCA	CTCGTAGGAGCTCAGGATGG	114	60
GAPDH	TCACCACCGACTACATGACC	ACAGCAACCTCCTTCTCACC	121	60

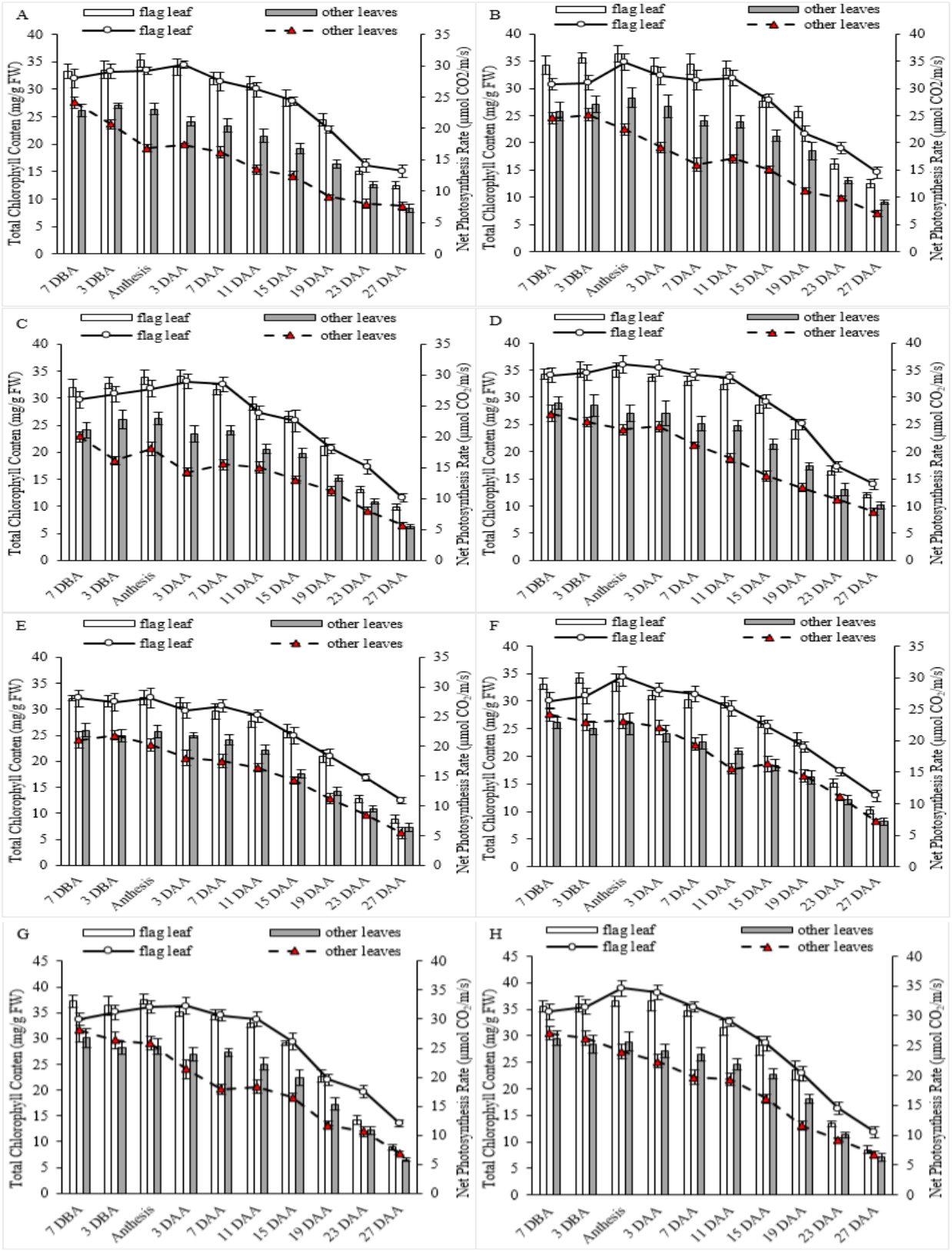

Fig I. Total chlorophyll content (columns) and net photosynthesis rate (Lines) of wheat flag leaf and other leaves; genotypes included: N-91-9 (A), N-91-17 (B), Tiregan (C), Nogal (D), Euclide (E), Agorazado (F), Lucullus (G), and Antonius (H); error bars represent twice the standard error and indicate significance and non-significance.

reverse transcription

RNA extraction was done using Biozol reagent (Bio PLUS, Japan). RNA samples were treated with RNAse-free DNase I (Biolabs) to eliminate any DNA contamination. Total RNA was checked by electrophoresis on 1.5% agarose gel, and RNA concentration was determined using a NanoDrop spectrophotometer BT-600 (Thermo Scientific). the first-strand cDNA was then generated from 1 µg of template RNA and tested by housekeeping gene primers using PCR. The specific function of primers used by cDNAs was evaluated in a standard polymerase chain reaction.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Primers were designed relying on the information available on the NCBI site using AlleleID7 software. RT-qPCR reactions were carried out by an IQ5 machine, Bio-Rad Company and Cyber Bio Pars Kit (Gorgan University of Agricultural Sciences and Natural Resources) that was able to evaluate in real-time. As housekeeping genes, GAPDH gene was used for normalization of target genes expression. The sequences of the primers used in the RT-qPCR analysis are listed in Table 1. At the end of the reaction, after receiving the charts, the information was transferred to the REST software and the data were analyzed. Gene expression was assessed by $2^{-\Delta\Delta CT}$ (Pfaf 2001) in relation to the control plants at the same stage with three biological replicates (each replicate consisted of four pooled plants). The illustrations were drawn using Excel 2016 software.

Results

Biochemical traits

The results of chlorophyll content and photosynthesis network rate in flag leaf and other leaves for the studied genotypes are shown in Fig. I. Most genotypes had the highest chlorophyll content in both flag leaves and other leaves between 7 DBA and 3 DAA. Also, the amount of chlorophyll in flag leaf was higher than other leaves in all stages and in all genotypes. The highest amount of chlorophyll (37.6 mg g^{-1} FW) belonged to Lucullus cultivar that was observed in anthesis stage and in flag leaf (Fig I. G). The general trend of changes in chlorophyll content in both flag leaf and other leaves in most genotypes after the anthesis stage was a decrease due to approaching the final stages of growth and yellowing of leaves due to aging. Of course, only in the case of Antonius and Tiregan cultivars on the flag leaf this upward trend continued until the 3 DAA stage. Meanwhile, the chlorophyll content of N-91-17 line and Nogal cultivar, in addition to being higher than most other genotypes, did not decrease significantly compared to the anthesis stage up to 11 DAA stage, indicating the superiority of chlorophyll content of these genotypes to other genotypes. On the other hand, in Nogal cultivar the chlorophyll content of other leaves (Fig. I. D) was similar to that of the flag leaf and was higher than that in other genotypes.

