J. Crop Sci. Biotech. 2019 (March) 22 (1) : 37 ~ 45
DOI No. 10.1007/s12892-017-0180-0

RESEARCH ARTICLE

The Effect of Exogenous Applications of Salicylic Acid on Drought Tolerance and Up-Regulation of the Drought Response Regulon of Iraqi Wheat

Fakhriya Kareem*, Hail Rihan, Michael P. Fuller

School of Biological and Marine Sciences, Faculty of Science and Engineering, Plymouth University, Plymouth, Devon PL4 8AA, UK

Received: December 28, 2017 / Revised: April 27, 2018 / Accepted: June 02, 2018

Abstract

Drought is a major limiting factor of wheat production globally. In this research, salicylic acid was used in order to increase drought tolerance in Tamooz 2 of Iraqi wheat. It was observed that the SA treatment had animportant positive impact on the final wheat biomass and on the yield components (spike drying weight, grain dry weight, 1000 grain dry weight). The significant positive role of SA treatment on the up-regulation of *CBF14* gene was confirmed. The expression of *CBF14* was significantly affected by the developmental stage of wheat plants and the period between SA spray and sampling. We believe that results obtained in this study help to understand more deeply the molecular mechanism of drought tolerance in wheat. It could also have important practical applications, especially for wheat growers in dry climates.

Key words : Salicylic acid, drought tolerance, CBF/DREB, wheat, abiotic stress and growth stages.

Introduction

Recently a lot of research has been focused on the influence of drought stress on plant growth and crop productivity and has revealed that crop plants vary in their response to water deficit depending on the severity of the stress and also the stage of plant development at which stress occurs (Claassen and Shaw 1970; Gupta et al. 2001). The sensitivity of crops to damage from low soil moisture supply at different stages of growth has previously been reported for maize (*Zea mays* L.) (Denmead and Shaw 1960; Li-Ping et al. 2006) and showed that plants were apparently least affected by moisture stress imposed during the vegetative growth season, and although the plants in this duration appeared to recover from damage, the imposed stress could still result in reductions in grain yield at a later period.

The physiological mechanisms of plant responses to drought stress in semi-arid conditions are characterized by reduction of the transpiration process through closing the stomata which in turn affects the movement of CO_2 into the plant and also it associated with a decrease of the leaf area in order to maintain high tissue water potential and protect the metabolic process functions from the damage effects caused by the stress (Chapin et al. 1993; Chaves et al. 2009; Flexas et al. 2006; Hsiao 1973). Plants ability to cope with abiotic stresses such as drought, salinity, and cold is not only associated with physiological mechanisms but is directed by molecular controls at the cellular level leading to biochemical changes which enhance the ability to survive under these stresses (Dubouzet et al. 2003).

The molecular mechanisms of stress tolerance in plants involve stress perception and signal transduction followed by the expression of specific molecules (gene products) that play a key role in protecting plant cells from dehydration and damage. The expression of some transcription factors (TFs) that are induced by drought stress varies according to the response system of upregulation to produce different gene products (Reddy et al. 2004). Many researchers have focused on the family of TFs which are the dehydration responsive element binding gene (*DREBs*) or C-repeat binding factor gene (*CBFs*). The *DREB/CBF* is one of the most important group of the several families of transcription factors and has been known to be induced by environmental constraints such

Fakhriya Kareem (✉)
Email: fakhriya.kareem@plymouth.ac.uk

as cold, salt and drought (Liu et al. 1998; Nakashima et al. 2000; Pellegrineschi et al. 2004; Sakuma et al. 2006) and have been determined as important in the regulation of the drought response in plants. *DREBs* and *CBFs* have been shown to have considerable homology and more and more frequently are being considered to be of the same gene family.

To elucidate the molecular mechanisms of the gene expression in response to drought stress, many studies have been conducted primarily on the model plant species *Arabidopsis thaliana* (Iuchi et al. 2000; Liu et al. 1998). Research has been conducted to identify *DREB1/CBF* expression in transgenic *Arabidopsis thaliana* which was resulted in overexpression of a number of cold responsive/drought responsive (*CRT/DRE)* genes eliciting a higher level of tolerance to salinity and/or drought stress through the abscisic acid (ABA) independent pathway (Kasuga et al. 1999; Kitashiba et al. 2004; Xianjun et al. 2011; Yang et al. 2011). This TF group upregulate the expression of many downstream genes for signal transduction leading to the expression of many stress-responsive gene products that control the protection of cells from stress by the production of important metabolic proteins and other cellular compounds (Agarwal et al. 2010).

