J Plant Mol Breed (2020) 8 (2): 38 - 49
DOI: 10.22058/JPMB.2021.537769.1240

RESEARCH ARTICLE

Evaluation the effect of senescence on the mineral remobilization in two bread wheat cultivars

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ABSTRACT: The main source of protein and micronutrients in wheat grains is the flag leaf and to a lesser extent the lower leaves. As healthy leaves reach the final stage of growth, senescence, they remobilize the nutrients necessary before tissue destruction and death. This experiment was carried out in Golestan province, and the Wheat cultivars studied were included Euclide and Antonius. Sampling was carried out from flag leaf, other leaves, stem, and grain at 7 stages, Anthesis, 7, 11, 15, 19, 23, and 27 Day After Anthesis (DAA). The total chlorophyll content in the Antonius cultivar was higher in both flag leaf and other leaves than Euclide cultivar. The expression of TaNAM-B1 and TaSAG12 genes, which have been identified as signaling genes for senescence in wheat, showed results consistent with the results of chlorophyll content in leaves. Increased expression of both genes after anthesis was observed earlier in Euclide cultivar than the Antonius cultivar and had higher expression in most stages. In light of the results, the change in concentrations of Cu, Zn, and Fe in the Euclid cultivar was more in all organs than in Antonius one. Also, given the importance of minerals in the food basket, it can be noted that Euclid cultivar, in which leaf senescence begins earlier and more minerals are stored, can produce grains with higher nutritional value than Antonius cultivar.

KEYWORDS: Anthesis, Minerals, Remobilization, Senescence, Wheat.

INTRODUCTION

There are enormous evidences on cereals implying that grains growth and development to some extent relies on mobilization of plant assimilates, which are mainly stored in the stem before flowering, which is referred to as remobilization of stem assimilates [1]. The potential of genotypes to remobilization of photosynthates from stem to grain during grain filling using the monitoring of changes in dry weight and weight density at flowering to physiological maturity to estimate more accurately the contribution of stem assimilates during grain filling [1, 2]. The main source of protein and micronutrients in wheat grains is the flag leaf and to a lesser extent the lower leaves [3]. When healthy leaves reach the end stage of their growth, they turn yellow and in other words, aging begins. They distribute the nutrients necessary for tissue destruction before tissue death. Remobilization of absorbed nutrients through roots stored in the shoot, especially key nutrients from senescence tissues, can be essential for the overall readiness of the plant, especially in environments where there is a lot of energy to absorb, is vital. Therefore, the efficiency of nutrient remobilization from old leaves to younger ones is important [4]. *NAM-B1* in wheat has been shown to be involved in leaf senescence. Reducing the expression of *NAM-B1* homologs using RNAi delays senescence by more than 3 weeks and reduces wheat grain protein, zinc, and iron content by more than 30% [5]. To date, dozens of Senescence-Associated Genes (*SAGs*) genes have been identified in the model plant Arabidopsis [6-8]. The SAG12 gene, which encodes cysteine protease, is

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Received: 29 August 2021/ Revised: 13 November 2021
Accepted: 27 November 2021

specifically activated during the later stages of developmentally controlled senescence and is widely used as a molecular marker for leaf senescence. General assessments of the remobilization of most minerals are relatively low during plant growth and are only well known for their abundant nutrients such as nitrogen, sulfur, and phosphorus, and have often been studied separately [10]. The remobilization of iron (Fe), copper (Cu), and zinc (Zn) has been studied in a detailed manner. In wheat, the concentrations of Fe and Cu in all plant organs decreased due to their remobilization during the grain filling period (77 and 40-62%, respectively) [11]. Zn remobilization from leaf to grain was significant in wheat [12] and barley [13] but was affected by the availability of Zn in the post-pollination period [12]. When radioactive labeling is used at the tip of the leaf, Zn is observed to be effectively mobilized to the growing wheat grain [14]. Improving wheat grain protein content (GPC) over the past 20 years has been one of the major goals of plant breeding because GPC is one of the determinants of bread and pasta quality [15]. Under normal culture conditions, grain protein content is reported to be between 10 and 14%. GPC is a complex trait that is not only influenced by hereditary factors but also by environmental factors [16]. According to the researches, gene loci for GPC have a pleiotropic effect on grain nitrogen remobilization, mineral content, and senescence during wheat development [17-19]. The remobilization of trace elements (such as Zn, Fe, etc.) across plant membranes is carried out by different families of carrier genes. Different families of carrier genes have specific and overlapping abilities to carry different metal cations that potentially interfere with control to regulate the remobilization of trace elements to developing grains [20]. This study aimed to investigate the role of remobilization of some minerals to the grain after pollination as well as evaluation of expression of some genes during the senescence stage in two bread wheat cultivars that have different senescence habitats.

MATERIALS AND METHODS

plant material

This experiment was carried out in Golestan province, Kordkoy city, Georgian neighborhood 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 in 2018-2019. The applied Wheat cultivars were grown using common agriculture practice and included Euclide and Antonius (Foreign cultivars that were first cultivated in this area and different in terms of the beginning of senescence). For Sampling, which was done with the Zadoks scale, over 35 plants of any genotype were labeled. Sampling was carried out from flag leaf, other leaves, and stem at 7 stages, Anthesis, 7DAA, 11DAA, 15DAA, 19DAA, 23DAA, and 27DAA which were carried to the laboratory in liquid nitrogen and stored at -80 °C freezer. grain Samples harvested from 7DAA. For measured mineral content, Part of the plant samples was carried with a paper pocket and incubated in oven at 80°C for 48 hours. The analysis of morphophysiological data was conducted using SAS 9.2 with proc GLM, and the comparison of means was performed using LSD at a 5% probability level.