The increasing trend of photosynthesis in flag leaf up to anthesis stage can be seen in all genotypes (Fig. I). This upward trend continued in Line N-91-9, Tiregan, and Lucullus cultivars until the 3 DAA stage, and a decrease in photosynthesis rate was observed in other genotypes. The highest photosynthesis rate was related to Nogal cultivar (Fig. I. D) with 36 µmol $CO_2/m^{-1}/s^{-1}$ in anthesis stage. In Nogal and Lucullus cultivars, despite the increasing-decreasing trend, the amount of photosynthesis was observed in the period of 7 DBA to 11 DAA, but the difference between the observed values at different times was not significant, indicating more stability of photosynthesis in these cultivars. Photosynthesis rate had different conditions compared with changes in chlorophyll content in different genotypes and at different stages. This is because the amount of photosynthesis, in addition to being a function of the amount of chlorophyll, also requires sufficient light. The rate of photosynthesis in the flag leaf had an increasing-decreasing trend while in other leaves in almost all genotypes the rate of photosynthesis showed a decreasing trend. The lower rate of photosynthesis and its decrease in other leaves since the appearance of flag leaf is due to the expansion of the flag leaf which limits the light received by the other leaves. As Fig. (II) shows, different trends were observed in sucrose content

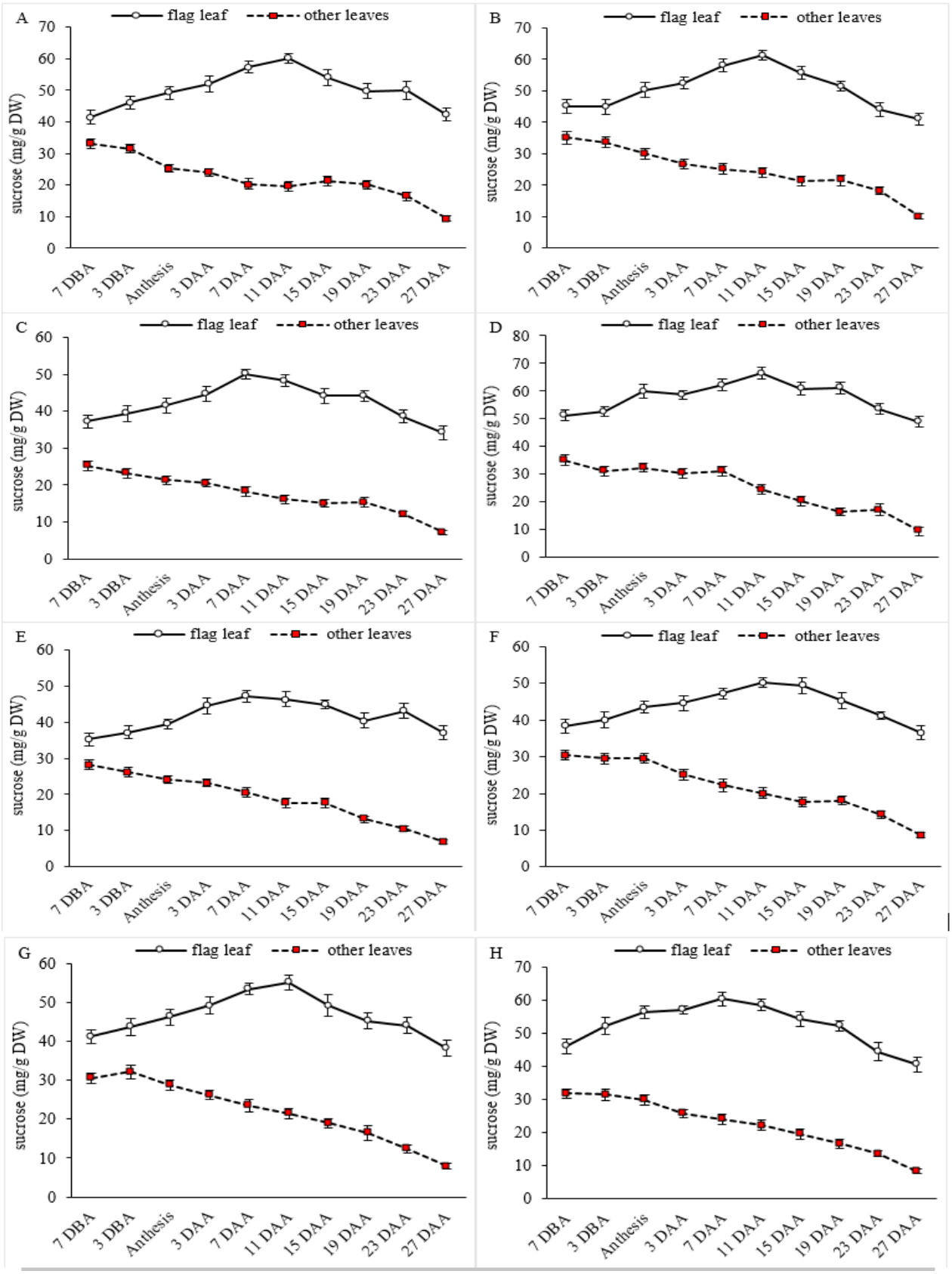

Fig. II. Sucrose content of wheat flag leaf and other leaves; genotypes included: N-91-9 (A), N-91-17 (B), Tiregan (C), Nogal (D), Euclide (E), Agorazado (F), Lucullus (G), and Antonius (H)- Error bars represent twice the standard error and indicate significance and non-significance.

of flag leaves in different genotypes. In Tiregan, Euclide, and Antonius cultivars, the highest amount of sucrose was observed in 7 DAA stage while in other genotypes, the highest amount of sucrose was observed in 11 DAA stage. In Nogal and Tiregan cultivars, the amount of sucrose in the flag leaf did not decrease in stage 19 DAA compared to stage 15 DAA, but its increase was not significant. Also, in Line N-91-9 and Euclide cultivars similar conditions were observed in stage 23 DAA compared to stage 19 DAA. Nogal cultivar (Fig II. D) had higher sucrose content in both flag leaves and other leaves compared with other genotypes in most of the studied stages. The sucrose content in other leaves showed a decreasing trend for all genotypes since the 7-DBA stage. On the other hand, in some stages where a slight increase was observed in the sucrose content, the difference with the previous stage was not significant. In most genotypes, sucrose content in other leaves did not decrease significantly until anthesis and in Nogal cultivar (Fig. II. D) this condition was observed up to 7 DAA.

