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Alterations in the expression of senescence-associated miRNAs and their target genes in the mature flag leaf of wheat under drought stress

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Targeted mRNAs cleavage is directed by a class of tiny RNAs known as microRNAs (miRNAs). Different plant processes are regulated by miRNAs, including senescence, responses to environmental stressors, and development. The current study identified the senescence regulation of miRNAs. It evaluated the impact of miRNA-directed modifications in expression of target genes of *Triticum aestivum* cultivars in response to drought. Two cultivars of spring wheat, Sahel-1and Gemiza-10, were used in this study. The Fv/Fm ratio, indicative of the maximum quantum efficiency of photosystem II, and the Performance Index (PI), an indicator for assessing sample vitality, exhibited significantly elevated values in the drought-tolerant cultivar relative to the drought-sensitive cultivar.Furthermore, quantitative PCR analyses for the miRNAs families; miR156, miR172, miR164, miR171, miR156, and miR319 and their putative target genes at reproductive phase under drought conditions in the two genotypes were performed. The up-regulation of AP2 in the Sahel-1genotype played a crucial role in leaf wilting and delaying aging under stress of drought. The miR164 down-regulation and up-regulation of NAC1 contributed to the delay of leaf wilting and senescence.

Keywords: microRNA, Drought stress, Senescence, *Triticum aestivum*

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INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the main staple crop worldwide. Increasing the population leads to an increase in global demand for bread wheat (Dubcovsky and Dvorak, 2007; Shewry and Hey, 2015). Drought affects plant development, productivity and growth that in turn initiate the drought-resistance strategies in plant (Zhang et al., 2021). The microRNAs (miRNAs) are small regulatory RNA molecules that are found throughout various organisms. They regulate gene expression by binding to messenger RNA (mRNA), leading to either the degradation of the mRNA or the inhibition of protein translation, depending on the sequence similarity between the miRNA and its target mRNA (Dong et al., 2022). The miRNAs regulate the plant response to environmental stressors, including development and growth (Ma and Hu, 2023; Samynathan et al. 2023; Abdelsattar et al., 2024). In *Arabidopsis thaliana*, the first plant miRNA was discovered where 16 miRNAs were identified (Reinhart et al., 2002). In plants, miRNAs have been shown to be involved in drought stress response and have been identified in various studies. For example, a study by Kumar et al. (2024) identified 216 drought-responsive miRNAs in rice, which were co-localized with quantitative trait loci (QTLs) governing drought tolerance. Another study by Meher et al. (2022) found that miR169 was inhabited by drought stress, while miR398 was induced by drought stress in Arabidopsis. Additionally, Das et al. (2021) reported that drought affected miRNAs expression in maize. MiR156-mediated regulation of *SPL* gene exerts influence on transitions phase in wheat, rice, maize, tomato, and malus (Liu et al. 2017; Chen, 2010; Niu et al., 2019). Furthermore, flowering regulation in plants, in addition to development and growth, requires the involvement of miR156 and its SPL target genes (Aung et al., 2015; Wang et al., 2015). Additionally, Wang et al. (2009) and Han et al. (2013) reported that miR156 and its target gene *SPL* improved drought resistance in Arabidopsis. Abiotic and biotic challenges affect plant floral organ identity through *APETALA2* (*AP2*) genes, which are regulated by miR172 targeted *AP2* genes (Rubio-Somoza and Weigel, 2011; Debernardi et al. 2022). Plants employ senescence, which is best observed in leaves, to mitigate the consequences of stress. To manage the rice senescence process; abscisic acid (ABA) controls miRNA in the leaves, including miR172. These miRNAs target *AP2* transcription factors, which are overexpressed in plant cultivars resistant to senescence (Pomeranz et al., 2010).

Sun et al. (2022) and Yan et al. (2022) indicated that many plant species, including *Morus alba*, *Oryzasativa, Liliumpumilum*, *Solanumlycopersicum*, *Arabidopsis thaliana*, *Hordeum vulgare* and *Triticum aestivum* harbor the conserved 21-nt miRNA called miR171 (Hou et al. 2020). Plant stress responses, development and growth can be affected by miR171 functions (Um et al., 2022). By using the GA-DELLA signaling SCL module as a reaction to environmental

cues, miR171 regulates Arabidopsis growth (Mahale et al., 2014). The miR171 expression varies depending on plant species and genotypes and stage of growth. In *Triticum dicoccoides* the stress of drought decreases the expression level of miR171. In Arabidopsis, either miR156 or miR171 were downexpressed under drought (Liu et al., 2008). In rice exposed to drought stress, miR171 is variably expressed in different developmental stages (Zhou et al., 2010). miRNA played a crucial role in transcriptional profiling in drought responses (Ferdous et al., 2015; Chung et al., 2016). The up regulation of miR171 in *Solanum lycopersicum* led to a phenotypic development in flowering time, occurring earlier than which observed in the wild genotype.