Total Chlorophyll

Poura et al [21] method was used to measure total chlorophyll content. 0.5 g of leaf sample (stored frozen at -80 °C) was completely crushed and mixed with 10 ml of 80% acetone at room temperature until the tissue was completely bleached. After centrifugation at 5,000g for 10 min, the absorbance (A) was recorded by spectrophotometer at 646.6 and 663.6. The amount of total chlorophyll was calculated based on the following formula described by Chen et al [22].

Total chlorophyll content = 20.21A645 + 8.02A663

Total RNA isolation, DNase Treatments, and Reverse transcription

RNA extraction was done P-Biozol (Bio Flux). RNA samples were treated with RNAse-free DNase I (Biolabs) to eliminate any DNA contamination. Total RNA was checked by electrophoresis on a 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 in a standard polymerase chain reaction was evaluated.

RT-qPCR

Primers were designed relying on the information available on the NCBI site using the AlleleID7 software (PREMIER Biosoft, Palo Alto, CA, USA). RT-qPCR reactions were carried out by the IQ5 machine was used

by 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, the *GAPDH* gene was used for normalization of target genes expression. The sequences of the primers used in the RT-qPCR analysis are listed in Table1. 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 $2^{-\Delta\Delta CT}$ [23] about 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.

Mineral measurement

To measure the minerals, the plant samples were placed in a Chinese mortar after grinding and placed in an electric oven at 550 °C for 2 hours to turn to ashes. Then, extraction was performed using hydrochloric acid 2 Normal, and the concentration of mineral elements (Fe, Zn, N, and Cu) in the extract was measured using an atomic absorption spectrometer [24].

RESULTS

Chlorophyll content

In general, the results of chlorophyll content evaluation (Fig. 1) of leaves in both cultivars showed a decreasing trend after pollination. The total chlorophyll content in Antonius cultivar was higher in both flag leaf and other leaves than Euclide cultivar and the cultivars had a significant difference in the level of 5% probability of LSD test except in stages 23 and 27DAA. As can be seen in Figure 1, the decreasing trend of total chlorophyll content in Euclide flag leaf intensified from the 15DAA stage and showed a significant difference with the previous stage in the 5% probability level of LSD test. In Antonius cultivar, however, a significant decrease was observed during 19DAA. This difference was also evident in the reduction of chlorophyll content of other leaves of the two cultivars, so that in Euclide cultivar at 11DAA and in Antonius cultivar as flag leaf at 19DAA, a significant decrease in total chlorophyll content was observed. One of the first signs of senescence in plants is a decrease in chlorophyll content in the leaves, due to which we see yellow color in the leaves. Chlorophyll decomposition is an integral process of senescence and is also the last part of leaf growth. As the leaves age, the plants break down chlorophyll to form colorless linear tetrapyrroles that are stored in the vacuum of senescence cells [25, 26].

Table 1. Primers sequences used in the RT-qPCR analysis

Primer	Primer sequence (5' → 3')	ACC. No.
TaNAM-B1	cgccggaacaactagaagaacatc tccacggagtcctcgcactc	HQ872050
TaSAG12	gagactcggaagtgactgtcac gtcgtgatgcaaatgtttacgcg	AB267407
GAPDH	ccagtacatcagccaccact acagcaacctccttctcacc	EF592180

Gene expression

Fig. 2 illustrates the results of the evaluation of *TaNAM-B1* gene expression. In the Euclide cultivar, the expression of this gene in flag leaf at 15DAA was significantly increased compared to 11DAA and its difference with Antonius cultivar was significant at a 5% probability level of LSD test. In Antonius cultivar, expression of *TaNAM-B1* gene in flag leaf at 19DAA showed a significant increase in 5% probability of LSD test compared to 15DAA, which was consistent with the results of decreasing total chlorophyll content in flag leaf at the same time. The results of expression of this gene in other leaves (Figure 2) showed that Euclide cultivar had more expression than Antonius cultivar in all measurement times, which differed in 11, 15 and 23DAA at a 5% probability level. LSD was significant. As can be seen in Fig. 3, the expression level of *TaSAG12* gene in leaf leaves of Euclide cultivar was higher than Antonius cultivar in other phases except for Anthesis stage and this difference was significant from 15DAA time at 5% probability level of LSD test. Also, in relation to the expression of this gene in other leaves, the results show that the expression of this gene in the Euclide cultivar was higher than Antonius cultivar except at 27DAA. The difference between cultivars in terms of expression of this gene from 7DAA to 23DAA was significant at the 5% probability level of LSD test. A Significant increase in *TaSAG12* gene expression was observed in other leaves in the Euclide cultivar from 11DAA and in Antonius cultivar from 15DAA compared to the previous stages.