The activity of sucrose synthase (Fig. III) in flag leaves of different genotypes was different. In line N-91-9 (Fig. III. A) the activity of this enzyme increased up to the anthesis stage and after a significant decrease in the 3 DAA stage again had an increasing trend up to 15 DAA. The highest activity of this enzyme was observed at 15 DAA (12.3 $mg/g^{-1}FW/ h^{-1}$). In line N-91-17 (Fig. III. B) the activity of sucrose synthase enzyme increased up to 11 DAA, when the highest enzyme activity was recorded (14 $mg/g^{-1}FW/h^{-1}$). Also, after decreasing the amount of enzyme activity at stage 15 DAA, an increasing trend up to 23 DAA was observed. Tiregan cultivar (Fig. III. C) showed the highest activity related to sucrose synthase at 23 DAA, so that an increasing trend for the activity of this enzyme was observed up to this stage, although it showed a significant decrease at 27 DAA stage. Nogal cultivar (Fig. III. D) showed similar conditions to line N-91-17 (Fig. III. B) for sucrose synthase activity and the highest enzyme activity was observed at 11 DAA stage (15.1 $mg/g^{-1}FW/h^{-1}$). Euclide (Fig. III. E), Aorazado (Fig. III. F), and Lucullus (Fig. III. G) cultivars had similar conditions to Tiregan cultivar (Fig. III. C) in terms of sucrose synthase activity. In Antonius cultivar, the activity of this enzyme increased up to 11 DAA and then a decreasing trend was observed, which was not significant until 19 DAA compared to the activity of the enzyme at 11 DAA stage. The results of sucrose synthase activity in other leaves showed that the activity of this enzyme in all studied genotypes except Lucullus cultivar (Fig. III. G) was maximum at 7 DAA and showed a decreasing trend. In Lucullus cultivar, the highest amount of this enzyme's activity was in other leaves during 3 DBA, and then a decreasing trend was observed. Also in line N-91-17 (Fig. III. B) and Tiregan cultivar (Fig. III. C) at 19 DAA, the activity of this enzyme in other leaves showed a significant increase compared to 15 DAA. Interestingly, in N-91-9 and N-91-17 lines as well as in Euclide and Agorazado cultivars, the activity of this enzyme in other leaves in 7 DBA stage was more than its activity in flag leaf. A possible reason for this could be the lack of full spread of the flag leaf of these figures at this stage.

The results of sucrose phosphate synthase activity (Fig. IV) shows that the activity of this enzyme in the leaves of Line N-91-9 (Fig. IV. A) and Euclide cultivar (Fig. IV. E) at 15 DAA was the highest and in other genotypes. The highest activity of this enzyme was observed during 19 DAA. As observed, the activity of this enzyme in N-91-9 and N-91-17 lines and also in Antonius cultivar was higher in flag leaf compared with the other leaves in all measurement times. In Nogal and Agorazado cultivars, the amount of enzyme activity in flag leaf was higher than other leaves in all stages, with the difference that their difference was not significant at 7DBA. In Tiregan cultivar (Fig. IV. C), sucrose phosphate synthase activity in other leaves was higher than in flag leaf at 7DBA, but it was not significantly different. Flag leaf was not significantly different from enzyme activity in other leaves. In Euclide cultivar (Fig. IV. E) the activity of this enzyme in other leaves up to Anthesis stage was higher than its activity in flag leaf. Sucrose phosphate synthase enzyme activity in other leaves showed a significant difference with its value in the flag leaf with increasing 3 DBA time, but at two stages of 7 DBA and anthesis, their differences were not significant.