Analysis of the expression of dehydration-inducible genes has shown the expression of *DREB*/*CBF* genes induced in a variety of plants for example in rice (*Oryza sativa*) where the overexpression of *OsDREB* improved the level of tolerance to drought (Chen et al. 2008). Because many DNA markers are linked to drought tolerant traits in cereals, wheat and barley plants have been used in the creation of transgenic lines to monitor the expression of *DREB*/*CBF* factors from grains in response to severe drought conditions (Morran et al. 2011). Some of these reports have recognized the functional role of salicylic acid (SA) as a signalling molecule in regulating physiological mechanisms in plants that are exposed to abiotic stress. Previous investigations carried out on wheat plants showed that treating plants with SA could result in a reduction of the negative effects of water deficit on plants under stress (Aydin and Nalbantoglu 2011; Janda et al. 1999).

The relation between application of SA and the gene regulation of the drought response regulon has not been reported in the literature to date. This study aimed to invitigate the effect of spraying plants with salicylic acid (SA) at various growth stages on thedrought tolerance and on the up-regulation of *CBF 14* in wheat.

Materials and Methods

One variety of wheat (*Triticum aestivum* L.) which was shown to exhibit drought stress tolerance (Kareem 2017) was used in this study. Seeds were incubated at 25°C in a growth chamber (Sanyo Gallenkamp PLC LE 3202, England) for 3 days. Germinated seeds were then transplanted into plastic pots (28.5 cm width, 26 cm height) with 16 seedlings per pot, four pots per treatment (total n = 64 plants per treatment), and the plants were grown in the greenhouse (Skarden Garden, University of Plymouth) with an average temperature of 17°C and with supplementary lighting (sodium vapour lamps) to maintain a photoperiod of 12 h.

One month later, after three leaves had been formed (GS13) (Zadoks et al. 1974) the pots were irrigated to full field capacity (FC). The moisture content of pots was then regularly monitored with a Theta Probe (Delta-T Devices Ltd) and two watering regimes established when the seedling plants had four leaves (GS14). Irrigation of pots was carried according to Theta probe readings with the intention that the well-watered treatment was brought back to 100% FC after it had dropped to 70% FC whilst droughted pots were brought back to 70% FC after they had dropped to 50% FC.

The wheat plants were foliar sprayed with 1.44 mM SA at three times during the experimental period. The spraying treatment of salicylic acid 1.44 was prepared from the stock solution 1 g/1litre by dilution with distilled water according to Amin et al. (2008). The first spray was made for seedlings at two months after sowing once the plants had five leaves (GS15 Zadoks scale). The second application of SA was made during stem extension stage (GS32) 1 month after the first spray, while, the third spray was applied after a further 4 weeks later at flowering stage once the ears started to emerge from the sheath (GS51). The stomatal conductance of fully expanded leaves was measured using an automatic Porometer at 2 and 10 days after spraying with SA at stem extension and flowering stages.

At stem extension, plants were fertilized with 7.5 g pot^{-1} Gromore fertilizer (NPK 7:7:7). Two months after sowing, all the plants were sprayed with fungicide against powdery mildew (Fungus Clear Ultra, Westland Horticulture limited, Germany), and also sprayed with insecticide against aphids (Bug Clear Ultra, Westland Horticulture limited, Germany). Both sprays were repeated twice at 1 month intervals.

The experimental design was a completely randomized block (RCBD) with 16 treatments (2 water conditions x 8 SA spray treatments (none, leaf, stem extension, flowering, leaf+ stem, leaf+flower, stem+flower, leaf+stem+flower) in each of four replicate blocks. Destructive leaf samples were collected at 2 and 10 days after SA spraying for molecular analysis of *DREB/CBF* gene from plants of each replicate (block). At each interval, three leaves were detached from each treatment and placed immediately into plastic bags and placed on ice and then transferred to the -80°C freezer for the future molecular analysis. Plants in all four blocks were allowed to grow until the harvest stage.

The trace of soil moisture content under both watering regimes was plotted and showed a cyclical pattern of soil drying (Fig. 1). Once grain had set and the wheat plants had begun to showing yellowing, the irrigation was terminated to let the plants mature naturally. Three weeks from ending the watering, the plants were ripe for harvest and all the plants were pulled and the roots removed and discarded. The harvested plants were collected in paper storage bags and transferred to the laboratory for measurement of the yield components.