Mineral concentration

The concentration of Cu in different organs is shown in Fig. 4. A significant decreasing trend of Cu concentration

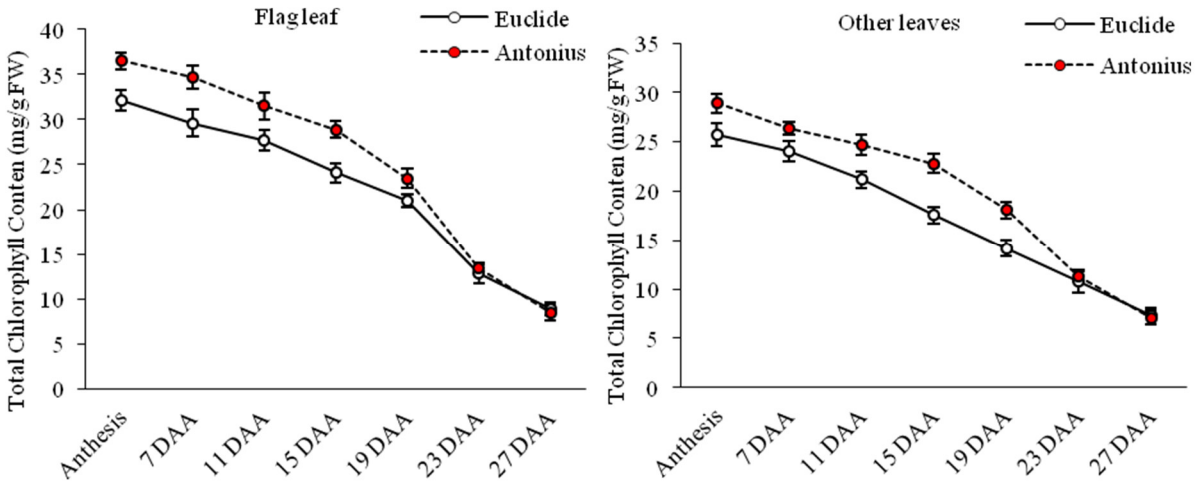

Figure 1. chlorophyll content in Flag leaf and other leaves at anthesis and after anthesis in wheat. Error bars: Show different significant between stage and cultivar.

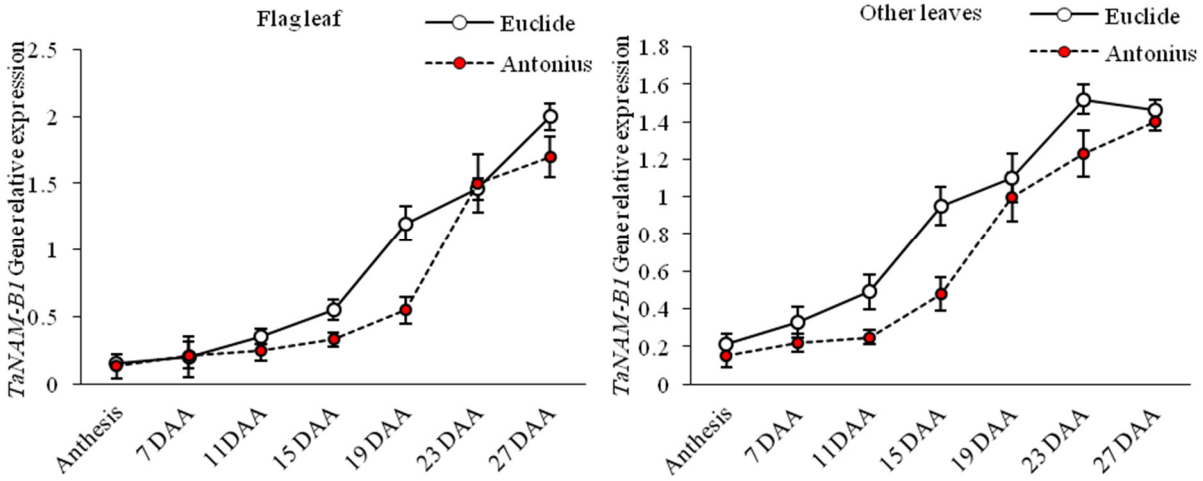

Figure 2. Relative expression of *TaNAM-B1* in Flag leaf and other leaves at anthesis and after anthesis in wheat. Error bars: Show different significant between stage and cultivar.