Fan et al. (2015) mentioned that HAM1, HAM2, HAM3, and HAM4 which affect leaf morphology and heading date in rice were influenced by the level of miR171c expression. In *Arabidopsis thaliana*, SCARECROW-LIKE6-II transcription factor is regulated by miR171; and miR171 up-regulation resulted in increasing plant height and decreasing branching (Wang et al., 2010). According to Huang et al. (2017) GRAS members' roles shown in growth of shoots, chlorophyll, and roots by regulating gibberellic acid-auxin signaling.

The miR164 targets NAC genes, which negatively regulate tolerance to drought and are up regulated to delay plant senescence (Chen and Yu, 2023). MicroRNAs included in the biosynthesis of osmoprotectants, including trehalose and proline, were also up regulated, revealing that the plants possess inherent mechanisms to safeguard apposite tomolecular and cellular damage.The miRNA regulation lessens the strains brought on by drought. This suggests that all plant systems share these stress responses, which is critical to plant life and homeostasis (Sade at al., 2018). Salicylic acid-induced protein 19, or SIP19, is negatively regulated by miR164, which was up-regulated in rice leaves showing slowed senescence (Xu et al., 2017). In Arabidopsis, increased expression levels of miR164 lead to hindrance of leaf senescence through *NAC1* and *NAC21/22* expression down-regulation (Guo et al., 2004).

In rice, miR164 also targets *NAC* genes to adversely affect drought tolerance and control rice senescence (Fang at al., 2014). In Arabidopsis, it has been indicated that miR164 affected the *NAC* to regulate developmental processes (Guo et al., 2005). The miR164 family in rice regulates nine genes related to*NAC* family which negatively impacted the development process and drought tolerance (Fang et al., 2014; Chen and Yu, 2023). In mature wheat grains, previous studiesreported that members of the miR164 family target genes involved in phytosulfokine precursor and various *NACs* genes (Geng et al., 2020).The miR159 expression as a result of drought stress was observed to differ based on both the species and the specific tissue type in Arabidopsis, maize, cotton, and potatoes(Liu et al., 2008; Wei at al., 2009; Xie at al., 2015; Yang at al., 2014). Li et al. (2017) reported that drought stress up regulated miR159 in leaves, but in barley and lucerne, the root showed that miR159 less expressed. Targeting transcription factors (TFs) like *MYB, ARF*, and *NFY-A* is how miRNAs is typically involved in this biological process. In Arabidopsis, ABA increases miR159 accumulation during the stage of germination under drought, and acting as adverse regulator, cleaves its target RNAs, MYB33 and MYB101, to decrease signaling of hormone (Reyes et al., 2007). Drought causes down-regulation of miR159, which raises *MYB* expression and slows development and affects apoptosis (Reyes et al., 2007). The miR159 family members regulate *MYB* genes expression in plant (Allen et al., 2007). In wheat, it has been demonstrated that miR159 was regulated of *MYB33*, which is important for development of plant and signaling pathway (Qiu et al., 2016).

The drought stress response regulation, floral organ development, root morphogenesis and leaf are mediated by the regulation of miR156, miR172, and miR159; in addition to their target genes *SPL, MYB33*, and *AP2*, respectively (Liu et al., 2017). According to Fan et al. (2020), in Chinese white poplar, the family miR319 is involved in several biological processes, such as trichome initiation, assembly of cell wall, leaf development, in addition to resistance to stress either abiotic or biotic. The TFs of TCP class is the main targets for miR319 (Koyama et al., 2017; Zhang et al., 2016). Fan et al. (2020) reported that the regulation of trichome initiation in *Populus tomentosa* is regulated through the signaling pathway of miR319 and TCP, which enhances the defenses of insects. The cell wall thickening and cell elongation in cotton (*Gossypium hirsutum*) fibers are controlled by miR319 signaling (Cao et al., 2020). In *Panicum virgatum*(switchgrass), it mediates ethylene biosynthesis and signaling and the response to stress of salt (Liu et al., 2019). Hu et al. (2019) and Wang et al. (2021) have demonstrated that miR319 targets

transcription factor (GAMYB)(Jian et al., 2022). The ongoing study was designed to assess how miRNA signaling affects the expression of target genes in response to drought stress in two wheat cultivars.

