IMAM KHOMEINI INTERNATIONAL UNIVERSITY
IJGPB
Iranian Journal of Genetics and Plant Breeding
Print ISSN : 2251-9610
Online ISSN : 2676-346X

Assessment of physiological characteristics and the expression of *CLsHSP18.1A* and *CcNAC2* genes by RT-PCR in seeded watermelon genotypes (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) under drought stress

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ABSTRACT INFO

Research Paper

Received: 08 Jul 2022

Accepted: 18 Dec 2022

ABSTRACT

Drought stress is one of the most important limiting factors, decreasing the production of vegetable crops in the global scale. The aim of this study was initially to examine two extreme seeded watermelon genotypes (C6 and C12) using physiological and biochemical characteristics in response to drought stress. The expression of key genes against drought stress (*CLsHSP18.1A* and *CcNAC2*) were also investigated using RT-PCR in the same condition. The highest concentration of proline content occurred in C6 (tolerant genotype) with 9.93 µmol/g fresh leaf after 5 days water stress. Malondialdehyde (MDA) and electrolyte leakage were significantly higher in C6 during drought stress. The result of gene expression levels for *CLsHSP18.1A* and *CcNAC2* indicated that there was a significant increase of expression in both genes in response to drought stress. In C6, drought stress after four days caused 2.5 fold up-regulation of *CLsHSP18.1A* compared with the control ($p \leq 0.05$), but in C12 (susceptible genotype), the expression decreased 1.4 fold compared to the relevant control. Semi-quantitative measurement of *CcNAC2* expression in both genotypes showed that maximum up-regulations occurred after two days of stress with the highest level in C6. Furthermore, four days after stress, a significant decrease occurred in the expression of this gene in both genotypes, but there was not a significant expression difference between susceptible C12 and its relative control. Overall, the expression levels of both targeted genes were higher in tolerant (C6) than that of susceptible genotype (C12) in all time points of drought stress. It seems that these genes may play a role in mechanisms involving in the drought tolerance in watermelon which could be important in molecular breeding of drought tolerant watermelons.

Key words: *CLsHSP18.1A, CcNAC2*, Drought, Gene expression, Proline, Watermelon.

How to cite this article:
Ghaemi Rad Z.-S., Vessal S., Babaeian Jelodar N., and Bagheri N. (2021). Assessment of physiological characteristics and the expression of *CLsHSP18.1A* and *CcNAC2* genes by RT-PCR in seeded watermelon genotypes (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) under drought stress. *Iranian Journal of Genetics and Plant Breeding,* 10(1): 107-115.
DOI: 10.30479/IJGPB.2022.16893.1313

INTRODUCTION

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is a monoecious and diploid (2n=22) plant belonging to curcurbitaceae family. This herbaceous creeping plant, mainly propagated by seeds and thrives best in warm areas. It is a tropical plant, requiring lots of sunshine and high temperature over 25 °C for optimum growth. Watermelon thrives best in a drained fertile soil with fairly acidic nature (Gupta *et al.,* 2018). Its fruit contains high amounts of water, having a rich source of vitamins C, A and, important minerals such as calcium, potassium, and iron, along with abundant fibers (Sultana *et al.,* 2004; Namdari, 2011). Iran stands after China and India in terms of cultivation area of watermelon, however it ranks the 17th position from yield perspective in Asia, with about 101000 hectares cultivation area, as its cultivation is mostly located in dry areas in Iran (FAO, 2020).

Drought is one of the most important abiotic stresses that limits the production of crops in arid and semi-arid climates such as Iran. This stress affects adversely plant growth by changing the biochemical and physiological processes such as enzyme activity, cell membrane permeability, leaf water status, and photosynthesis (Hussain, 2006; Jaleel *et al.,* 2009). Drought stress has a significant influence on morphological properties such as shoot and root length and dry weight as well as changes of biochemical and gene expression products (Mensah *et al.,* 2006).

There are a few studies on effective parameters related to evaluation drought. In some studies, physiological factors of watermelon plants have been investigated under drought conditions (Sarker *et al.,* 2005; Nasr, 2013). It was reported that proline synthesis is rapidly increased under drought stress in watermelon plants, which reverses to normal level after compensation of water deficit, indicating the ability of proline in osmotic adjustment under drought conditions (Sarker *et al.,* 2005; Nasr, 2013). Moreover, drought stress caused significant increase in malondialdehyde (MDA), as an indicator for peroxidation of membrane lipid (Nasr, 2013).