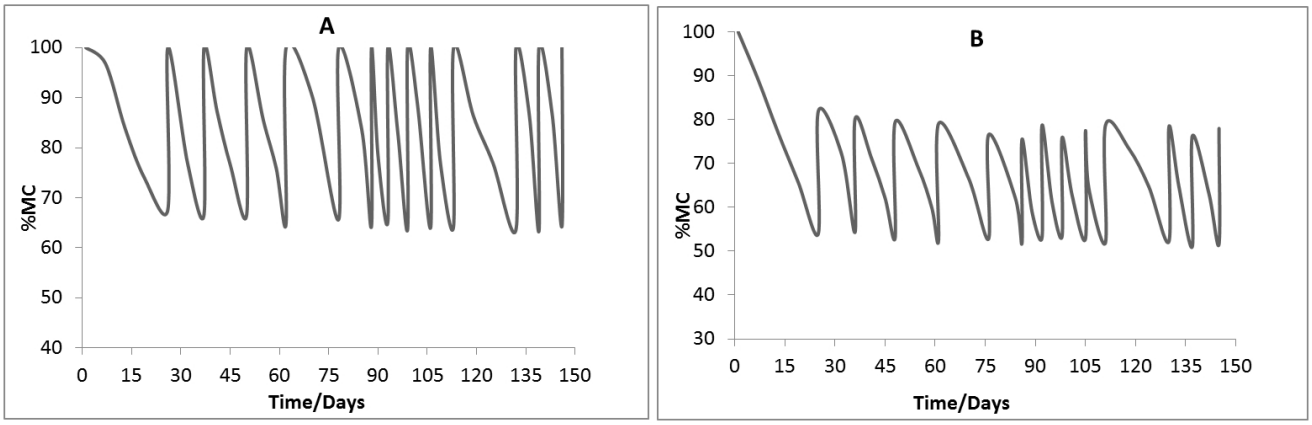

Fig. 1. The Moisture content of pots over the duration of the experiment as monitored by Theta Probe- A well-watered, B droughted.

Stems and leaves from plants for each pot were placed in paper storage bags, and dried in a drying oven (Gallenkamp Economy Incubator size two, model IH-150, England) at 80°C for 2 days until constant weight (Singh and Usha 2003). Then, the shoot dry weight was recorded (Precisa balances, Swiss quality, model 400M NO 13909, Switzerland). Each pot was dealt as one unit (i.e. the plants in a pot were pooled). Spikes were cut from the stems of plants, counted and kept in a separate paper storage bag, one per pot. Then, spike samples were dried in the oven at 80°C for 2 days until constant weight. The spikes were then weighed (Sartorius balance, model I8400P NO 35039, UK.). Total grain dry weight was determined by threshing the spikes so as to separate the grains from the chaff, and then the number of grains for each spike was counted. The grain from all spikes were then pooled and the weight recorded (Sartorius balance, model I8400P NO 35039, UK). From these measurements, the average 1000 grain dry weight per pot, the grain yield per pot and the average grains per spike were obtained (Forno 1972).

Molecular analysis for gene expression of *DREB/CBF*

Frozen samples (100 ± 10 mg) were ground to a powder in liquid nitrogen with a mortar and pestle and the total RNA was isolated using the Spectrum plant total RNA kit (Sigma Aldrich: spectrum plant total RNA kit, Cat # STRN50) according to the manufacturer's instructions. The total extracted RNA was quantified by Nano drop 1000 spectophotometer to estimate its concentration. The purity of the RNA was assessed by examining the absorbance ratio at 260 and 280 nm. Reverse transcription was done using using M-MLV Reverse Transcriptase (Sigma: M1302) in 20 µL volume. Sequence specific primers for *CBF/DREB CBF* 14 was used as the indicator for Transcription Factors *CBF/DREB* because they are identical and have homologous sequences. Primers used were as follows:

CBF14-Int-(F 5′-CCGTTCAGCACCGCCAAGGC-3′) and *CBF14*-Int-(R 5′- CCATGCCGCCAAACCAGTGC-3′) and for the endogenous control, Actin 1 mRNAs () (F 5-CCC AAAGGCCAACAGAGAGAAG-3) (R 5-CACCAGAGTC CAGCACAATACC-3) and were designed using Primer-BLAST (Ye et al. 2012) and synthesized by Eurofin MWG/ Operon (Germany). *Actin1* was selected as an endogenous control gene in order to normalise the gene expression obtained of *CBF14* in plants (Jain et al. 2006). The primers of *Actin 1* as described by Al-Issawi et al. (2013) and Rihan (2014) were designed to specifically bind the extremities of the DNA fragment to be amplified and were obtained from (Eurofins MWG/operon. Germany) (Forward primer (F 5-C CCAAAGGCCAACAGAGAGAAG-3) and reverse primer (R 5-CACCAGAGTCCAGCACAATACC-3)).

The cDNA for the samples was used as a template for Real time PCR reaction (Applied Biosystem, StepOne Pluse) which was prepared with SYBR Green JumpStart Taq ReadyMix (Sigma kit Cat. #S4438-100RXN). The PCR thermal cycle was optimized to be as follows:

1- Starting step at 94°C for 10 min.
2- Cycling step, which consisted of 40 cycles of (95°C for 15 s, followed by 60°C for 1 min.
3- Melting step, this consisted of 95°C for 15 s followed by 60°C for 1 min followed by 95°C for 15 s.