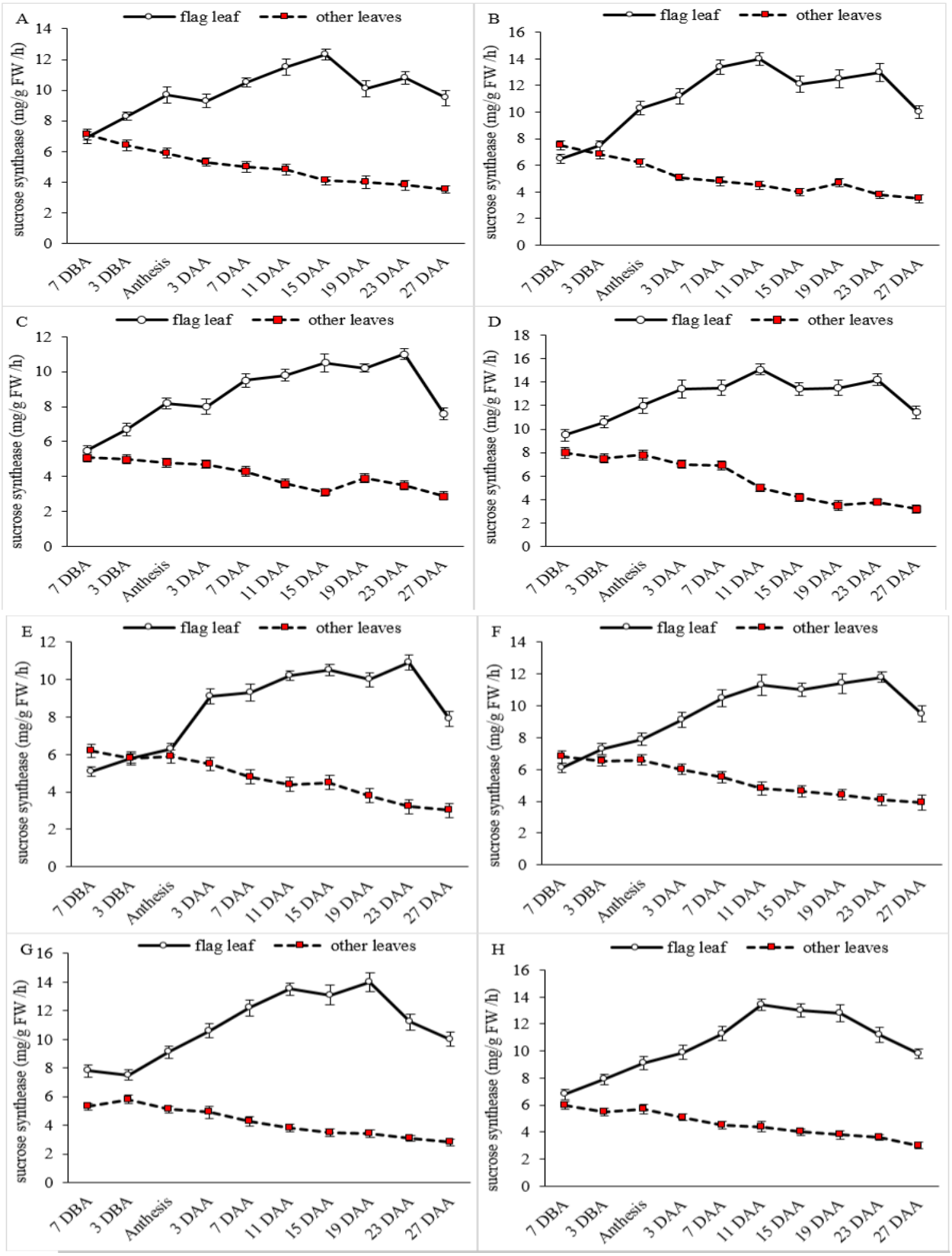

Fig. III. Sucrose synthase activities of wheat flag leaf and other leaves; genotypes included: N-91-9 (A), N-91-17 (B), Tiregan (C), Nogal (D), Euclide (E), Agorazado (F), Lucullus (G), and Antonius (H). Error bars represent twice the standard error and indicate significance and non-significance

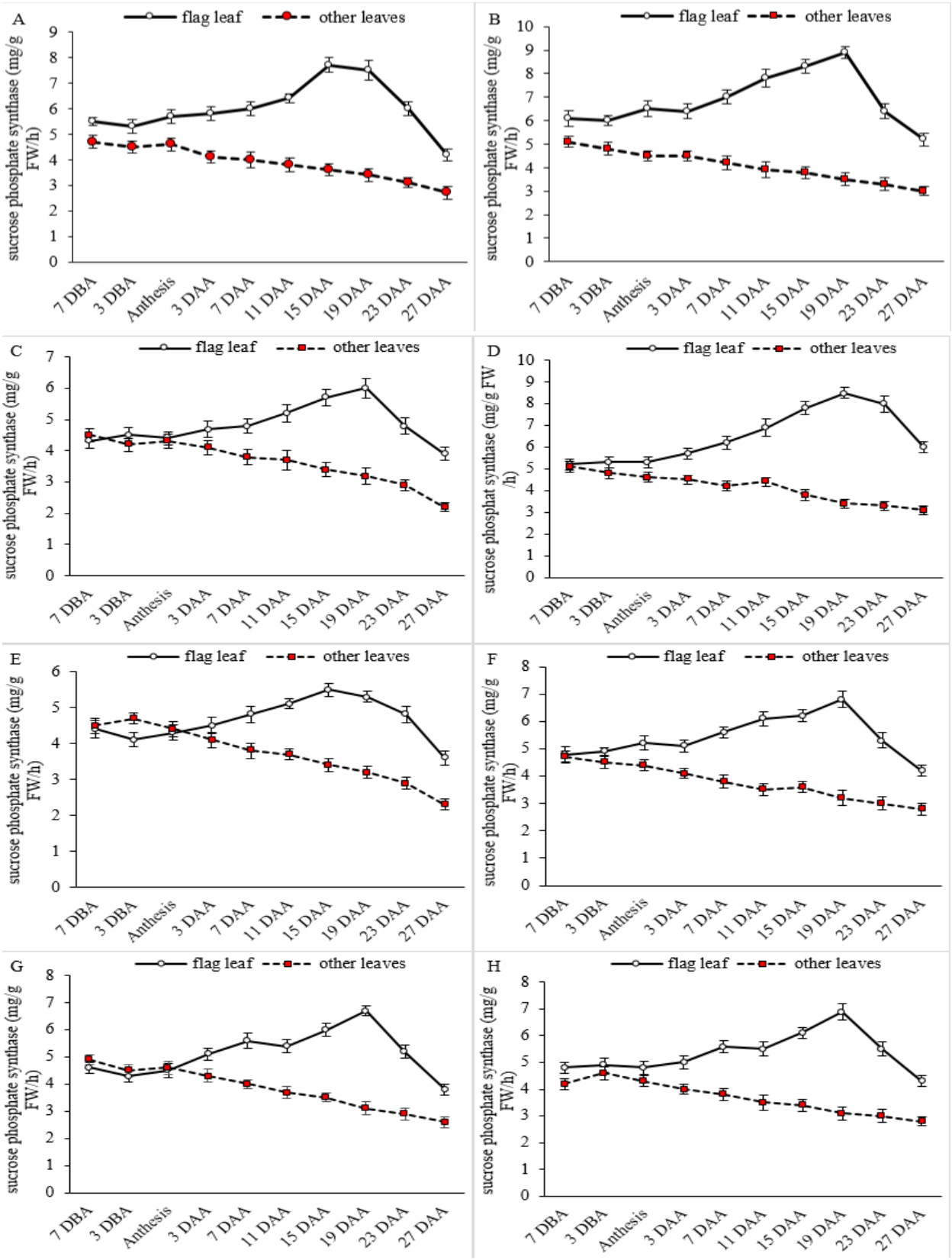

Fig. IV. Sucrose phosphate synthase activities of wheat flag leaf and other leaves; genotypes included: N-91-9 (A), N-91-17 (B), Tiregan (C), Nogal (D), Euclide (E), Agorazado (F), Lucullus (G), and Antonius (H). Error bars represent twice the standard error and indicate significance and non-significance.