Plants initially respond to water deficit at molecular and cellular level through the expressional adjustment of a set of genes according to their performance (Shinozaki *et al.,* 2003; Hong *et al.,* 2017; Todaka *et al.,* 2017). Despite the large number of studies about the influence of stress on the gene expression in large scale such as microarray, individual analysis of the genes seems to be necessary to confirm this information. Therefore, evaluation of the expression of selected genes associated with drought responses in tolerant and sensitive watermelon genotypes, may also lead to the functional identification of effective genes in creating drought tolerant plants through appropriate crosses or genetic engineering.

CLsHSP18.1A is one of the key genes in drought tolerance in watermelon, encoding small heat shock proteins. Expressioin of sHSPsin plants can inhibit abnormal aggregation of other proteins and protect normal proteins through interacting with folding target proteins, protection of cell integrity, resulting in resistance under various adverse conditions (Wu *et al.,* 2022). Overexpression of *sHSP* in response to drought stress was reported in watermelom (Akashi *et al.,* 2011; Altunoğlu *et al.,* 2019; Zheng *et al.,* 2021). The expression of two transcription factors *CcNAC2* in *Citrullus colocynthis* and some other plants in response to various stresses were evaluated by (Wang *et al.,* 2014; Dong *et al.,* 2019; Yang *et al.,* 2021). These transcription factors have an important role in the growth and development of plants and their response to biotic and abiotic stresses. Different stresses such as drought, salinity, wound, abscisic acid and other hormones can cause significant upregulation of *NAC* and *CcNAC2* (Wang *et al.,* 2014).

To our knowledge, few studies are available about the seeded watermelon plant (*C. lanatus*), in terms of accurate evaluation of the expression key genes in response to drought stress. So, in this experiment, the expression of genes related to drought stress were evaluated for Iranian native watermelon genotypes. To achieve this, two contrastive watermelon genotypes for drought responsive were initially assessed by some physiological and biochemical characteristics in response to drought stress. Then, the expression of *sHSP* and *NAC* in these two genotypes were investigated under the same drought condition.

MATERIALS AND METHODS

Plant material

Based on the initial study done on the farm (unpublished data, private communication), two tolerant (C6; the code refers to *Citrullus* accession number in our seed bank) and sensitive (C12) genotypes to drought stress were selected for this study. For fast imbibition and removing the slimy material from seed coat, the seed were soaked in water for 12 h and rinsed further 3 times with tap water. The seeds were germinated in germinator and planted in 2 litters pots containing soil: sand: leaf compost (1:1:1 ratio), and grown in greenhouse with 25±3 °C and a 16/8 h (light/dark) photoperiod

condition under 50±5% relative humidity. The soil moisture content was preserved at approximately 75% until the stress was applied. Samples from young leaves were collected from 3-week-old seedlings at 0, 2 and 4 days exposure to progressive dehydration by irrigation withdrawal for gene expression analysis. Samples were immediately frozen in liquid nitrogen, followed by storing at -80 °C for further use.

Measurement of physiological and biochemical characteristics

Physiological and biochemical properties were measured at the starting time of stress (control) and the fifth day after stress.

The relative water content (RWC) was assessed according to (Barr and Weatherley, 1962). In brief, the fresh weight (FW) of separated leaves from each plant were measured. Then the leaves were dipped in a conical flask containing deionized water. After 16 hours, the leaves removed from the water and dried using tissue paper, and turgid weight (TW) were measured. To determine the dry weight (DW), leaves were then placed in the oven at 70 °C for 2 days and then weighed. Relative water content was calculated according to the following equation:

$$\text{RWC} = \frac{FW - DW}{TW - DW} \times 100 \quad (1)$$

Membrane Stability Index (MSI), was calculated as previously described by Premachandra *et al.* (1990). The first test tube containing 10 ml deionized water and 0.1 gr leaf tissue were placed in water bath at 40 °C for 30 min. The second test tube with the same content were placed in 100 °C water bath for 10 min. Then the tubes were moved to the refrigerator to reduce the temperature up to the ambient temperature. The rate of electrolyte leakage was read using EC meters. Membrane stability index (MSI) were assessed according to the following equation:

$$\text{MSI} = 1 - \frac{EC40}{EC100} \quad (2)$$

The proline content was determined according to an established method (Bates *et al.,* 1973). Briefly, 0.2 gr leaf tissue were grounded and homogenized in 4 ml 3% (W/V) hydrated sulfosalicylic acid. After centrifuging at 10000 rpm for 20 min at 4 °C, 2 ml of the supernatant solution were mixed with 2 ml Nin hydrine reagent and 2 ml glacial acetic acid. The mixture was placed in the 100 °C water bath for one hour. The reaction mixture is separated using 4 ml toluene, and the absorbance was read using spectrophotometer (OPTIMA sp-3000 plus) at 520 nm. Finally, using the standard curve, proline concentration was calculated according to the following formula:

$$\text{Proline } (\mu mol/gFW) = \left(\frac{\mu g\ proline}{ml} \times \frac{ml\ toloen}{115.5(\mu gl\mu mol)}\right) / \frac{gr\ sample}{5} \quad (3)$$

The lipid peroxidation was measured as described earlier (Heath and Packer, 1968). In brief, 0.2 g fresh leaf tissue was pulverized with 5 ml 0.1% trichloroacetic acid and centrifuged at 10,000 rpm. The supernatant was mixed with 20% trichloroacetic acid and 0.5% thiobarbituric acid solution (1:4 ratio), and then moved into 95 °C water bath for 30min. The malondialdehyde was calculated using the following equation.

$$\text{MDA}(\mu mol/gFW) = \text{A532-600}/1.55\times10^{-5}\,Mcm^{-1} \times \text{b} \quad (4)$$

Analysis of gene expression

After the initial assessment of the watermelon genotypes for evaluation of the stress tolerance of the genotypes through physiological and biochemical evaluation, the expression analysis for two candidate genes, *NAC* and *sHSP,* were done at 3 time points (0, 2 and 4 days after drought stress) using semi-quantitative RT-PCR.

Total RNA was extracted using column Total RNA Extraction Kit (Jena Bioscience). Briefly, 50 mg fresh tissue samples (from the third true leaves from growing point) were ground into the powder in liquid nitrogen and transferred to 1.5 ml tubes immediately. 10 µL/mL 2-Mercaptoethanol were added to the samples, followed by 500 µL Lysis buffer. After severe vortex (15-30 s) and centrifuging (10000 g for 10 min), 500 µL chloroform was added to the solution and centrifuged again. Then, 300 µl isopropanol was added to the supernatant, and transferred to spin column and then centrifuged. After washing with primary and secondary buffers containing 20 and 80% v/v of ethanol 96%, respectively, 38 µl elution buffer was added to extract the total RNA. In order to remove the genomic DNA, DNase treatment was done using DNaseI fermentase according to the manufacturer's and stored at -80 °C. RNA concentration for each samples was determined by NanoDrop ™ 1000 spectrophotometer, and then were loaded on to 1% agarose gel to measure quantity and quality of extracted RNA samples.

The complementary strand of extracted RNA, was synthesized using two-step RT-PCR kit (Vivantis), and M-Mulv enzyme. According to the manufacturer's

Table 1. Details of primers used for amplification of drought responsive genes (*CLsHSP18.1A* and *CcNAC2*) and a house keeping gene (*CcActin154*).

Gene	Gene ID	Accession	Description	Product length (bp)	Primer sequences
CLsHSP18.1A	315932717	HQ681894.1	Heat shock protein 18.1 *[Citrullus lanatus]*	364	5′ GCAACACTCTGCCTTCTTCC 3′ 5′ GCATCGACTGGAAAGAGACC 3′
ccNAC2	585231897	KC814687.1	NAC domain transcription factor 2 *[Citrullus lanatus]*	106	5′ GTGCCGGATTTACAACAAGAA 3′ 5′ AATCTTCGGCTTCTCGCTTC 3′
ccActin 154	111463272	XM_023147331.1	Housekeeping gene	118	5′ CACCATCACCAGAATCCAGCACGA 3′ 5′ GGCTCCACTCAACCCAAAGGCTAAC 3′

Table 2. Analysis of variance for physio-biochemical features.