Finally, the results obtained were analyzed to get the relative quantitation of the expression of the target gene (*Cbf14*) against the endogenous standard gene (18s rRNA). The melting curve was set up at the end of the 40 cycles for 15 s at 95°C, 1 min at 57°C and 15 s at 95°C in order to be sure that only the gene of interest and the control gene were amplified.

Each PCR treatment was replicated three times to ensure consistency of results.

Statistical analysis

Results are presented as means ± standard error (SE). All data were analysed using the statistical software Minitab (version 17) and balanced analysis of variance (ANOVA). Significant differences between means were assessed by the least significant difference test (LSD) at the probability of 95%.

Results

The effect of SA treatments on the final biomass of wheat

There were highly significant effects of both watering conditions ($P = 0.043$) and SA treatments ($P \leq 0.001$) on shoot dry weight. Moreover, there was a significant interaction between SA treatments and watering conditions ($P \leq 0.001$). The best significant impact was observed when plants were treated with SA three times (leaf + stem + flower) under both well-watered and drought conditions (Fig. 2)

The effect of SA on the yield components of wheat

No significant effect of watering conditions was observed on the number of spikes per pot ($P = 0.515$). However, SA treatments had significant impact on the number of spikes ($P \leq 0.001$). A significant interaction between the watering conditions and SA treatments was observed ($P \leq 0.001$). SA treatments at stem+flower stages was found to have the best effect on the number of spikes per pot under both well watered and drought conditions (Fig. 3).

No significant effect of the watering conditions was observed on the dry weight of spikes ($P = 0.682$). However, SA treatments had highly significant effects on the weight of spikes ($P \leq 0.01$). Moreover, a highly significant interaction between watering conditions and SaA treatments was observed ($P \leq 0.01$). The best SA treatment was when plants were treated with SA at stem and flowering stages under both well-watered and drought conditions (Fig. 4).

Drought significantly increased the dry weight of grain ($P = 0.006$). SA treatment also had a sigificant impact on the grain weigh ($P \leq 0.001$) and there was a significant interaction between the watering conditions and SA treatment ($P \leq 0.001$). The best significant effect was observed when plants were sprayed with SA at both stem and flowering stages ands for plants grown under both well-watered and drought conditions (Fig. 5).

The average 1000 grain dry weight was not affected as much as other yield components but there were significant differences caused by the SA treatments ($P \leq 0.001$) and a significant interaction between SA treatments and watering conditions was observed ($P = 0.010$). SA treatment at stem+ flower and leaf+stem+flower stages showed a significant increase in average 1000 grain dry weight for plants under both well watered and drought conditions (Fig. 6).

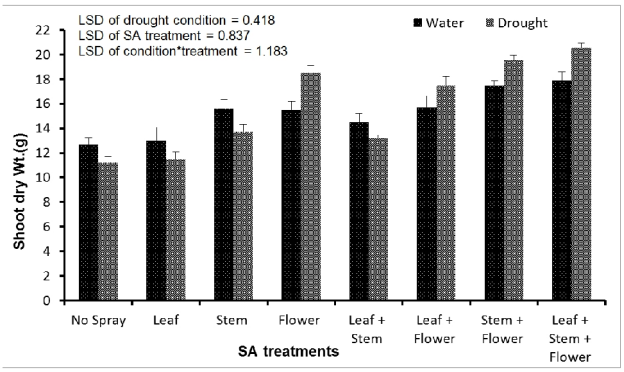

Fig. 2. The effect of SA sprays on the final shoot dry weight of wheat plants under watered and drought conditions at harvesting stage.

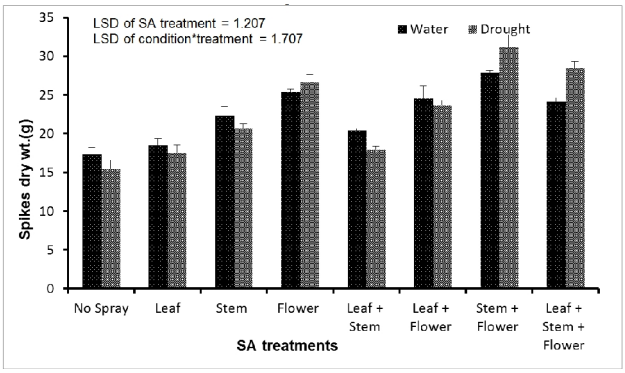

Fig. 4. The effect of SA on spike dry weights of wheat plants under watered and drought conditions at harvesting stage.