MATERIALS AND METHODS

Experimental protocol

Two spring wheat cultivars, Sahel-1 (drought-tolerant) and Gemiza-10 (drought-sensitive), were used in this study (Ahmed et al. 2014; Sanad et al. 2019). It was kindly provided by (Field Crops Research Institute (FCRI), Agriculture Research Center (ARC), Egypt. The seeds of both cultivars were sown in pots each containing three kilograms of soil (two seeds per pot). A pot experiment was conducted at the greenhouse (day/night, 24/14°C; 16/8 h light/dark; 56.0 ± 14.2% RH; 20.0 klux) of Agricultural Genetic Engineering Research Institute (AGERI), (ARC), Egypt. After 30 d of sowing, the pots were divided into three groups, each group received a specific treatment for threemonths. Group T0 (Control) was watered every two days; group T1 (short-term stress) (was exposed to drought for one week and group T2 (long-term stress) was exposed to drought for two weeks. At the end of experiment, the mature flag leaves were harvested and immediately frozen at −80°C until used.

Chlorophyll fluorescence measurements

Parameters of Chlorophyll fluorescence were quantified using a Plant Efficiency Analyzer (Pocket-PEA, Hansatech, Norfolk, UK). The Performance Index (PI) and Fv/Fm were recorded after both the short-term and the long-term drought stress, as well as for the untreated plants. Measurements were taken from the flag leaves of 10 plants for each treatment during dark hours, using weak light from a green lamp for orientation and illumination of the samples.

mRNA and miRNA extraction and the first strand cDNA synthesis

TRI-reagent was used for total RNA extraction (Sigma-Aldrich Inc., MO, USA) following the manufacturer's instructions. NanoDrop™ 2000 was used for quantification of RNA concentration (Thermo Fisher Scientific). Total RNA was treated with RQ1 DNase (Promega, USA) following the manufacturer's protocols. According to Varkonyi-Gasic et al. (2007), miRNAs were reverse-transcribed into complementary cDNAs by MMLV reverse transcriptase (Promega, USA) using stem-loop primers (Tables 1 and 2). For the expression analyses of mRNA genes, MMLV reverse transcriptase was used to reverse transcription of total RNAs into first-strand cDNA (Promega, USA). The used specific primers were designed using PrimerQuest^{TM}Tool (https://www.idtdna.com/PrimerQuest/Home/Index) (Table 1).

Quantitative PCR

The miRNAs and mRNAs expression level were assessed using qPCRs according to the manufacturer's instructions (Agilent Stratagene Mx3005p), The BioEasy Master Mix of SYBR Green (BIOER, Hangzhou, China) was used. For the evaluation of miRNA expression, 20 µl reaction volumes were prepared, each containing 1 µl of 1:10 diluted cDNA template. The reactions also included 1 µl of a 10 µM miRNA-specific forward primer, 1 µl of 10 µM universal reverse primer, 10 µl of 2× SYBR Green Master Mix, and 7 µl of RNase-free water.The target genes of miRNAs were evaluated. Each reaction included 1 µl of diluted cDNA, 0.3 µl of each forward and reverse primer (10 µM), 10 µl of 2×SYBR Green Master Mix). The PCR condition was pre-denaturated at95°C/60s followed by 40 cycles of at denaturation at 94°C for 5 s, annealing/extension at 60°C for 40s), the melting curve analysis was carried out to check the product amplification (single peak). As an internalreference gene for normalization, U6small nuclear RNA (snRNA)-specific primer was used for miRNAs and Actin-specific primer for mRNA genes (Tables 1 and 2). According to Schmittgen and Livak (2008), the relative expression levels were calculated.

Statistical analysis

One-Way ANOVA was performed Using SPSS v23.0 (Chicago, IL, USA) to compare the data, followed by Duncan's multiple range (Duncan et al., 1953) test. The data was represented as (Means ±SE) and significant differences were tested at $p<0.05$.

RESULTS AND DISCUSSION

Chlorophyll fluorescence parameters

Photosynthetic performances were assessed for Sahel-1 and Gemiza-10 cultivars; Figure (1) represents the performing index andFv/Fm chlorophyll fluorescence parameters. Figure (1A) shows these parameters, such as wheat leaves' maximum PSII quantum yield (Fv/Fm). The Chlorophyll fluorescence evaluation reflects the status of plant photosynthetic apparatus instantly. The results show a significant difference between Sahel-1 and Gemiza-10 in relation to the Fv/Fm values. The Fv/Fm ratio for Sahel-1 was higher than Gemiza-10 by values of 0.83 and 0.82, respectively, in the control plants.