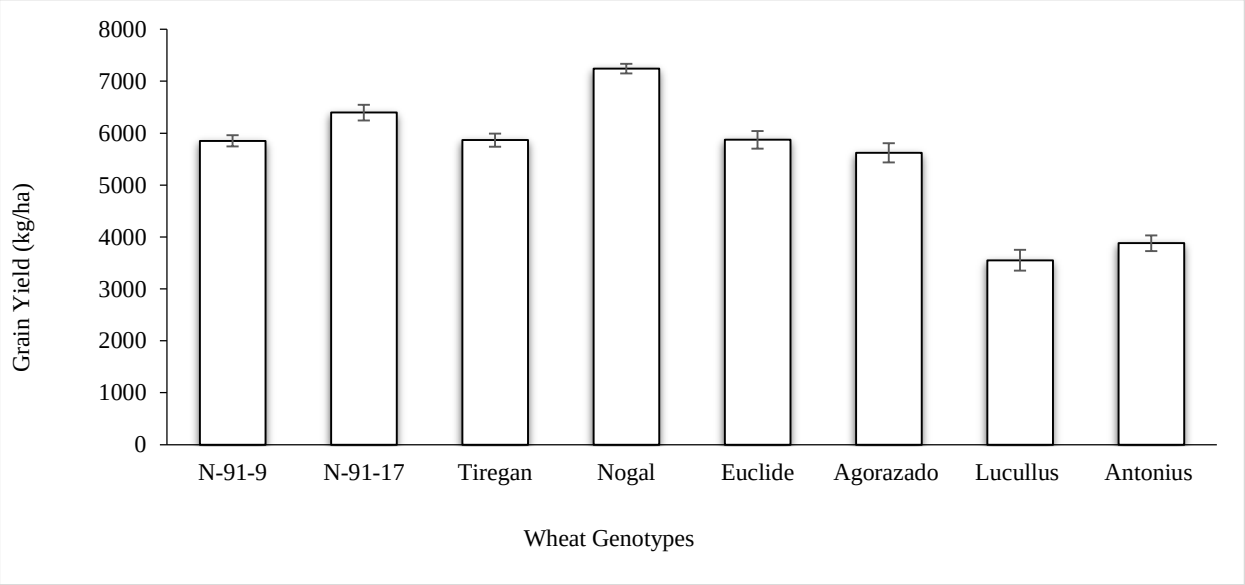

Fig. V. Grain yield of wheat genotypes; error bars represent twice the standard error and indicate significance and non-significance.

Like in Euclide cultivar, the activity of sucrose phosphate synthase in other leaves of Lucullus cultivar was higher than its value in flag leaf up to anthesis stage, but the difference between them was not significant.

The results of grain yield (Fig. V) showed that Nogal cultivar had the highest grain yield and its difference with other genotypes was significant. Line N-91-17 was in second place in terms of grain yield and its difference with other genotypes was significant. Loculus cultivar had shown the lowest grain yield among all genotypes and its difference was not significant only with Antonious cultivar. N-91-9, Tiregan, Euclide, and Agorazado genotypes did not differ significantly in grain yield. Nogal cultivar and line N-91-17 were superior to other genotypes in terms of photosynthesis rate and sucrose content of leaves as well as enzymes involved in sucrose storage and transport, which indicates the important role of measured traits in the filling process. Their measurement can also be used as an important factor in the selection of cultivars.

Gene expression

***RbcL* gene**

Since Tiregan cultivar is one of the common cultivars cultivated in the region, the results of gene expression in different genotypes compared to this cultivar were studied and analyzed.

The expression level of *RbcL* gene in line N-91-9 (Fig. VI. A) showed an increase in expression both in flag leaf and in other leaves and in all stages compared to Tiregan cultivar. The difference in expression of this gene in flag leaf up to anthesis stage and also from 15 DAA to 27 DAA was higher than other cultivars compared to other times. However, in relation to the difference in expression of this gene in other leaves compared to the control cultivar, the results showed more difference in gene expression during 7 DBA stage and also less difference in expression of this gene from 7 DAA to the final stages of sampling. Line N-91-17 (Fig. VI. B) showed the highest expression of *RbcL* gene compared to the control cultivar in the flag leaf during 3 DBA and the least difference in the expression of this gene in the flag leaf was observed during 3 DAA. Also, the difference in expression of this gene in other leaves was more in the early stages and in 7 DBA stage, the highest amount of expression of this gene was observed compared to the control cultivar. In Nogal cultivar (Fig. VI. C) the expression of *RbcL* gene was higher than the control cultivar in all studied times and also this difference was more in flag leaf than other leaves. The highest difference in the expression of this gene in Nogal cultivar compared