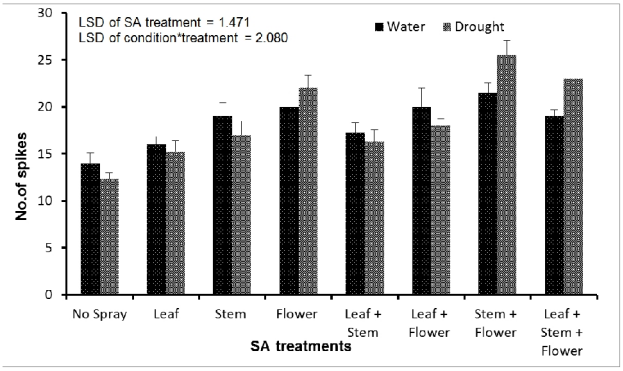

Fig. 3. The effect of SA spray on the number of spikes at the harvesting stage of wheat plants under watered and drought conditions.

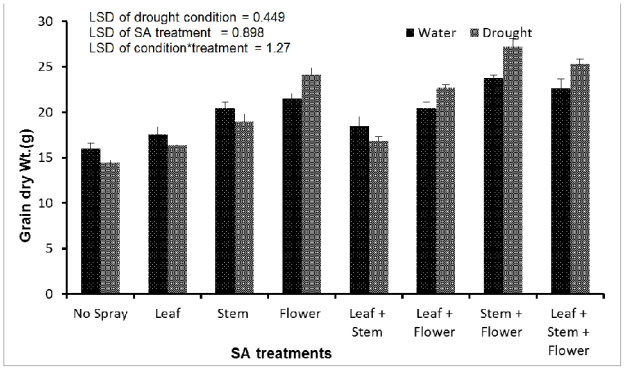

Fig. 5. The effect of Salicylic Acid (SA) spray on grain dry weight of wheat plants under watered and drought conditions at harvesting stage.

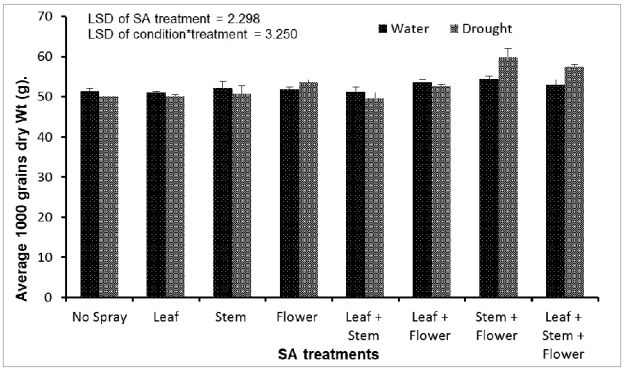

Fig. 6. The effect of SA spray on average 1000 grain dry weight of wheat plants under well-watered and drought conditions at harvesting stage.

The effect of SA treatment on the up-regulation of the CBF

Early spraying (GS15) of SA

Watering condition had a significant effect on the expression of *CBF14* after 2 days of SA treatments. In the control treatment, *CBF14* expression under the well-watered conditions was negligible and this increased under drought. There was also a significant effect of SA increasing the gene expression of *CBF14* for the wheat plant seedlings 2 days after spraying at both watering treatments ($P = 0.02$) (Fig. 7A).

Although there was no significant increase in the expression of *CBF*14 under the effect of SA in plants grown under drought conditions after 10 days of SA treatments, SA significantly increased the expression of this gene under the well-watered regime. Both drought ($P \leq 0.001$) and SA treatment ($P \leq 0.001$) significantly increased the expression of *CBF14*. Moreover, a significant interaction between watering condition and SA treatments on the expression of *CBF14* was observed ($P = 0.002$) and the best increase was observed when plants were grown under well-watered conditions and sprayed with salicylic acid (Fig. 7B).

Mid-spraying (GS32) (stem stage) of SA

There was a positive significant effect on the up-regulation of *CBF* gene 2 days after spraying with SA at GS32 ($P \leq 0.001$). However, SA treatment with plants sprayed earlier at GS15 did not significantly increase the expression of *CBF*14 gene.

A highly significant interaction between watering condition and SA treatment was obseved at this developmental stage ($P = 0.031$) and the best effect was observed when well-watered plants was sprayed with SA at the stem extension stage only. No significant effect of watering conditions on the upregulation of *CBF*15 gene was obseved at this developmental stage ($P = 0.18$) (Fig. 8A).

Drought had a significant effect of the up-regulation of *CBF14* gene after 10 days of spraying with SA during stem extension stage. SA had a significant effect on the up-regulation of this gene ($P \leq 0.01$). The best significant effect was obseved when plants were treated with SA at the stem stage only and stem + leaf stages. A significant high interaction between SA treatment and water regime was observed ($P \leq 0.01$) and well-watered plants treated with SA at stem stage showed the highest level of *CBF14* expression (Fig. 8B)

Late spraying at GS 51 (flowering stage)

The expression level of *CBF14* gene was significantly increased by SA treatments ($P \leq 0.01$) after 2 days of spraying treatment with SA and a highly significant interaction was observed between SA treatment and watering condition ($P \leq 0.01$). The highest *CBF14* gene expression was observed when plants were grown under drought and were treated with SA at leaf+stem+flowering stages (Fig. 9A).