Table 1. List of Primer sequences for miRNA qRT-PCR validation

miRNA	Forward	Stem-loop
Mature-miR172	5′-agaaucuugaugaugcugcau-3′	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgtgcag-3′
Tae-mir172	5′-TCGCTtgaatcttgatgatg-3′	
Mature-miR171	5′-ugauugagccgugccaauauc-3′	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgatatt-3′
Tae-mir171	5′-TCGCTtgattgagccgtgcc-3′	
Mature-miR319	5′-uuggacugaagggagcucccu-3′	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACagggag-3′
Tae-mir319	5′-TCGCTttggactgaagggag-3′	
Mature-miR156	5′-ugacagaagagagugagcac-3′	5′- GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACgtgctc-3′
Tae-mir156	5′-TCGCTtgacagaagagagtg-3′	
Mature-miR164	5′-uggagaagcagggcacgugca-3′	5′-GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACtgcacg-3′
Tae-mir164	5′-TCGCTtggagaagcagggca-3′	
Universal reverse primer	5′-gtgcagggtccgaggt-3′	

Table 2. List of Primer sequences used for cDNA synthesis for target genes

Gene	Sequence
Ta_*NAC*(F)	5′-GGAATCCCTGAGCAAATGATA-3′
Ta_*NAC*(R)	5′-CACAAACTGCAACAACTACTG-3′
Ta_*SPL3*(F)	5′-CCCTATTACGCACATCAACTC-3′
Ta_*SPL3*(R)	5′-GAAATCGCCGTCCTTCTTT-3′
Ta_*AP2*(F)	5′-GAGAGGGACTAACATGGAACTG-3′
Ta_*AP2*(R)	5′-ACAGAACCTTGTCAGGAAGTAAA-3′
Ta_*GAMYB*(F)	5′-CGTGTGATACTACGGTGGTTAG-3′
Ta_*GAMYB*(R)	5′-GTGAGTGAATTGCCACTGAAAG-3′
Ta_*U6 snRNA*(F)	5′-TTGGAACGATACAAAGAAGATTAGC-3′
Tae_*U6 snRNA*(R)	5′-GCATATAAGAAGTGCGTGTCATC-3′

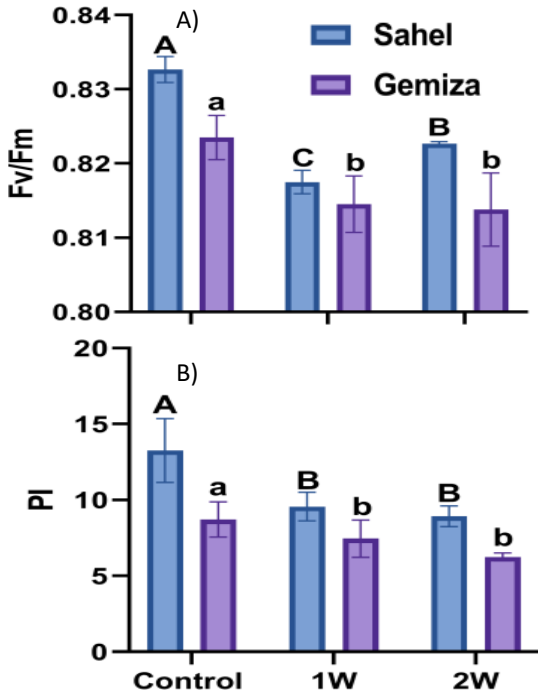

Figure 1. Drought stress effect of on (A) PSII efficiency (Fv/Fm) and (B) performance index (PI) in *Triticum aestivumL*. Data represented as (means ± SE) of three replicates. Different letters indicate significant difference ($p < 0.05$) for Sahel-1 by capital letters; and Gemiza-10 are followed by small letters.