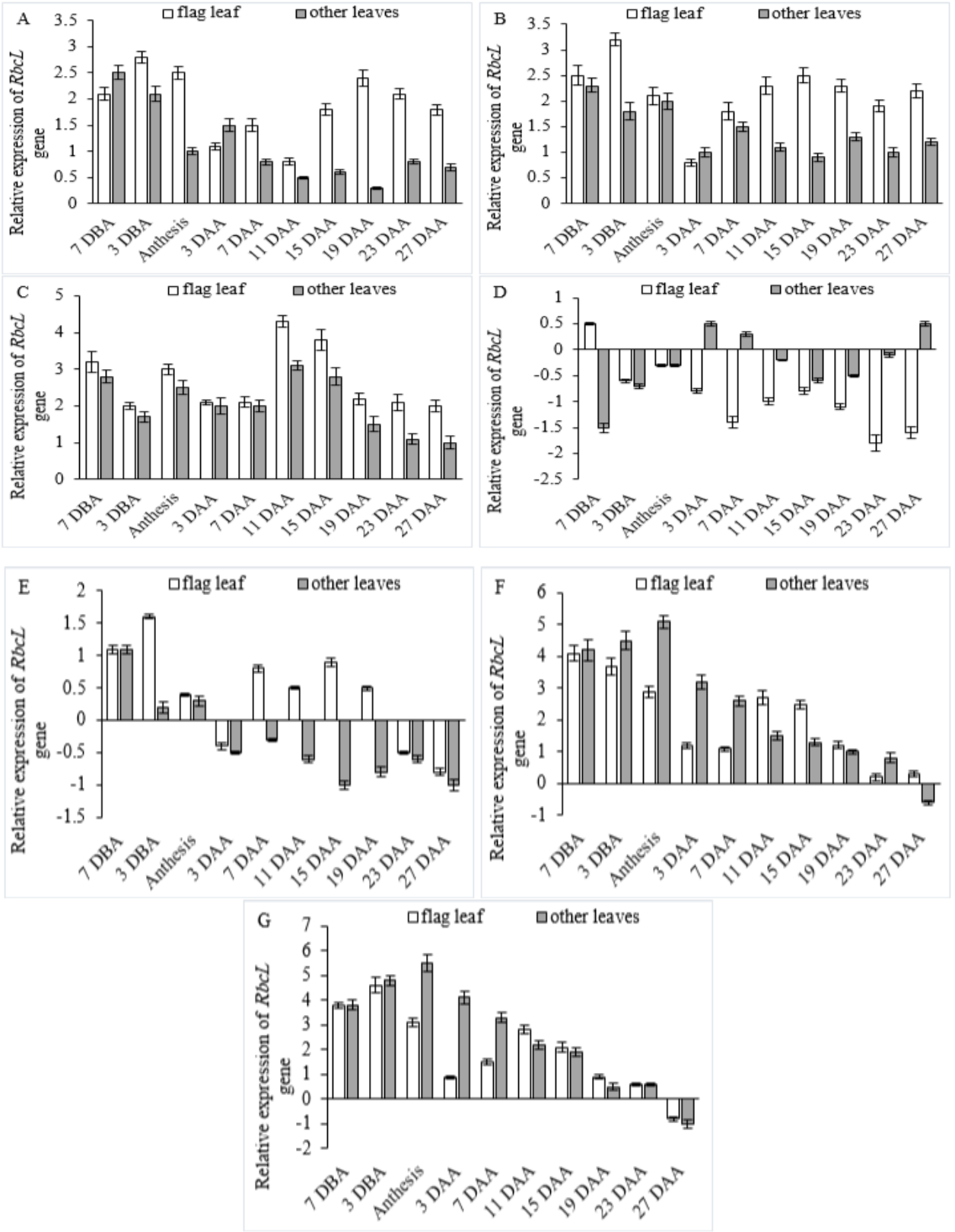

Fig. VI. *Rcbl* gene expression of wheat flag leaf and other leaves; genotypes included: N-91-9 (A), N-91-17 (B), Nogal (C), Euclide (D), Agorazado (E), Lucullus (F), and Antonius (G). Error bars represent twice the standard error and indicate significance and non-significance.

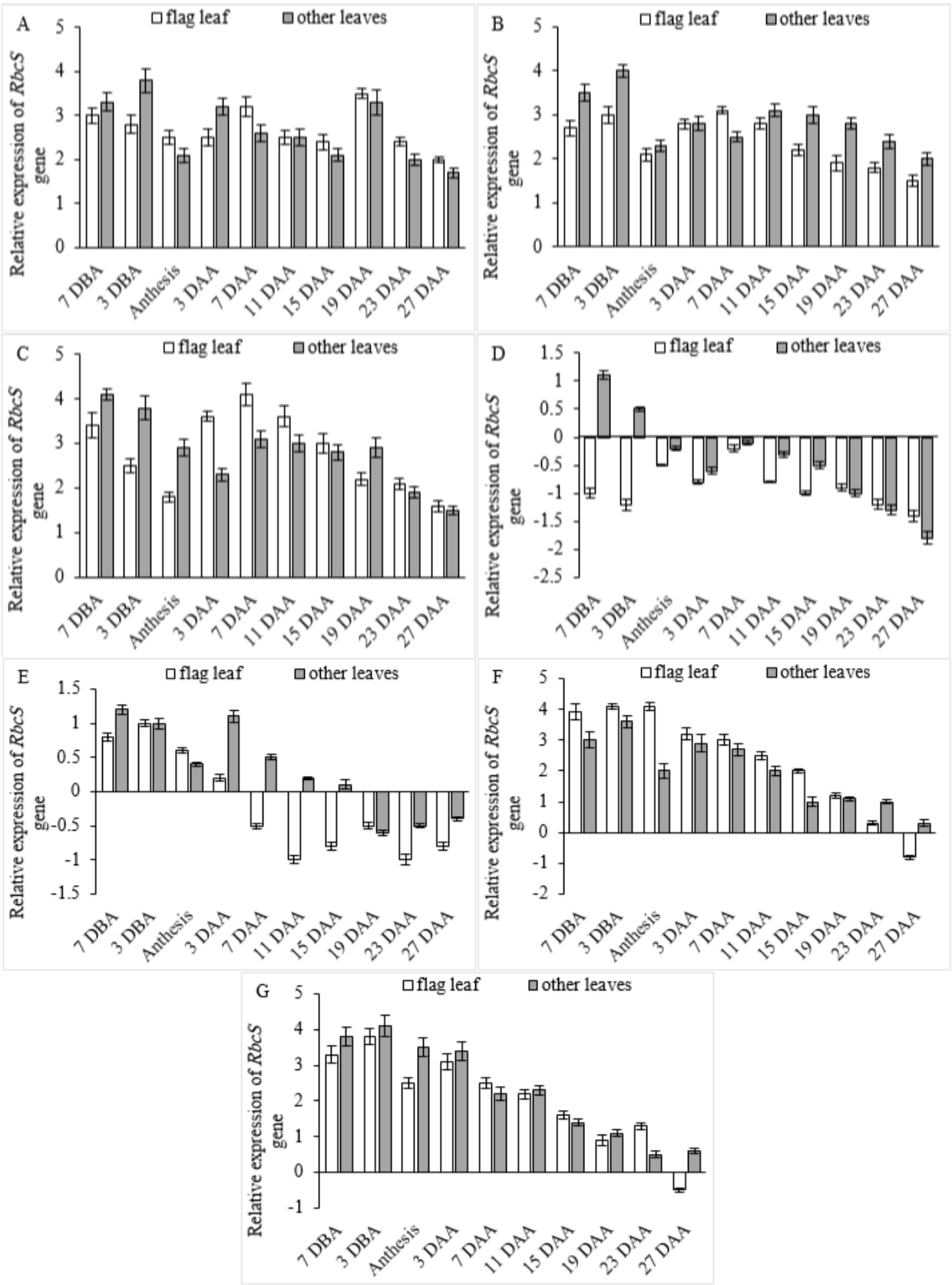

Fig. VII. *Rcbs* gene expression of wheat flag leaf and other leaves; genotypes included: N-91-9 (A), N-91-17 (B), Nogal (C), Euclide (D), Agorazado (E), Lucullus (F), and Antonius (G). Error bars represent twice the standard error and indicate significance and non-significance.

to the control cultivar was observed in both flag leaves and other leaves at 11 DAA stage. In Euclide cultivar (Fig. VI. D) the expression of this gene in flag leaf was higher only in 7 DBA compared with the control cultivar, showing a significant decrease in other stages, especially at 23 DAA. In other leaves, only in 3, 7, and 27 DAA the expression of this gene in Euclide cultivar was slightly higher than the control cultivar, and in other stages lower expression was observed.

In Agorazado cultivar (Fig VI. E) the expression of *RbcL* gene in flag leaf was lower than the control cultivar only in 3, 23, and 27 DAA, but different conditions were observed in other leaves and less expression for this gene was observed after anthesis stage, compared to the control cultivar. In the case of Lucullus cultivar (Fig. VI. F), except for 27 DAA in other leaves, more expression was observed in all other stages as compared with the control cultivar for *RbcL* gene. Antonius (Fig. VI. G) cultivar was similar to Lucullus in terms of *RbcL* gene expression, except that 27 DAA in flag leaf, it had less expression, like other leaves, compared with the control cultivar for this gene.