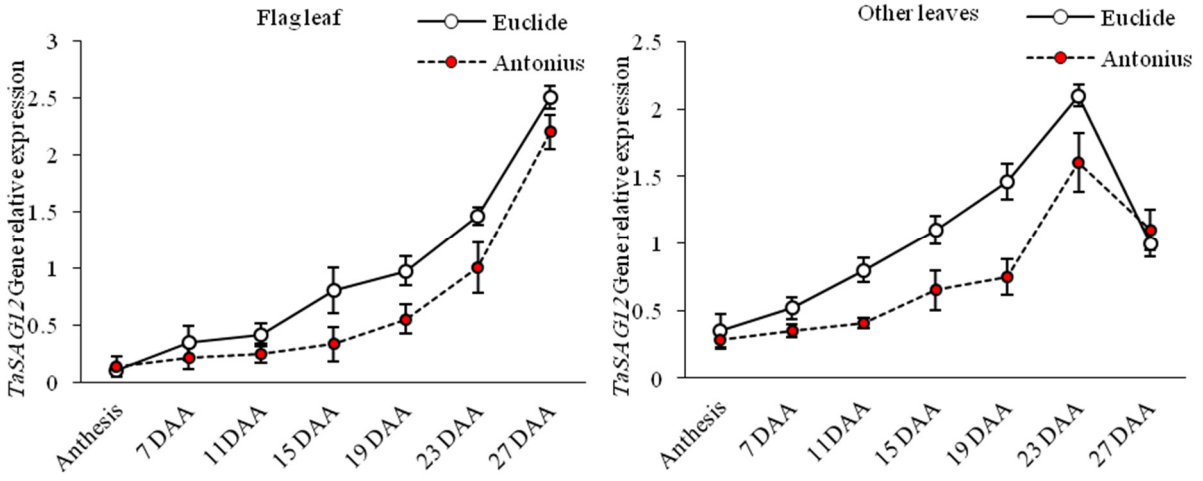

Figure 3. Relative expression of *TaSAG12* in Flag leaf and other leaves at anthesis and after anthesis in wheat. Error bars: Show different significant between stage and cultivar.

concentration was observed in flag leaf for Euclide cultivar from 15DAA and Antonius cultivar from 19DAA. Also in other leaves, the amount of Cu concentration in both cultivars had a decreasing trend in other stages, except for the insignificant increase observed in the Euclide cultivar at 15DAA and Antonius cultivar at 11DAA. The stem Cu concentration of Antonius cultivar was higher than Euclide cultivar and except for 11DAA at other stages, this difference was significant at the 5% probability level of the LSD test. In general, the amount of Cu in the stem was less than in the leaves. The highest grain Cu concentration was related to Euclide cultivar which was observed at 7 days after pollination and there was a significant difference in the level of 5% probability of LSD test with grain Cu concentration in Antonius cultivar at this stage. In general, during grain filling in Euclide cultivar, a decreasing trend was observed in the amount of Cu concentration, which was the highest decrease at 15DAA. In Antonius cultivar, the conditions were different in terms of Cu concentration and except in 15 and 27DAA at other stages, it had a lower concentration than Euclide cultivar. The conditions regarding the Zn concentration in different organs (Fig. 5) were somewhat similar to the Cu concentration. The results showed that the concentration of Zn in the leaf of Euclide cultivar had a decreasing trend up to 11DAA and after a slight increase at 15DAA again had a decreasing trend. The highest concentration of Zn (34.6 mg/kg) in the flag leaf belonged to the Euclide cultivar, which was observed at the time of anthesis. In the flag leaf of Antonius cultivar, after anthesis, Zn concentration was lower than in the previous stage except at the three times of 11,15 and 27DAA. Zn concentration increased significantly in these times at the level of 5% probability of LSD test. Failed. In other leaves, Zn concentration in Euclide cultivar was higher than Antonius cultivar in all stages and their differences were significant at 5% LSD test at other times except 27DAA. The highest Zn concentration in the stem was 24.1 gr/kg which was observed in the Antonius cultivar at the time of anthesis and its difference with the Zn concentration in the stem of Euclide cultivar at the same time was significant at a 5% probability level of LSD test. Zn concentration in the stem of Antonius cultivar decreased from the time of pollination to 19DAA and then increased. Zn concentration in the stem of Euclide cultivar showed an increasing-decreasing trend up to 15DAA and then significantly decreased up to 23DAA. In the Euclide cultivar, the changes in Zn concentration at grain filling time were decreasing and, in all stages, Zn concentration in the grain of this cultivar was higher than Antonius cultivar. The results of the N concentration study in the studied organs of the plant (Fig. 6) showed that in both cultivars, changes in N concentration in all organs (flag leaf, other leaves, stem, and grain) had a decreasing trend. Antonius cultivar had a higher N concentration than Euclide cultivar in both flag leaf and other leaves up to 19DAA. At 23 and 27DAA cultivars, N concentration was higher in flag leaf and other leaves in Euclide cultivar, and its difference with cultivar Antonius was significant at the 5% probability level of the LSD test. Also, the concentration of N in the stem and grains of the Antonius cultivar was higher than Euclide cultivar in all measurement stages. As can be seen in Fig. 7, the results of Fe concentration measurement showed that Euclide cultivar had higher Fe concentration than Antonius cultivar in flag leaf, other leaves, and stem and this difference in flag leaf and other leaves in all stages at the surface. The 5% probability of the LSD test was significant. Also, in the days after pollination, the concentration of Fe in the flag leaf of both cultivars had an almost increasing trend, in other leaves and stems up to 19DAA, and then decreased except at 27DAA in other leaves of Euclide cultivar. Fe concentration in Euclide cultivar decreased during filling and its highest amount (30.2 gr/kg) was observed in the anthesis stage. In the Antonius cultivar, except for 11and 23DAA, the trend of changes in Fe concentration was decreasing.