Under drought conditions, in Sahel-1and Gemiza-10, Fv/Fm significantly decreased under short- and long-term drought, but remained higher in Sahel-1 than that in Gemiza-10 (Figure 1A). Performing index exhibited a similartrendas that ofFv/Fm in both cultivars. The Fv/Fm values were significantly different in the current resultsbetween Sahel-1 and Gemiza-10. Consequently, the performingindex exhibited a similar manner to Fv/Fm in both cultivars (Figure 1B). Chlorophyll fluorescence gave accurate data on the responses of photosynthetic performance in wheat flag leaves for both cultivars (sensitive and tolerant) exposed to drought stress. In wheat, leaf senescence is correlated with decreased photosynthesis activity due to stress of drought (Sommer et al., 2023). In accordance to these results, previous researchers have postulated that photochemical efficiency of PSII(Fv/Fm) may be significant decrease resulting from leaf senescence (Sommer et al., 2023; Yang et al., 2023). Regarding these reports, our study is consistent with these results, whereas in the tolerant cultivar, Fv/Fm was significantly increased in comparison to the sensitive cultivar. Interestingly, The Fv/Fm values confirm that under control conditions, Fv/Fm shows significant high values in comparison to Gemiza-10, revealing that Fv/Fm is genotype-dependent regardless of responses to sensitivity against stress of drought.

Gene expression analysis

The relative gene expression assessment was performed using qPCR for study the expression of some common miRNAs families involved in leaf development and senescence including miR156, miR172, miR164, miR171, and miR319 and their putative target genes at reproductive phase under drought conditions in two wheat cultivars. Three replicates of each leaf sample were used quantification of five miRNAs in addition to their target genes.

The miR156 and miR172 expression level were significantly down-expressed under both drought stress levels in Sahel, but the miR156 expression level was increased significantly in Gemiza-10 under short-term drought by 4-fold and sharply down-regulated at two weeks of drought (Figure 2A and B). The miR172 expression level increased at short- and long-term drought stress in Gemiza-10 by 1.3 and 2-fold, respectively (Figure 2 A1 and B1). The *SPL3* and *AP2* gene expression levels proposed asmiR156 and miR172 targets were significantly up-regulated by 2 and 1.7-fold at each drought stress condition in Sahel cultivar. Contrarily, in Gemiza-10, the expression level of *SPL3* and *AP2*genes did not show significant changes at both drought stress levels compared with the untreated plants, except at 1w treatment, where the *AP2* expression level was significantly up-regulated by 1.7-fold (Figures 2A1, B1, A2, B2). In Sahel, the miR171 expression level was down-regulated significantly compared to Gemiza-10, where there was a significant up-regulation of its target gene at 1w and 2w of drought conditions by 6 and 52-fold, respectively. The *DELLA* gene, proposed as miR171 target, was slightly up-regulated at 1w drought condition by 1.2 and 1.5-fold in Sahel-1and Gemiza-10 cultivars, respectively; but at 2w drought conditions, there were no significant changes compared to the untreated plants (Figure 3A, B).

The miR164expression levels showed significant up-regulation in Sahel-1wheat cultivar at both drought stress conditions, while the *NAC1* gene expression level, potentialmiR164 target, showed a significant up-regulation in at both drought levels by an average of 2-fold. Contrary, in the Gemiza-10 cultivar, the expression level of miR164 was significantly up-regulated by 27 and 12-fold at 1w and 2w, respectively. However, its expression level for *NAC1* was slightly up-regulated by 1.7-fold at 1w drought condition in comparison to 2w drought condition, which did not show any significant change compared to the control (Figure 4A, B).

The miR159expression level showed a significant inhibition in Sahel-1cultivar at both drought condition. Interestingly, in Gemiza-10 cultivar, the miR159 expression level was significantly up-regulated by 20 and 43- fold at 1 and 2w drought conditions, respectively, compared to the control plants (Figure 5A1 and B1). The *GAMYB33* in Sahel-1cultivar, that is proposed as a miR159 and miR319 target was significantly up-regulated by an average of 2-fold at both drought conditions. On the other hand, the expression level of GAMYB33 in the Gemiza-10