***RbcS* gene**

As shown in Fig. VI, the N-91-9 and N-91-17 (Figs. VII. A and B) and Nogal cultivar (Fig. VII. C) lines at all stages evaluated had higher expression for the *RcbS* gene in comparison with the control cultivar. The remarkable point is the high expression of this gene in other leaves. Euclide cultivar (Fig VII. D) for Rcbl gene showed more expression than control cultivar only in 7 and 3 DBA and had less expression than control cultivar in all other stages both in flag leaf and other leaves.

Agorazado cultivar (Fig. VII. E) had higher expression for *RbcS* gene than flag control until 3 DAA for flag leaf and up to 15 DAA for other leaves, but at other stages the expression of gene in this cultivar was lower than control. Lucullus (Fig. VII. F) and Antonius (Fig. VII. G) cultivars showed similar conditions for expression of this gene than control cultivar, so that in flag leaf except in 27 DAA, they had more expression than control cultivar. Also, in other leaves they had more expression than the control cultivar in all evaluated stages.

***Rca* gene**

Results of *Rca* gene expression showed that the expression of this gene in N-91-9 and N-91-17 (Fig. VIII. A and B) and Nogal cultivar (Fig. VIII. C) lines were higher compared to the control cultivar both in flag leaf and in the other leaves. In flag leaf, all three genotypes had higher expression than control cultivar at the time of anthesis and 3 DAA. Euclide cultivar (Fig. VIII. D) had higher expression for *Rca* gene than flag control until anthesis stage in flag leaf and 3 DBA in other leaves. In other stages, the expression of this gene was lower. Also, Agorazado cultivar (Fig. VIII. E) showed higher expression than *Rca* gene for *Rca* gene up to 11 DAA in both flag leaf and other leaves. Lucullus (Fig. VIII. F) and Antonius (Fig. VIII. G) cultivars showed similar conditions for expression of this gene than control cultivar, so that in flag leaf in all evaluated stages they had more expression than control cultivar and in other leaves except for 27 DAA, they also showed more expression than the control cultivar. The largest difference in expression of this gene in these two cultivars compared to the control cultivar was in the flag leaf 7 and 3 DAA.

Discussion

The chlorophyll content of leaves is one of the key factors in determining the rate of photosynthesis and dry matter production, so that the concentration of chlorophyll in the leaves as an indicator is not strong enough for evaluation (Liu et al., 2019). In this regard, Zhang et al (2012) concluded that the chlorophyll content of wheat flag leaves has a positive relationship with photosynthetic capacity. Photosynthesis is also a comprehensive physiological process that is interrelated with, and limited to, the reactions of light and darkness, and the rate of photosynthesis is an important indicator of photosynthesis. As the findings of this study suggest, the amount of chlorophyll in the flag leaf of all cultivars was more than other leaves and in almost all genotypes. Increase and decrease in photosynthesis is consistent with the chlorophyll content, which is in line with the results of Zhang et al (2012). In

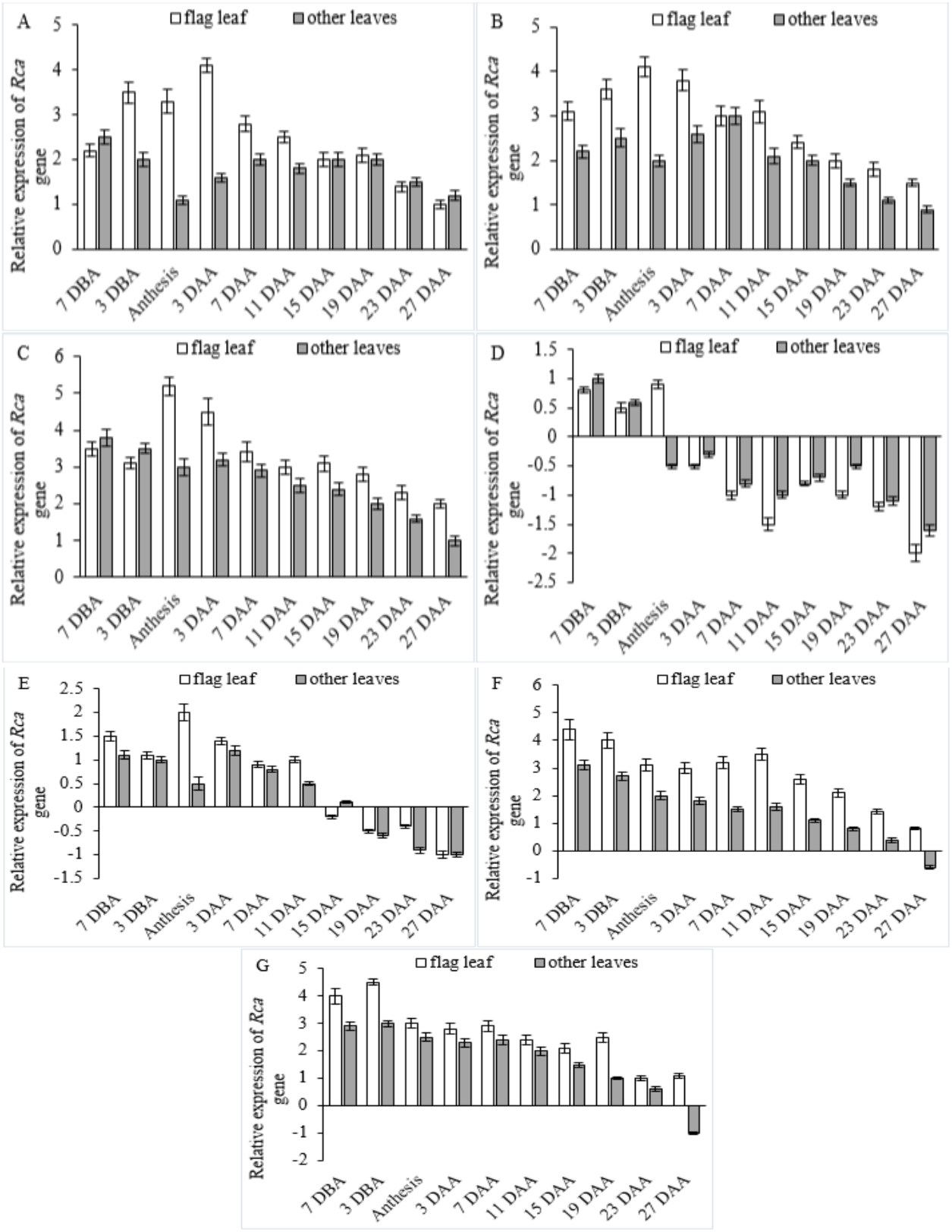

Fig. VIII. *Rca* gene expression of wheat flag leaf and other leaves; genotypes included: N-91-9 (A), N-91-17 (B), Nogal (C), Euclide (D), Agorazado (E), Lucullus (F), and Antonius (G). Error bars represent twice the standard error and indicate significance and non-significance.