The results of simple correlation between the studied parameters are given in Table 2. The highest positive and significant correlation was observed at a probability level

Table 2. Simple Correlations between the studied parameters

	Cu	Zn	N	Fe	Chlorophyll	TaNAM-B1	TaSAG12
Cu	1	0.480**	0.827**	0.592**	0.866**	-0.757**	-0.735**
Zn		1	0.709**	0.191	0.663**	-0.644**	-0.551**
N			1	0.322*	0.938**	-0.882**	-0.805**
Fe				1	-0.317	0.501**	0.489**
Chl.					1	-0.929**	-0.862**
TaNAM-B1						1	0.912**
TaSAG12							1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

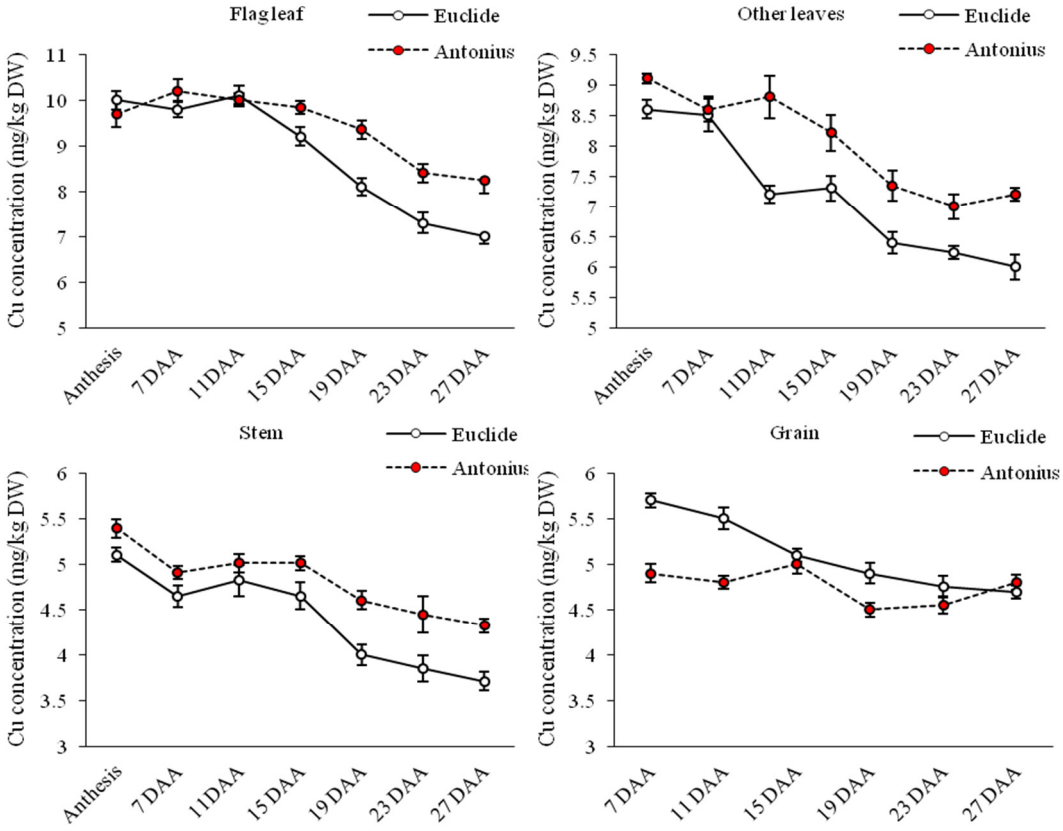

Figure 4. Cu concentration in Flag leaf, other leaves, stem and grain at anthesis and after anthesis in wheat. Error bars: Show different significant between stage and cultivar.