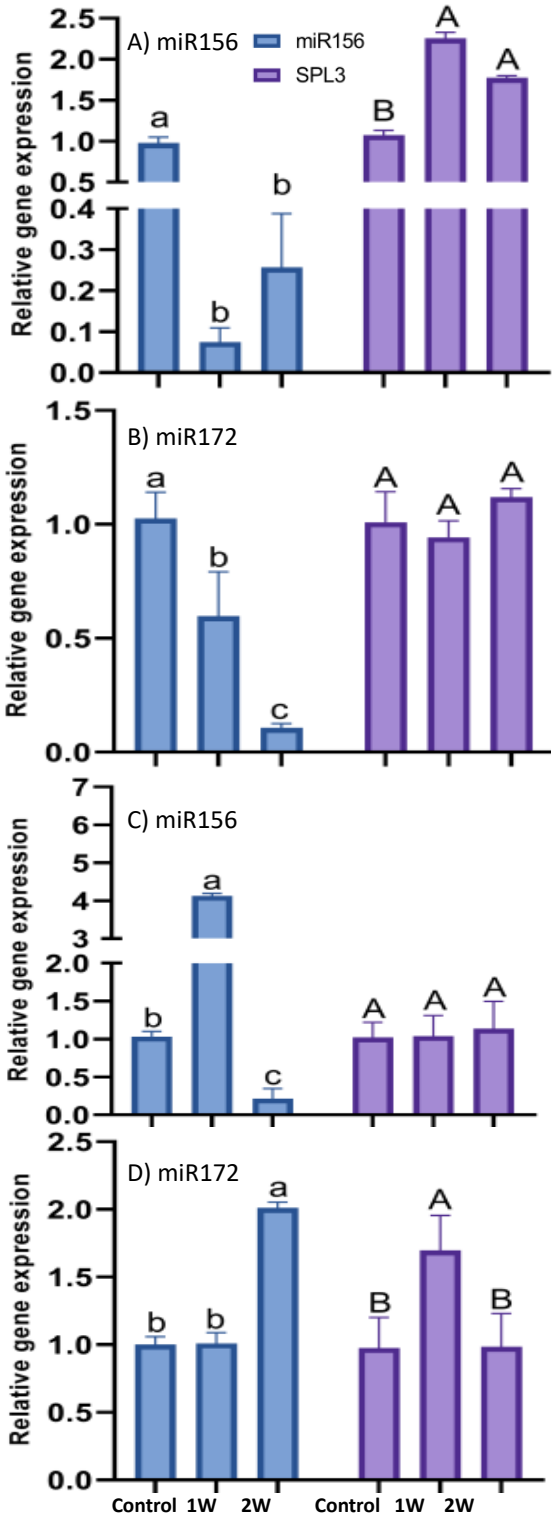

Figure 2. The miR156 and miR172 expression levels and their target genes *SPL3*and*AP2* involved in plant vegetative/reproductive phase transition and senescence in flag leave of Sahel-1 (A and C) and Gemiza-10 (B and D) under one (1w) and two weeks (2w) of drought stress. Different letters indicate significant difference ($p < 0.05$) for miRNA and its target gene is followed by small and capital letters, respectively.

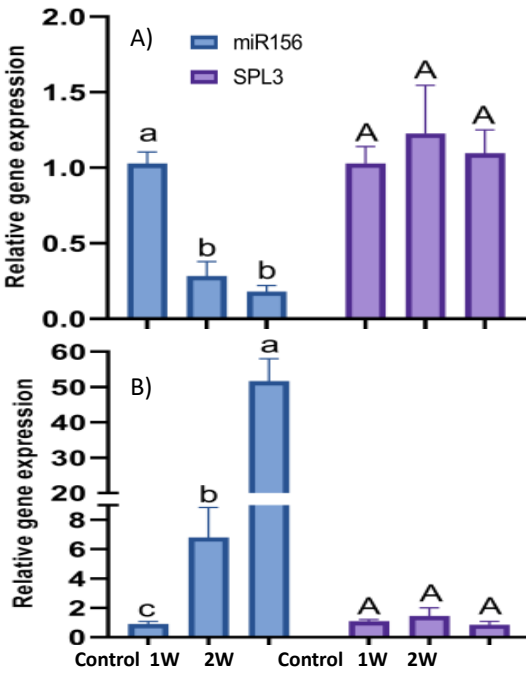

Figure 3. The miR171 expression levels and its target gene *DELLA* involved leaf growth regulation & chlorophyll biosynthesis in flag leave of Sahel-1(A) and Gemiza-10 (B) under one (1w) and two weeks (2w) drought stress conditions. Different letters indicate significant difference ($P < 0.05$) for miRNA and its target gene is followed by small and capital letters, respectively.