SA treatments had a very significant positive effect of the expression of *CBF14* gene ($P \leq 0.001$) after 10 days of spraying treatment with SA. Watering condition had a significant effect of the expression of this gene ($P \leq 0.001$) and interestingly, the expression of this gene was higher in watered plant plants compared to those grown under drought conditions.

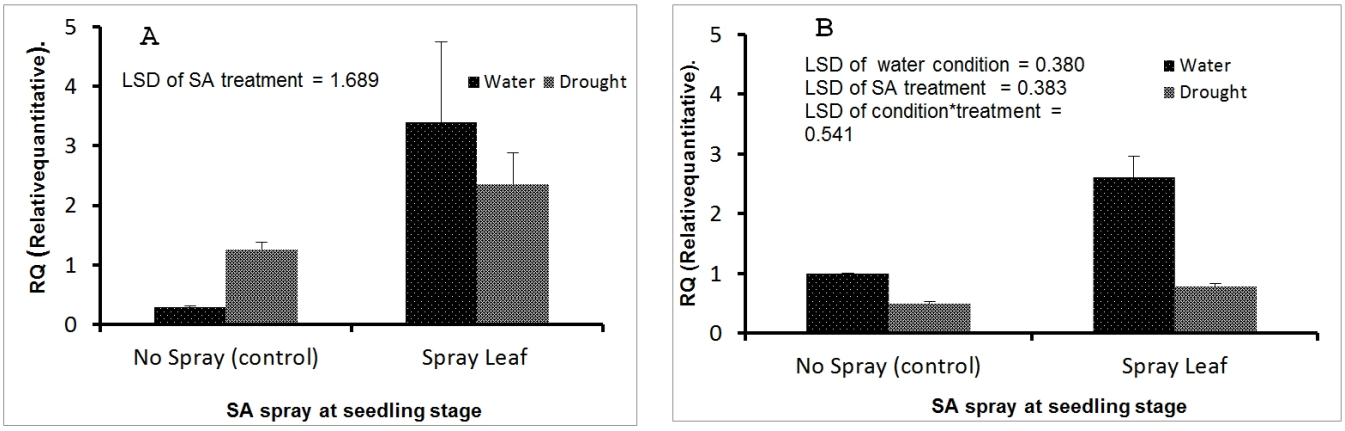

Fig. 7. The effect of SA on the up-regulation of the CBF gene 2 days (A) and 10 days (B) after spraying of wheat plants (VAR.Tamooz 2) at GS15 under both watered and drought conditions.

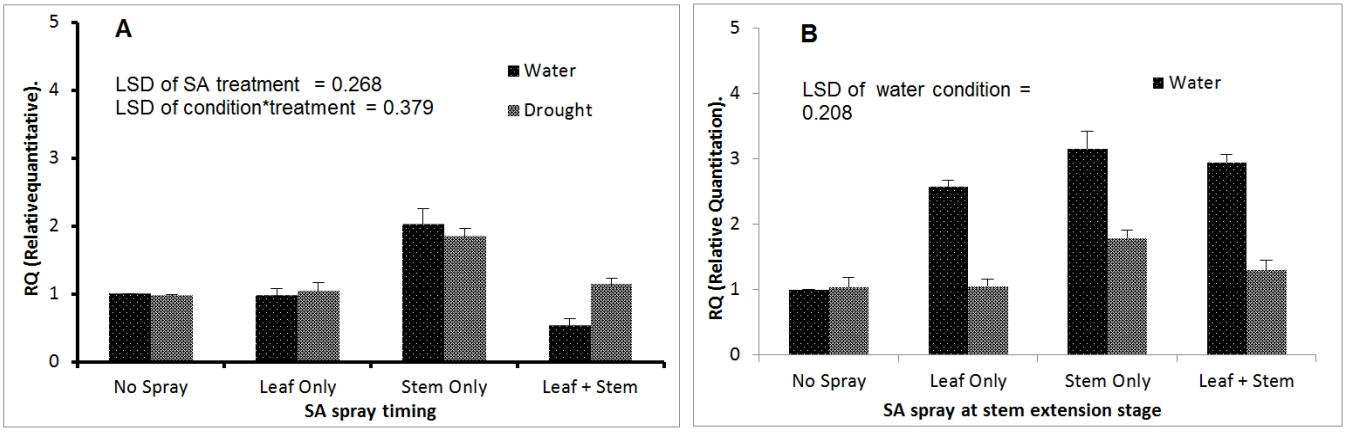

Fig. 8. The response of *CBF* gene to SA spray of wheat plants variety Tamooz 2 at GS32 two days (A) and ten days (B) after spraying under water and drought conditions during stem extension stage.