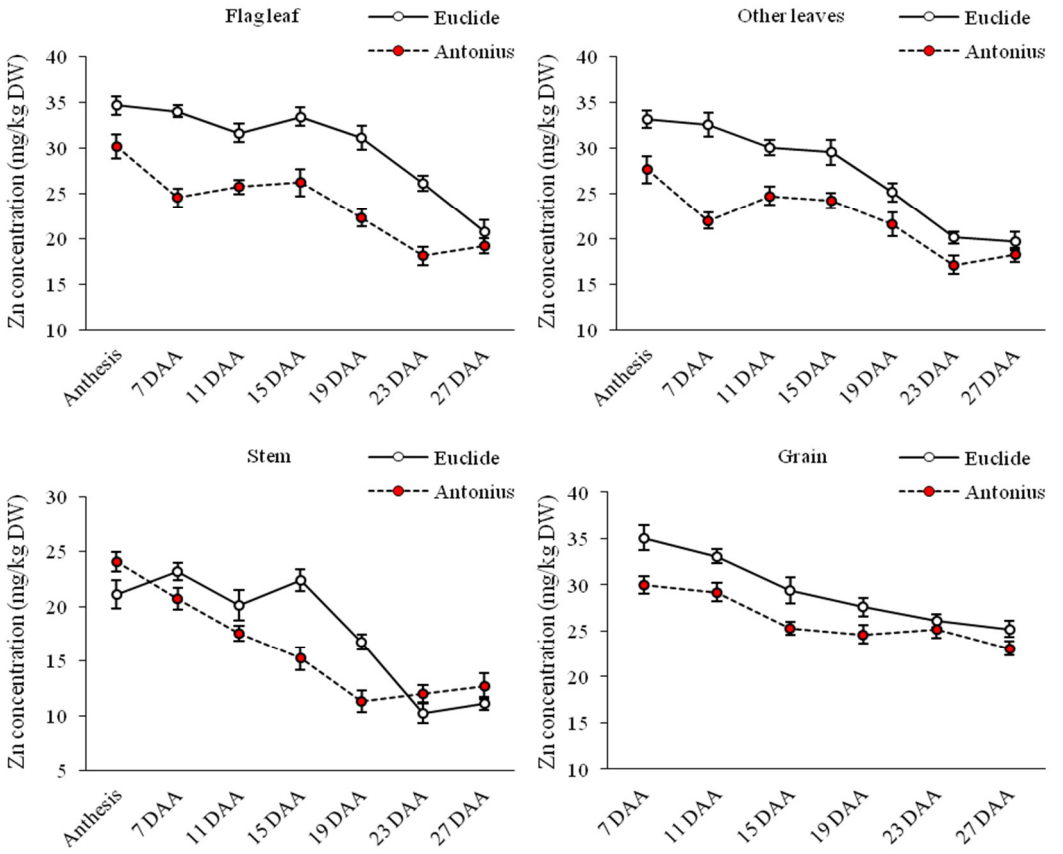

Figure 5. Zn concentration in Flag leaf, other leaves, stem and grain at anthesis and after anthesis in wheat. Error bars: Show different significant between stage and cultivar.

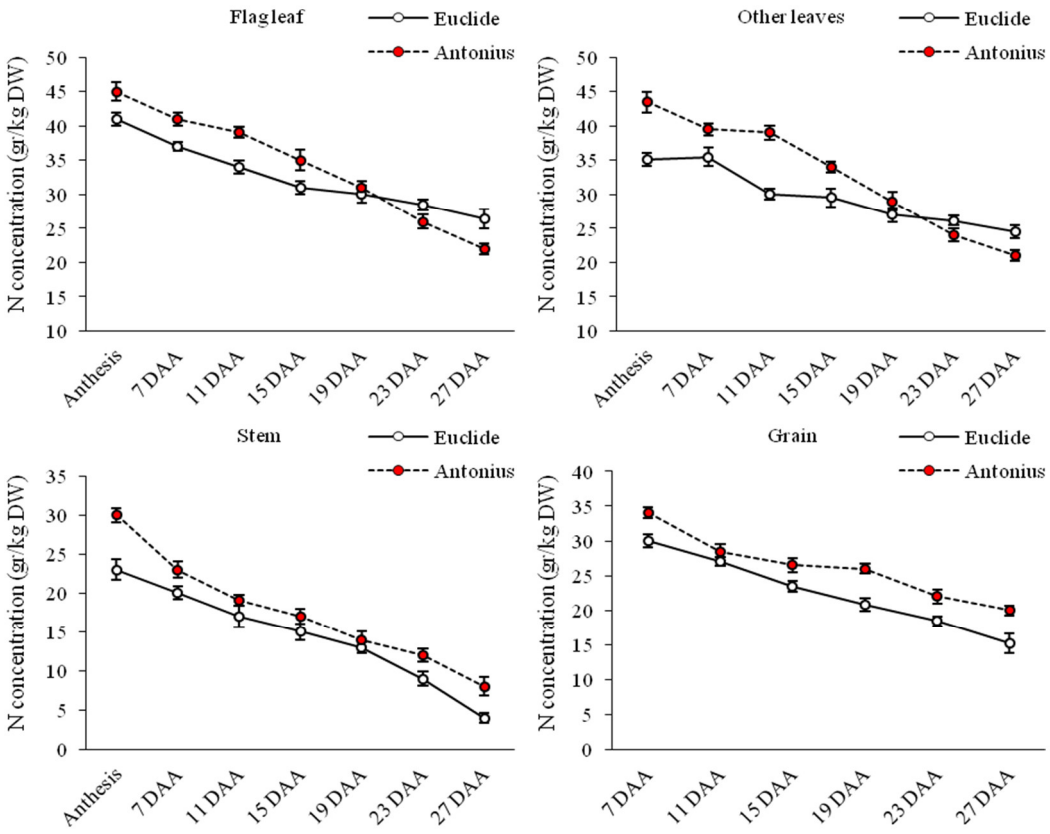

Figure 6. N concentration in Flag leaf, other leaves, stem and grain at anthesis and after anthesis in wheat. Error bars: Show different significant between stage and cultivar.