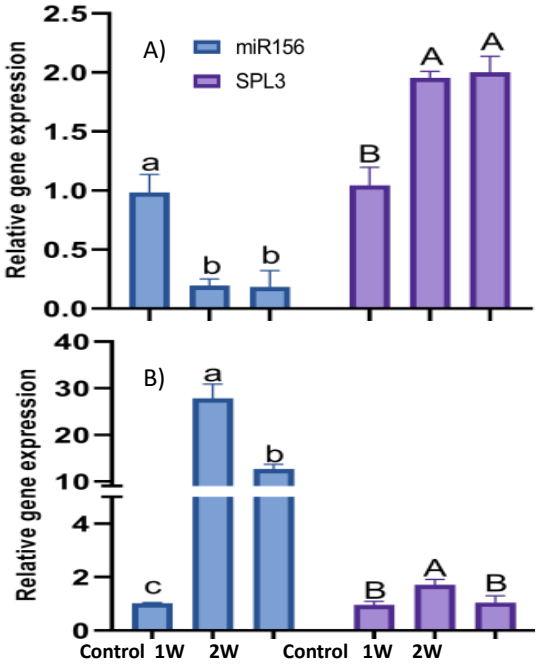

Figure 4. The miR164 expression levels and its target gene *NAC1* involved in leaf senescence of Sahel-1(A) and Gemiza-10 (B) flag leave under one (1w) and two weeks (2w) drought stress. Different letters indicate significant difference ($P < 0.05$) for miRNA and its target gene is followed by small and capital letters, respectively.

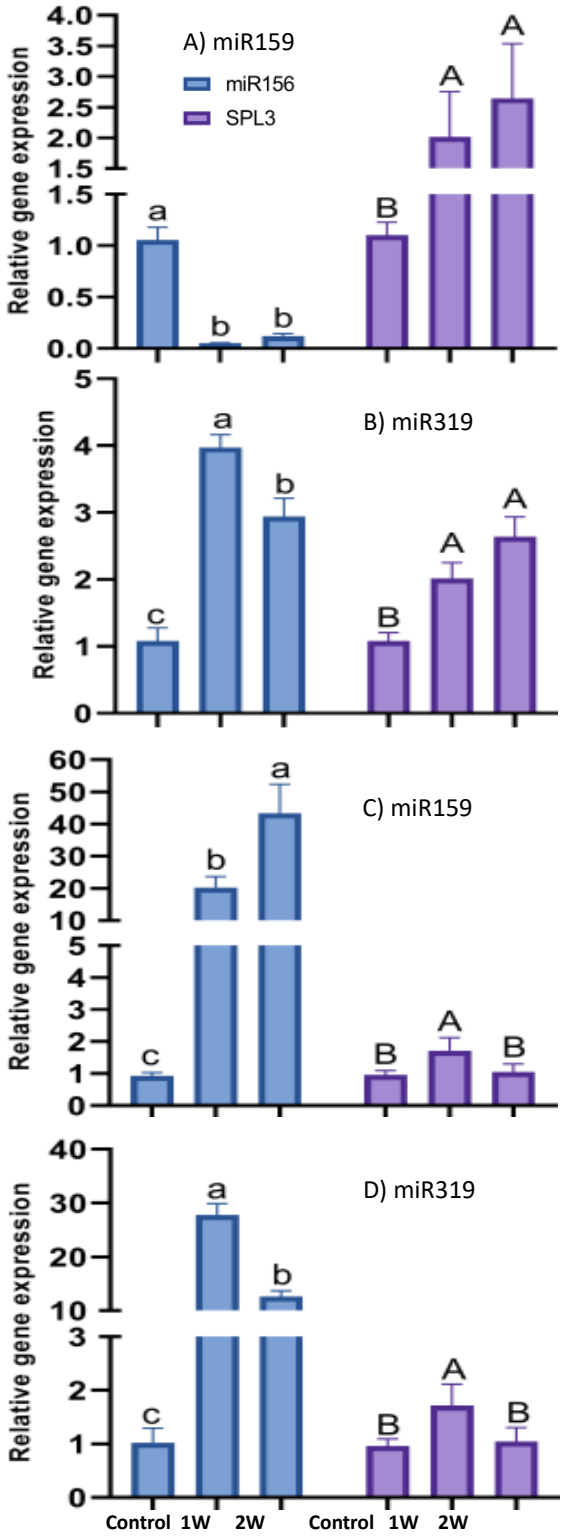

Figure 5. The miR159 and miR319 expression levels and their target gene *MYB33* involved in leaf senescence in flag leave of Sahel-1(A and B) and Gemiza-10 (Cand D) under one (1w) and two weeks (2w) of drought stress. Different letters indicate significant difference ($P < 0.05$) for miRNA and its target gene is followed by small and capital letters, respectively.

cultivar was up-regulated by 1.7-fold under 1 w drought condition, but no significant difference was observed at 2 w of drought compared to the untreated plants (Figure 5).The miR319 expression levelwas down-regulated significantly in Sahel-1cultivar at both drought conditions. Interestingly, in Gemiza-10 cultivar, the miR319 expression level showed significant up-regulation by 12 and 28-fold at 2 and 1w drought condition, respectively, in comparison to control group (Figure 5A2, B2).