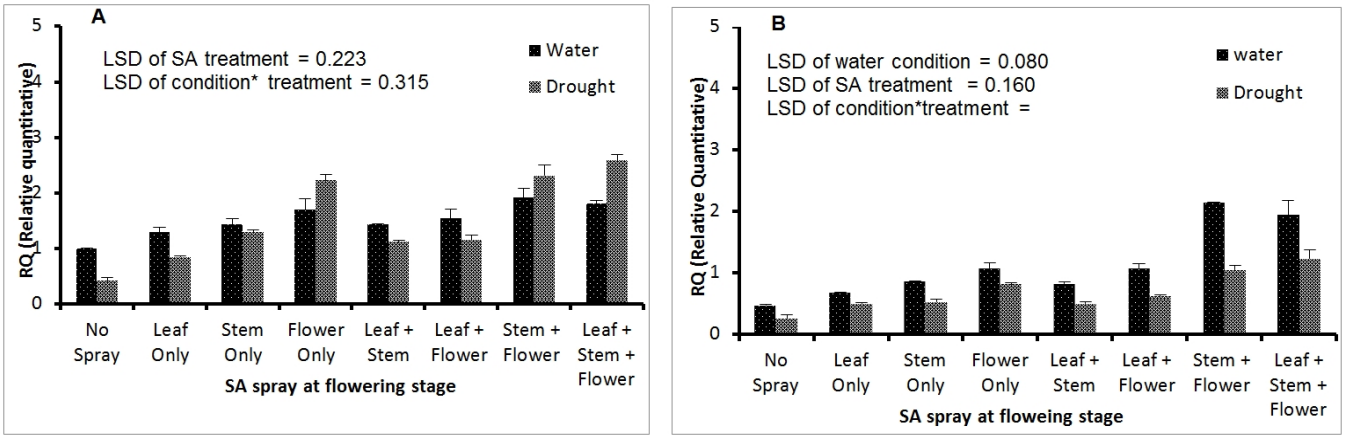

Fig. 9. The effect of SA on the up-regulation of the *CBF* gene 2 days (A) and after 10 days (B) after of spraying at GS51 wheat plants variety Tamooz 2 under well-watered and drought conditions.

A highly significant interaction between watering condition and SA treatments was observed ($P \leq 0.001$) and the highest CBF14 expression was observed when well watered plants were treated with SA at both stem+flowering stages (Fig. 9B).

Discussion

Spraying of wheat plants with SA had a highly significant effect on shoot dry weight and grain yield, under both well-watered and droughted conditions. In fact the spraying of SA not only counteracted the effects of drought it enhance yield so that it exceeded the well watered yields in most treatments. There was a small additional effect of spraying more than once especially if this was during stem extension and at the beginning of flowering. The exogenous application of SA appears to promote shoot growth and components of yield to counteract the adverse effect of abiotic stresses in a number of crop plants. In agreement with the current findings, Singh and Usha (2003) also found that SA-treated plants under water stress exhibited a higher dry mass in wheat (*T. aestivum* L.). Shakirova et al. (2003) observed a significant effect of SA applied at earlier growth stages on wheat plant growth under both salinity and water deficit stress. Khodary (2004) and Gunes et al. (2007) also reported the promotive effect of SA spray on the dry yield of Maize plants (*Zea mays* L.) grown under salinity stress (Habibi 2012). Aldesuquy et al. (2012), proved that foliar application of SA (grain pretreated at 0.05 M) and glycine betaine (GB, 10 mM) combined with SA had a positive impact on the dry mass of wheat shoots under stress conditions. It was also demonstarted that treatments of SA, (pre-soaking grain in 1 mM SA and leaf spray at pre-anthesis stage) significantly increased plant biomass and shoot dry weight in wheat under water deficit conditions (Abdelkader et al. 2012). Elgamaal and Maswada (2013) found that although the application of SA on yellow maize hybrid plants decreased the number of ears under water stress, it increased the productivity indicating an overall improved tolerance to stress. Hussain et al. (2008) have also demonstrated the positive effects of exogenous application of SA (0.724 mM) combined with GB (100 mM) on improving the yield of hybrid sunflower (*Helianthus annuus* L.) under

different irrigation regimes. Azimi et al. (2013) found that treating plants with SA at anthesis and during early grain filling improved the response of Zarrin wheat cultivar under drought stress whilst Grown (2012) revealed that growth and yield of two sunflower cultivars were improved by SA spraying. Azimi et al. (2013) reported a positive effect of SA on the physiological process of ovule fertilisation, resulting in a significant elevation of the grain dry weight of plants under moisture stress. It seems that spraying plants with SA before spike emergence and at the flowering stage can result in an improved weight of grains per spike as well as the number of spikes (Saini and Westgate 1999) which supports the findings of the current research.