Sources of variation	df	Mean of square			
		RWC	EL	Proline	MDA
Genotype (A)	1	18.575^{ns}	120.929*	4.201**	0.400*
Drought stress (B)	1	1626.108**	8817.690**	95.767**	8.217**
A×B	1	82.530^{ns}	81.777*	6.901**	0.249*
Error	8	19.361	7.913	0.315	0.041
Coefficient of variation (%)	5.629	5.295	9.745	11.570	

RWC**:** Relative water content, EL**:** Electrolyte leakage, MDA**:** Malondialdehyde.
ns: Non-significant, ***:** Significant at the 0.05 level of confidence; ****:** Significant at the 0.01 level of confidence.

instructions, initial denaturation was performed for 3 min at 94 °C, 25 s at 94 °C and extension was accomplished in 35 cycles for 1 min at 72 °C and 5 min at 72 °C for final extension.

The size and resolution of synthesized cDNA was examined on 1% agarose gel. Due to the steady expression of the actin gene, in all samples and both genotypes, *ccActin 154* was used as housekeeping gene for normalization. To determine the optimal number of cycles for each gene, the replications was evaluated individually in 20, 25, 30 and 35 cycles. By comparing the exponential and plateau phases of the genes, the best number of cycles was chosen. The best band was observed in 30 cycles for sHSP18.1A gene and 35 cycles for both Actin and NAC genes. Primers were chosen according to (Akashi *et al.,* 2011) and (Wang *et al.,* 2014) (Table 1). Finally, to ensure specific amplification of the studied genes, PCR products were separated on 1% agarose gel. In this case, 4 ml cDNA, 2 ml Green viewer and 1 ml loading buffer were placed in each well and electrophoresed for 50 min at 86 v.

Statistical analysis

Amplified PCR products on the gel images were analyzed using TotalLab software (ver. 1.10). Relative expression levels of target gene in each sample were normalized by *ccActin154* as an internal control. After removing the background from the image, the resolved gel image was introduced into TotalLab software to measure the band intensity. Statistical analysis and drawing the diagrams was done using SPSS (ver. 19) software and the figures plotted by Microsoft Excel 2013. After confirming the normal distribution of data, analysis of variance was performed and significant differences of means were calculated by Duncan multiple range test ($p \leq 0.05$).

RESULTS

The results of physiological features showed that there was no significant differences in term of relative water content between two genotypes (Table 2). However, C6 genotype lost lower water of leaves as compared with C12. ANOVA for RWC attribute showed that the effect of genotype (A) and the interaction between genotype and treatment (A×B) was not statistically significant, but the simple effect of drought stress treatment (B) were significant ($p \leq 0.01$; Table 2). Therefore, seedlings at the start of stress had higher relative water content relative to stress conditions in both genotypes (Table 2).

In the present study, electrolyte leakage was increased

significantly ($p\leq0.05$) in both genotypes under drought condition (Table 2 and Figure 1) (C12=56.29% vs. C6=49.94%, compared to the controls). The membrane stability index (MSI) of C12 genotype was about 10% lower compared with C6 genotype under drought stress. Moreover, the interaction between genotype and treatment was statistically significant in terms of electrolyte leakage. Sensitive genotype (C12) had the highest amount of electrolyte leakage (86.01%) after 5 days, but both tolerant (25.44%) and sensitive (26.57%) genotypes showed lowest electrolyte leakage at the beginning of imposing drought stress (Figure 1). Compared to controls, the electrolyte leakage increased by 66% under stress conditions in C6 genotype, while it was 70% in C12 genotype (Figure 1).

Analysis of variance for proline indicated that the effect of genotype (A), drought stress (B) and the interaction of genotype and drought stress (A×B) were significantly different ($p\leq0.01$) (Table 2). Proline content in drought stress conditions was increased significantly in both genotypes of watermelon compared to respective controls. As it is shown in Figure 2, the highest proline content was observed in C6 genotype (9.93 µM/g fresh leaf tissue), which was 3.3 times more than its control. However, drought stress caused 2.4 times increase of proline content in genotype C12, which the rate of increase was lower than C6 genotype.