The leaf maturity final stage (leaf senescence) is governed by intricate regulatory mechanisms (Sultana et al., 2021). Španić et al. (2023)reported that the senescence of the flag leaf in wheat is changed significantly throughout drought stress status. miRNA plays a role as a small regulatory RNA that regulates the gene expression pathways for a plant during developmental stages and environmental stresses (Samynathan et al., 2023). The current research assesses the role of some miRNA-directed alterations in their target genes' expression during drought stress in wheat after one week or two weeks. Some miRNAs in addition to their target genes that exhibited responses to stress of drought as reported in previous research were identified in current findings, as indicated in miR156/*SPL3*, miR164/*NAC1*, miR172/*AP2*, miR171/*DELLA*, and miR159/*MYB33*. The miR156 and miR172 controlling plant vegetative/reproductive phase transition and leaf senescence following response to stress of drought (Ma et al., 2015).

In Sahel-1, miR156 displayed an opposite expression pattern to that of miR172, with their target genes *SPL3* and *AP2* as decreasing and increasing, respectively, under drought conditions for two weeks. In contrast, Gemiza-10 exhibited contrasting results, indicating that the up-regulation of miR156 and *AP2*may be involved in drought resistance in wheat. Additionally, we found that the up-regulation of *AP2* gene in the Sahel-1indicated that the plant delayed aging and leaf wilting under drought stress, otherwise miR172 level increase during leaf aging and mortality under stress conditions (Xu et al., 2019). Moreover, miR164 and its target gene *NAC1* exhibited a distinct role in controlling the *DELLA* wheat plant exposed to drought stress, in which the downregulation of miR172 and up-regulation of *NAC1* delayed the leaf wilting and senescence under stress of drought (Zhao et al., 2015; Sultana et al., 2021; Iqbal et al., 2022). In wheat, miR171 regulates genes expression (Qin et al., 2012). In Gemiza-10 cultivar, the up-regulation of miR171 and its negative regulation of the target gene *DELLA* under drought stress conditions, particularly during a two-week drought period, indicate its sensitivity to drought stress. In contrast, Sahel-1cultivar exhibited normal expression levels for *DELLA* under drought stress. Additionally, Hou et al. (2020) mentioned that *DELLA* genes play a crucial role in mediating leaf growth regulation in addition to chlorophyll biosynthesis. Moreover, we found that *DELLA* could control the expression level of *SPL3* in Sahel-1cultivar that confers its role to delay leaf senescence.

The results indicate that miR319 and miR159 may affect common target genes. One of these genes is *MYB33,* which has positive impact on tolerance regulation against drought (Qin et al., 2012). The tolerant cultivar showed*MYB33*differential high expression at two drought levels in comparison to sensitive cultivar. Additionally, miR159 downregulation in Sahel-1and the up-regulation in Gemiza-10 prove *MYB33* role in tolerance against drought. This confirms that miR159 has positive regulation for tolerance against drought in wheat (Wyrzykowska et al., 2022). Moreover, miR319 expression levels showed altered patterns, with the high expression level of miR319 potentially affecting plant architecture and flag leaf expansion, which in turn can impact the photosynthesis rate. Although miR319 was up-regulated in Sahel, Gemiza-10 exhibited a up-regulation rate, indicating that the low expression level of miR319 was important for plant improvement under drought stress in Sahel-1 as a drought-tolerant cultivar (Jian et al., 2022).

CONCLUSION

*AP2*up-regulation in Sahel-1cultivar might play a role in delaying aging and wilting of leaf exposed to stress of drought. Conversely, increased of miR172 levels during leaf aging and senescence under stress conditions suggested a contrasting effect. Additionally, the interaction between miR164 and its target genes *NAC1*emerges to have a significant impact on controlling wheat exposed to stress of drought, with the down-expression of miR164 and up-regulation of *NAC1* contributing to the delay of leaf wilting and senescence. Furthermore, *DELLA* plays an intrinsic role in *SPL3* expression level in the Sahel-1cultivar highlights its ability to stall leaf senescence. These insights highlight the sub-cellular and signaling mechanisms involved in response of plants against stress of drought, emphasizing the importance of regulatory pathways in ameliorative effects of environmental challenges against plant health & longevity.

CONFLICT OF INTEREST

There are no conflicts to declare

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