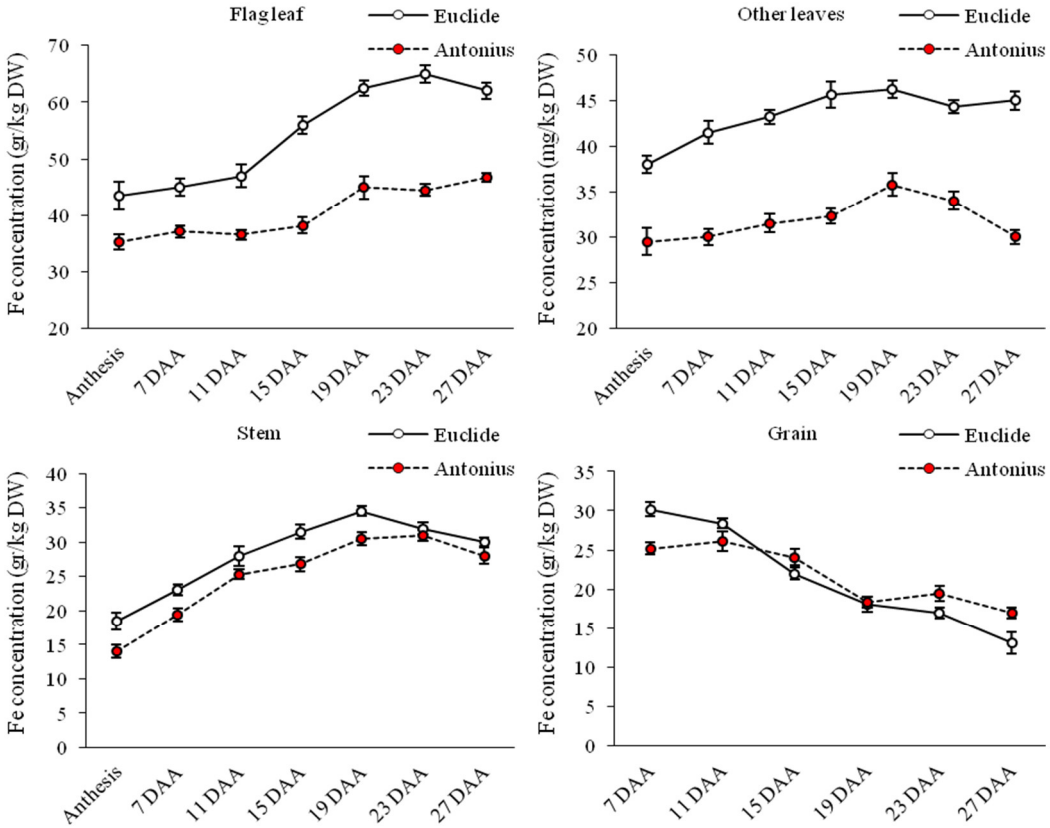

Figure 7. Fe concentration in Flag leaf, other leaves, stem and grain at anthesis and after anthesis in wheat. Error bars: Show different significant between stage and cultivar.

of one percent (0.912) between the two genes TaNAM-B1 and TaSAG12, which indicates that the expression of these two genes is similar in the occurrence of senescence. The results also showed that these two genes had the highest negative and significant correlation with chlorophyll content, which also indicates the occurrence of senescence by reducing the amount of chlorophyll and increasing the expression of genes involved in senescence. Cu concentration showed a positive and significant correlation with chlorophyll content, Zn, N, and Fe concentrations at the level of 1% probability and its relationship with the studied genes was negative and at the level of 1% probability. Zn concentration showed a positive but insignificant correlation with Fe but its relationship with other traits was similar to Cu. The concentration of N, except that it was signed with the concentration of Fe at the 5% probability level, had a similar correlation with other traits as Cu and became significant at the 1% probability level. Fe concentration showed a negative but not significant correlation with chlorophyll content, and its correlation with genes was positive and significant at the level of one percent.

DISCUSSION

Senescence and Chlorophyll content

According to the results, the amount of chlorophyll in both cultivars was reduced after pollination, with the difference that Antonius cultivar had higher chlorophyll content in both flag leaf and other leaves than Euclide cultivar. For this reason, the green leaves were more in this cultivar than the Euclide cultivar. Basically, chlorophyll depletion in the senescence process is one of the most important indicators of the diagnosis of senescence in plants [27]. The use of stored assimilates in vegetative tissues to replenish the grains of monocotyledonous plants such as wheat requires the initiation or stimulation of the senescence phenomenon in the whole plant [28]. Significant signs of leaf senescence include a very regular and controlled process of physiological interactions, including cessation of photosynthesis, decomposition of chloroplasts, dramatic reduction of chlorophyll, and breakdown of proteins and other large molecules [29]. Lack of decomposition and higher chlorophyll content in the leaves indicate more greenery of Antonius cultivar indicates a later occurrence of senescence and biochemical interactions in this cultivar. The results of Zhao et al [30] showed that the chlorophyll content in cultivars in which senescence-dependentgenes were silenced by RNAi decreased later than in wild cultivars. They stated that cultivars with more greenery had higher chlorophyll content, which was consistent with the results of this study.

Senescence and genes expression

The expression of *TaNAM-B1* and *TaSAG12* genes, which have been introduced as signaling genes for senescence in wheat, showed results consistent with the results of chlorophyll content in leaves. Increased expression of both genes after anthesis was observed earlier in Euclide cultivar than the Antonius cultivar and had higher expression in most stages. *TaNAM* transcription factors play an important role in controlling senescence, which in alternation affects the delivery of nitrogen, iron, and other elements to wheat grains and mediate the redistribution of nutrients in the growing grain during leaf senescence. Podzimska-Sroka et al [31] stated that *NAM* genes showed more expression during leaf senescence, and silencing of genes with RNAi resulted in delayed senescence of transgenic plants. The trend of increasing the expression of this gene was consistent with the decreasing trend of chlorophyll content in both flag leaves and other leaves, and the results of the correlation study between them also confirm this. The only significant difference was the expression of this gene at 15DAA and 11DAA in other leaves, which was not observed in leaf chlorophyll content. In this regard, Pierce et al stated that *NAM* transcription factors act on a specific subset of genes in the early stages after pollination, before the appearance of signs of senescence, which confirms the results of this study.