The amount of malondialdehyde (MDA) was also affected by genotype (A) ($p\leq0.05$), drought stress (B) ($p\leq0.01$) and the interaction between genotype and treatment (A×B) ($p\leq0.01$; Table 2). MDA was increased after five days of stress, in both genotypes ($p\leq0.05$). The MDA concentration in C12 genotype was some 30% more than C6 after drought stress, although the similar amount were observed at controls in both genotypes (Figure 3).

Gene expression analysis

In this study, gene expression levels in tolerant and sensitive genotypes of watermelon seedlings was measured after 0, 2 and 4 days of drought stress. There were no changes in the expression of *CcActin154* at different time points, so this gene could be used as an appropriate internal control to normalize the intensity of expression of other genes in RT-PCR experiment (Figures 4 and 5). The results indicated that the primers were so specific in amplification of the target genes, which no additional bands were observed. Also, in positive and negative control samples, there was no additional band. Analysis of gene expression showed that genotypes responded differently in terms of expression amount to drought stress (Figure 5).

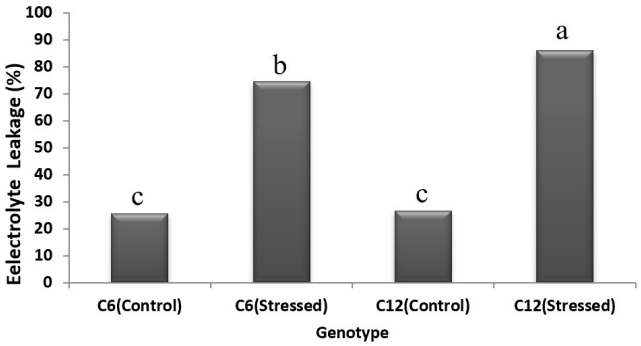

Figure 1. The effect of drought stress on electrolyte leakage after 5 days in two genotypes of seeded watermelon. The control treatment indicates the status of seedling electrolyte leakage at the start of stress. Means in columns with letters in common are not significantly different ($p\leq0.05$).

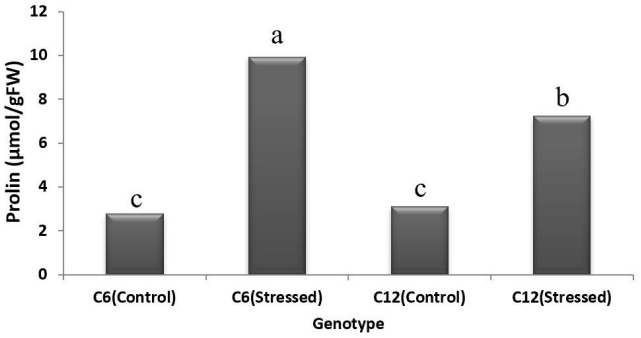

Figure 2. Changes of proline (µmol per gram fresh weight of leaf tissue), in two genotypes of seeded watermelon under drought stress after 5 days. Means in columns with letters in common are not significantly different ($p\leq0.01$).

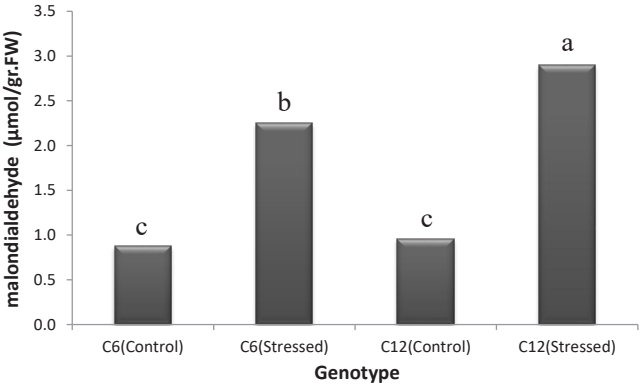

Figure 3. Changes of malondialdehyde amount (micromolar per gram fresh weight of leaf tissue) in two genotypes of seeded watermelon under drought stress after 5 days. Means in columns with letters in common are not significantly different ($p\leq0.01$).