In contrast with our findings, Pancheva et al. (1996) observed a negative effect of exogenous application of SA (100 mM^{-1}) on the growth and photosynthetic rate of barley plants (*Hordeum vulgare* L.). Also, Nemeth et al. (2002) indicated that the pretreatment of young maize plants (*Zea mays* L., hybrid Norma) with 0.5 mM SA decreased net photosynthetic rate and drought tolerance. It is suggested that the improvement of growth parameters by the application of chemical agents such as SA depends on the plant species, the stage of development, the method of application and the concentration of SA applied (Gutierrez-Coronado et al., 1998; Horvath et al. 2007).

SA treatments at the stem flower and leaf stem flower stages significantly increased the number of spikes per pot in drought stressed plants. This agrees with the findings of Slatyer (1969), Boyer and Westgate (2004), and Gholamin et al. (2010), who all came to the same conclusion, i.e. that the developmental growth period (around anthesis and early grain filling) is frequently sensitive to drought stress and can be alleviated somewhat by exogenous applications of SA. Interestingly, Cleland and Ajami (1974) reported that treatment of SA stimulates the flowering process of cocklebur plants and Martin-Mex et al. (2005), also concluded that an increase in the number of flowers per plant was caused by SA spraying in African violet plants at the early flowering stage. This could explain the significant impact of the time of spraying and concentraion on the response to SA treatments obtained in our study (Cakir 2004; Flexas et al. 2006; Slatyer 1969). The effect of SA treatment on the up-regulation of *CBF*/*DREB* gene was also investigated in this study. Monitoring the upregulation of *CBF*/*DREB* is notoriously difficult and it is only possible to take "snap-shots" of its activity at standard or pre-set times. It is confirmed in the results presented in this study that upregulation varies temporally, i.e. over time, after having received a stimulus. Furthermore, that stimulus could be the immediate application of SA or it could be as a result of a residual level of SA from a previous application or from drought. Since drought is a dynamic stress, varying both diurnally and over longer periods of time, the actual amount of drought needed to elicit *CBF*/*DREB* upregulation is extremely difficult to determine.

The regulation of stress-inducible genes in plants by the dehydration-responsive element (DRE) binding protein was reviewed by (Wang et al. 2006). The role of the transcriptional factor *DREB*2 gene in the upregulation of the drought-responsive gene was demonstrated in the cereal crops wheat, barley and maize by (Egawa et al. 2006; Qin et al. 2007; Xue and Loveridge 2004). Similarly, a pattern of *CBF* gene up-regulation has been reported by Al-Issawi (2013), in which the expression of *CBF14* increased on the first day of exposure of vegetative wheat plants (European and Iraqi genotypes) to low temperature (4°C). It then declined during the next few days but remained above control levels. Additionally, Chu et al. (2014), analysed the up-regulation patterns of two *DREB1* genes in Black Poplar (*Populus nigra*) by quantitative RT-PCR under normal growth and abiotic conditions with ABA treatment. When salt stress NaCl was applied, the *PnDREB68* and *PnDREB69* showed elevated expression levels in stem tissues at 8 and 48 h after the stress was applied. Given the vagaries of expression patterns of *CBF*/*DREB* and the complexity of the experiment carried out in this study, it is not surprising that a simple picture did not emerge for *CBF*/*DREB* expression. Nevertheless, it is clear that both drought and, more importantly, SA could upregulate the *CBF*/*DREB* expression and this could explain the positive biomass/yield effects found.

SA (Agarwal et al. 2006: Lata et al. 2015) upregulated the expression of *CBF/DREB* after 2 days of spraying however, the expression level of *CBF* gene decreased after 10 days of spray treatment. Morran et al. (2011), analyzed stress-inducible genes in transgenic *Triticum aestivum* cv. Chinese Spring plants, by the inducible promoter Rab17 gene from maize. It was shown that the over up-regulation of both downstream genes *TaDREB2* and *TaDREB3* resulted in plants more tolerant to severe drought and cold stress. *DREBs* were also transferred into wheat plants and were manipulated by the promoter rd29A so as to become involved in plant stress signalling and the development of stress tolerance (Pellegrineschi et al. 2004). The reason for this might be related to the fact that DREBs have a different category of transcription factors, which act as regulators of drought-responsive gene expression in direct or indirect ways (Bray 2004).

Based on the results of different sprays with SA on yield components of wheat plants under low-watering regimes, SA treatments at both stem, flower and leaf, stem, and flower stages significantly improved the drought tolerance of wheat plants. Furthermore, spraying wheat plants with SA had positive effects on the up-regulation of the drought response gene *CBF*/*DREB*, and whilst there was variance in the gene expression at different growth stages, there was some consistency between treatments. It can be concluded that spraying the plants with SA during the growing period of stem extension plus flowering stages is important for the protection of crop yield subjected to drought. Furthermore, this improvement in drought tolerance in stressed plants is probably mediated through the upregulation of the *CBF/DREB* gene and its downstream regulon.

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