SAG family genes have been introduced as the most widely used reference genes in describing leaf senescence [32]. Examination of *SAG12* gene expression after pollination, like other researchers, showed increased expression of this gene during leaf senescence [32, 33]. The positive and significant correlation of the expression of this gene with the expression of the *TaNAM-B1* gene and its negative and significant correlation with the amount of chlorophyll was also consistent with the results of Zhao et al [30]. James et al [34], in another study examining transgenic varieties of Arabidopsis that lacked the *SAG12* gene, reported that the absence of this gene in plants reduces seed production and N levels in Arabidopsis seeds in nitrogen deficiency conditions. However, the role of this gene in N remodeling is not yet fully understood.

Senescence and Cu, Zn, N and Fe remobilization

In light of the results, the change in concentrations of Cu, Zn and, Fe in the Euclid cultivar was more in all organs than in Antonius one. Due to the difference between the two cultivars in terms of the onset of leaf senescence, it can be said that the incidence of senescence has increased the mobility of these elements in the plant. The relationship between the onset of leaf senescence and increased remobilization of Fe and Zn has also been reported in the results of other researchers [35]. Because several proteins contain Fe and Zn ions, significant levels of metals can be released during leaf senescence due to the high level of protein degradation [3]. Podzimska-Sroka et al [31] stated that in transgenic plants in which leaf senescence was delayed by silencing the *NAM* gene, the Fe and Zn content in the grain was reduced by more than 30%. Wang et al [36] stated that when the grains are filled, the leaves are the source of Cu and Zn, and by transferring them to the grains, the content of these ions in the leaves decreases. They also stated that the vegetative parts for Fe ion are also as a source and Hummer as a reservoir and this ion accumulates in the leaves and stems in addition to remobilization to the grains, which is the reason for the increase in Fe during grain filling in the vegetative organ. These results are consistent with the findings of the present study.

Most of the nitrogen in wheat grains is obtained from proteins in vegetative tissues that are broken down into amino acids and recycled by grain mobilization [37]. Delay in senescence, along with the breakdown of proteins, leads to a decrease in amino acids or minerals, followed by a reduction in their remobilization. Degradation of any protein with zinc and copper ions releases a significant amount of these ions, and similarly, iron due to the degradation of chloroplast proteins can be released during leaf senescence. As shown in the results, the difference between Fe in the leaves of the two cultivars was much greater than Cu, Zn, and N. Late degradation of chloroplasts, which leads to delayed leaf yellowing and senescence, may explain why iron remobilization is more inhibited than other minerals in green cultivars.

Given the higher value of N in cultivars with more greenness, as can be seen in the present research, it is stated that this increase is attributed to the greater ability of green plants to absorb this ion from the soil and its remobilization from organs in senescence is reduced.

CONCLUSION

As a whole, comparing cultivars in terms of senescence and remobilization of minerals allows us to have a better understanding of remobilization and the contents of minerals in the grain. The results showed that the remobilization of Cu, Zn, N, and Fe to grains intensified with increased expression of the *TaNAM-B1* gene and the incidence of senescence in leaves. High expression of the *SAG12* gene, which encodes the protein cysteine protease, is also involved in senescence and N remobilization in plants. Also, given the importance of minerals in the food basket, it can be noted that the Euclid cultivar, in which leaf senescence begins earlier, and more minerals are stored, can produce grains with higher nutritional value than Antonius cultivar.

ACKNOWLEDGEMENTS

This study has been supported by the Gorgan University of Agricultural Sciences and Natural Resources.

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بررسی اثر پیری بر انتقال مجدد مواد معدنی در دو رقم گندم نان

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چکیده

منبع اصلی پروتئین و ریزمغذی‌ها در دانه‌های گندم، برگ پرچم و تا حدی برگ‌های پایینی است. هنگامی که برگ‌های سالم به مرحله نهایی رشد یعنی پیری می‌رسند، مواد مغذی لازم را قبل از تخریب و مرگ بافت به حرکت در می‌آورند. این آزمایش در استان گلستان انجام شد و ارقام گندم مورد مطالعه شامل اقلید و آنتونیوس بودند. نمونه برداری از برگ پرچم، سایر برگ‌ها، ساقه و دانه در ۷ مرحله گرده افشانی، ۷، ۱۱، ۱۵، ۱۹، ۲۳ و ۲۷ روز پس از گلدهی (DAA) انجام شد. میزان کلروفیل کل در رقم آنتونیوس هم در برگ پرچم و هم در سایر برگ‌ها بیشتر از رقم اقلید بود. بیان ژن‌های TaNAM-B1 و TaSAG12 که به‌عنوان ژن‌های سیگنال‌دهنده پیری در گندم شناسایی شده‌اند، نتایجی مطابق با نتایج محتوای کلروفیل در برگ نشان داد. افزایش بیان هر دو ژن پس از گرده افشانی در رقم اقلید زودتر از رقم آنتونیوس مشاهده شد و در اکثر مراحل بیان بالاتری داشت. با توجه به نتایج، تغییر غلظت مس، روی و آهن در رقم اقلیدس در همه اندام‌ها بیشتر از آنتونیوس بود. همچنین با توجه به اهمیت مواد معدنی در سبد غذایی، می توان به این نکته اشاره کرد که رقم اقلیدس که در آن پیری برگ زودتر شروع می شود و مواد معدنی بیشتری در آن ذخیره می شود، می تواند دانه هایی با ارزش غذایی بالاتر نسبت به رقم آنتونیوس تولید کند.

کلمات کلیدی: گرده‌افشانی، مواد معدنی، انتقال مجدد، پیری، گندم