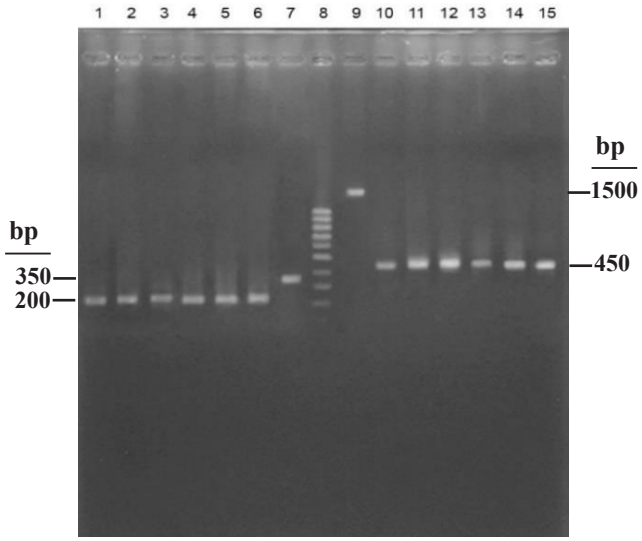

Figure 4. Semi-quantitative expression of sHSP gene under drought stress in tolerant (C6) and sensitive (C12) genotypes of watermelon. Wells 1-6 represent housekeeping actin gene in C6 (wells 1 to 3) and C12 (wells 4-6) genotypes at different time points (0, 2, 4 days of stress). Wells 7-9 are positive control (using DNA as a template in PCR) for actin genes (7) and the target gene (9) and molecular marker size (8). Wells 10-15 represent expression of the target gene (*CLsHSP18.1A*-) at different time points (0, 2, 4days of stress) in C6 (wells 10-12) and in C12 (wells 13-15) genotypes.

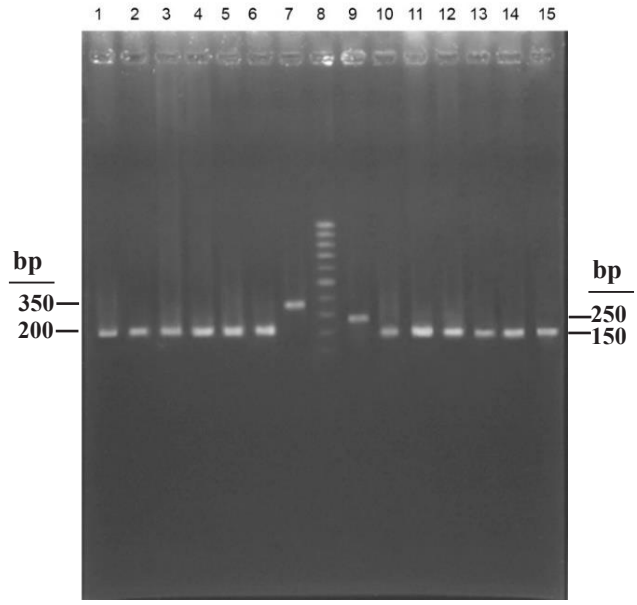

Figure 5. Semi-quantitative expression of *ccNAC2* gene under drought stress in tolerant (C6) and sensitive (C12) genotypes of watermelon. Wells 1-6 represent housekeeping actin gene in C6 (wells 1-3) and C12 (wells 4-6) genotypes at different time points (0, 2, 4 days of stress). Wells 7-9 are positive control (using DNA as a template in PCR) for actin genes (7) and the target gene (9) and molecular marker size (8). Wells 10-15 represent expression of the target gene (*ccNAC2*) at different time points (0, 2, 4 days of stress) in C6 (wells 10-12) and in C12 (wells 13-15) genotypes.

The gene encoding heat shock proteins in tolerant genotype (C6) significantly increased under drought stress with the progression of stress duration, compared to the control. As it is shown in Figure 4, the levels of *sHSP18.1A* expression in sensitive and tolerant genotypes are different in terms of band intensity (bands No. 10 to 15) at each time point, while no significant difference was detected in the expression of actin as internal control during progress of drought stress. The gene expression increased about 2 folds after 2 days and 2.5 folds after 4 days of stress imposed in tolerant genotype (C6), reaching the maximum, compared to control. The maximum expression of this gene in C12 (sensitive genotypes) was recorded after 2 days which increased about 65% and didn't change thereafter in site of increasing the stress level at 4 days (Figure 6). Accordingly, after 2 and 4 days of stress, the gene expression was increased 1.3 and 2.2 times more in C6 vs. C12, respectively (Figure 6). The pattern of *sHSP18.1A* expression constantly increased with continuation of stress time in C6, but there was no significant up-regulation in C12 after 2 days drought stress.