flag leaf has been reported as the photosynthetic capacity of the plant population. In their research, Lan et al (2003) reported that the yield of new cultivars could be greatly improved by expanding leaf photosynthesis capacity during grain filling. As a result, it can be argued that cultivars whose chlorophyll contents are more stable after flowering and decrease less will be more successful in filling the grain and achieving grain yield. As observed in this study, Nogal cultivar and line N-91-17 did not have a significant decrease in the amount of chlorophyll in the flag leaf until 11 days after flowering, and they had the highest rate of photosynthesis. Wheat is a saccharophyllous plant, meaning that from flowering to adulthood, the sucrose produced in the leaves, called the source, is transported by vessels to non-photosynthetic tissues such as roots, stems and grains (Ludewig and Flugge, 2013). Thus, the ability to synthesize sucrose in leaves and the release of photosynthesis reflects the ability of the source. The level of sugar in leaves is also crucial in regulating plant growth and development so that some researchers have stated that the accumulation of carbohydrates in leaves reduces photosynthetic activity and induces leaf senescence (Wang et al., 2015). However, several other studies support the theory that lower carbohydrate levels cause leaf senescence (Wang et al., 2015; Julius et al., 2017; Zhao et al., 2018). Results of this study showed that the amount of sucrose in the flag leaf of all cultivars was higher than other leaves and with the maximum amount, the amount of photosynthesis decreased, which is consistent with the first theory. On the other hand, the amount of sucrose in other leaves decreased from the stage 3 days before pollination and in some cultivars from the stage of anthesis and relatively in proportion to the reduction of photosynthesis rate in them. The second idea that induction of senescence in leaves due to reduced carbohydrate levels in relation to other leaves seems to be consistent.

The synthesis and decomposition of sucrose plays an essential role in food production and is the main source of carbon and energy in plants, so it is essential for agriculture. Carbohydrates derived from sucrose make up nearly 90% of plant biomass, and therefore sucrose is an important determinant of yield. The synthesis and degradation of sucrose are controlled by the two enzymes: sucrose synthase (SS) and sucrose phosphate synthase (SPS) (Koch, 2004, Ruan, 2014). SS reversibly converts sucrose to UDP-glucose and fructose in the presence of UDP. SPS is the major enzyme involved in sucrose synthesis and catalyzes the synthesis of sucrose-6-phosphate from UDP-glucose and fructose-6-phosphate. The activity of these two enzymes in leaves indicates the ability to produce sucrose through photosynthesis and conversion of photoassimilates (Ruan, 2014). The role of SS in seeds is mostly reported in sucrose degradation, but its activity is in leaves after anthesis and at the beginning of grain filling for sucrose synthesis (Wang et al., 2015), and as shown in Fig. (II), in all cultivars SS content of flag leaves increased after pollination with increasing photosynthesis and decreased approximately in the middle of grain filling. The amount of this enzyme in other leaves was at its highest level in the stages before anthesis and it seems that the photosynthetic flag was higher in other leaves before the appearance of leaves due to receiving enough light, and the activity of this enzyme rose in the direction of sucrose synthesis in other leaves. SPS enzyme is involved in converting sucrose to its transferable form from the reservoir to source (Julius et al., 2017). The activity of this enzyme has been reported during the filling stage in the leaves. The high activity of this enzyme indicates the high ability of the source to transfer sucrose to the source tissues (Li and Wang 2013). During the grain-filling period, the activity of this enzyme can play an effective role in grain filling and achieving high yield (Wang et al., 2015). As can be seen in the results (Fig. IV), the amount of this enzyme in the flag leaf increased with entering the grain filling stage and is at its highest value almost in the middle of the grain filling, which is in line with the results of other researchers.

Studies have shown that the level of *RbcL* mRNA is not completely correlated with the level of *RbcS* mRNA, but is directly related to the amount of *RbcS* protein synthesized. Also, the relationship between the expression of these two genes and the rate of photosynthesis has been reported, so that their expression is high in young

leaves and with the entry of leaves into the ageing stage, their expression and Rubisco enzyme levels decreases (Suzuki and Makino, 2013). Therefore, the expression of these genes can be considered as a criterion for the photosynthetic power of genotypes and they can be compared. The results of expression of these two genes (Figs. V and VI) compared to the control cultivar (Tiregan) were in accordance with the photosynthetic pattern of the cultivars and showed that the expression of these two genes was higher in genotypes that had higher photosynthesis. Researchers have shown that *Rca* levels in cells affect plant photosynthesis and crop yield and that in wheat, *Rca* gene expression has been reported to have a positive linear correlation with crop production. In this study, as shown in (Fig. VII), the amount of expression of this gene in genotypes with high photosynthesis increased compared to the control cultivar (Tiregan). The three photosynthetic genes *RbcL*, *RbcS*, and *Rca* had higher expression in cultivars with higher and more stable photosynthesis than the control, which increased grain yield in these cultivars. Another noteworthy point was that in Nogal cultivar and line N-91-17, the stability of chlorophyll content was more than other genotypes in addition to flag leaf in other leaves, and another reason can be higher performance of these two genotypes.

Conclusion

In this study, it was found that flag leaf in the post-anthesis stages has a significant role in photosynthesis and sucrose production in wheat, and cultivars with higher chlorophyll also had higher photosynthesis. Also, in genotypes with higher sucrose synthase enzyme activity, more sucrose is synthesized and due to the role of sucrose phosphate synthase, increasing its amount is involved in the transfer of sucrose from the leaves to other organs. In general, as observed in the results, higher chlorophyll content resulted in a higher amount of photosynthesis, which in the high amount of photosynthesis caused differences in the expression level of photosynthetic genes in different genotypes. Finally, photosynthesis further increased the sucrose content and increased grain yield. Among the studied genotypes, the results showed that Nogal cultivar and line N-91-17 were superior in terms of measured traits and can be recommended for use in future wheat breeding research.

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