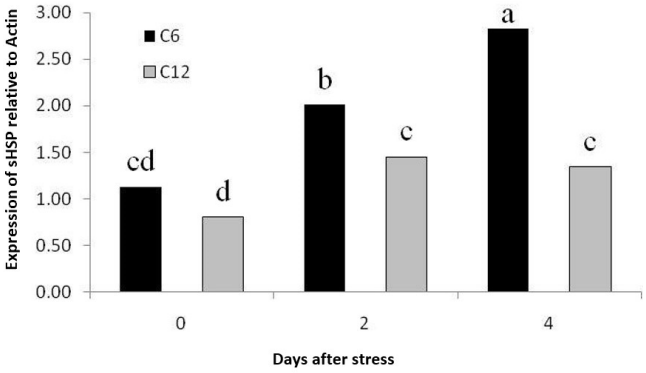

Figure 6. The relative intensity of *CLsHSP18.1A* expression compared with housekeeping gene (actin) in tolerant (C6) and sensitive (C12) genotypes of seeded watermelon in response to 0, 2 and 4 days of drought stress. Means in columns with letters in common are not significantly different ($p \leq 0.05$).

The RT-PCR analysis showed that the expression of NAC gene has also changed in response to drought stress, and the level of gene expression in sensitive and tolerant plants was significantly different in comparison to the respective controls ($p<0.05$). In all time points, the expression of this gene was significantly higher in tolerant genotype (C6) as compared to sensitive genotype (C12) ($p \leq 0.05$; Figures 5 and 7). After two days of stress, a significant up-regulation occurred

in NAC gene expression with 2.25 times more in tolerant genotype compared to control, reaching to its maximum level in the middle time point with a decrease later on. The similar pattern was also observed in the sensitive genotype (C12) but the expression level at 4 days of stress was not significantly different as compared to the beginning of the stress. Total increase in gene expression in tolerant genotype (C6) was 1.3 times more than sensitive genotype (C12) over stress period, while this ratio at 2 days after the stress was 1.8 (Figures 5 and 7).

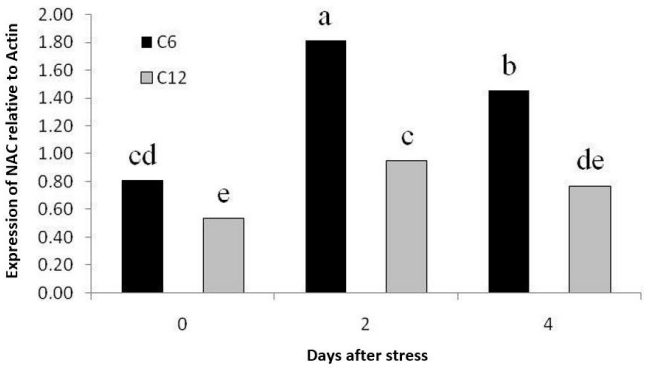

Figure 7. The relative intensity of *CcNAC2* compared with housekeeping gene (actin) in tolerant genotypes (C6) and sensitive (C12) genotypes of seeded watermelon in response to 0, 2 and 4 days of drought stress. Means in columns with letters in common are not significantly different ($p \leq 0.05$).

DISCUSSION

Drought tolerant plants employ some efficient mechanisms to conserve water. One of these mechanisms is the reduction of leaf area to reduce the evaporation and transpiration surface. Closing stomata also results in maintaing relative water content of leaf under drought stress (Wang *et al.,* 2014). It is reported that drought stress can cause 20 to 25% reduction in relative water content of watermelon leaves (Ferus *et al.,* 2011). In the present study, drought stress caused an increase in the electrolyte leakage in both genotypes (Table 2 and Figure 1). Moreover, compared to tolerant genotype, the MSI of sensitive genotype was lower under drought stress. This is due to drought stress damages to cell membranes by increasing the production of membrane destructive materials, such as ROS, causing membrane instability. Drought tolerant plants utilize mechanisms to deal with the destruction of membranes including decomposition of hydrogen peroxide by catalase and peroxidase enzymes (Fu and Huang, 2001). Significant increase in electrolyte leakage has been occurred in melon cultivars following abiotic stresses (Kavas *et al.,* 2013)

This study showed that the highest proline content was observed in both sensitive and tolerant genotypes by progressive drought stress but the rate of increase was significantly higher in tolerant genotype (C6). Similarly, higher levels of proline have been reported in drought and salinity tolerant genotypes compared to sensitive in melon (Kavas *et al.,* 2013). Proline accumulation in cells under stress, protect them from stress conditions and reduces toxicity effects. In response to drought stress, proline maintains the membrane structure by creating osmotic adjustment and preserving the structure of the enzyme in the cell (Ashraf and Iram, 2005).

Under drought stress conditions, MDA accumulation represents the accumulation of active radicals, protein and lipid oxidation, followed by degradation of cell membranes (Eraslan *et al.,* 2007), which increases in response to stresses (Gharibi *et al.,* 2019; Jiang *et al.,* 2019; AlKahtani *et al.,* 2021). In the current study, the amount MDA was increased after five days of stress, in both genotypes. However, as the MDA concentration in sensitive genotype was more than tolerant one after drought stress, it implies that more destruction of membrane lipids may be occurred in sensitive genotype (Figure 3). Previously, it was shown that lipid peroxidation increased under drought stress, leading to increase of MDA in the stressed cells (Eraslan *et al.,* 2007). It was also reported that drought stress caused significant increase of the MDA accumulation in watermelon leaves (Kiani and Jahanbin, 2006).

Analysis of gene expression showed that our genotypes responded differently in terms of expression amount to drought stress (Figures 6 and 7). The *sHSP18.1A* expression constantly increased for 4 days of stress induction in tolerant genotype, while the maximum expression of this gene in sensitive genotypes was recorded after 2 days. CcNAC2 and sHSP18.1A gene expressions in the genotype C6 were significantly higher than C12 in all time points of drought stress. In consistent with these results, (Akashi *et al.,* 2011) reported that sHSP gene expression had a significant increase in wild watermelon and other species (Dong *et al.,* 2019; Yang *et al.,* 2021) in response to drought stress. Moreover, Wang *et al.* (2014) reported a gradual increase of CcNAC2 gene expression at the beginning of drought stress, especially after 24 hours which is consistent with overall *CcNAC2* expression in the present study. It has been shown that the interaction of NAC proteins with some hormones

such as abscisic acid (ABA), JA, and salicylic acid (SA) affects both biotic and abiotic stress responses. Among phytohormones in NAC-associated pathways, a signaling crosstalk regulates the protective responses in plants via synergistic or antagonistic actions (Tuteja and Sopory, 2008). *CcNAC2* expression might be related to oxidative stress, and a drought related motif was also identified in the *CcNAC2* promoter (Wang *et al.,* 2014). In stressed condition, overexpressing of NAC gene exhibit significantly reduced stress-induced oxidative damage which can be confirmed by the lower level of MDA (Thirumalaikumar *et al.,* 2017). Furthermore, a strong transcriptional up-regulation of CLsHSP genes under water deficit was reported using RT-PCR (Akashi *et al.,* 2011). In consistent with this study, a gradual increase of CcNAC2 gene expression was reported at the beginning of stress, especially after 24 hours (Wang *et al.,* 2014).

In conclusion, based on examined physio-biochemical characteristics, including RWC, proline, MDA and electrolyte leakage, the response of tolerant genotype to drought stress was significantly higher and earlier compared to sensitive genotype that may indicate its relatively higher tolerance to drought stress. In this study, membrane stability of tolerant genotype (C6) was especially much higher under drought stress. On the other hand, due to the higher leaf relative water and accumulation of higher proline in tolerant genotype at early stage of drought stress, it seems that defense mechanisms may be activated earlier with high intensity. This could result in higher drought tolerance in responsive genotype (C6) under drought stress. Moreover, it seems that CcNAC2 and sHSP18.1A genes may also have a significant role in protection mechanism against drought stress in watermelon.

ACKNOWLEDGEMENTS

This research was supported by Sari Agricultural Sciences and Natural Resources University and Research Center for Plant Sciences, Ferdowsi University of Mashhad.

Conflict of Interest

The authors declare that they have no conflict of interest.

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