

**Research Article****Influence of Salicylic Acid on Plant Height, Protein Percent and Chlorophyll of Wheat under Drought Stress**Mostafa Khamar¹, Hamid Reza Mobasser^{1*} and Hamid Reza Ganjali¹¹Department of Agriculture, Islamic Azad University, Zahedan Branch, Zahedan, Iran

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Article History: Received: January 12, 2016 Revised: May 12, 2016 Accepted: June 12, 2016**ABSTRACT**

Salicylic acid (SA) is part of a signaling pathway that is induced by a number of biotic and abiotic stresses. It has been recognized as an endogenous regulatory signal in plants mediating plant defense against pathogens. The field experiment was laid out split plot with randomized complete block design with three replications. Treatments included drought stress (I₀: control, I₁: Irrigation cut in pod stage, I₂: Irrigation cut in flowering) as main plot and sub plot consisted of foliar application of salicylic acid (S₁: control, S₂: 4%Mm, S₃: 7%Mm and S₄: 1 Mm). Analysis of variance showed that the effect of drought stress and salicylic acid on all characteristics was significant.

Key words: Plant height, Protein (%), chlorophyll**INTRODUCTION**

Wheat is one of the major crops in the world, and its yield is significantly influenced by global climate change and water resources scarcity in the environment (Al-Ghamdi 2009). Salicylic acid (SA) is part of a signaling pathway that is induced by a number of biotic and abiotic stresses. It has been recognized as an endogenous regulatory signal in plants mediating plant defense against pathogens (Raskin, 1992). Salicylic acid (SA) is an endogenous growth regulator of phenolic nature, which participates in the regulation of physiological processes in plants. SA, for example, plays a role of natural inductor of thermogenesis in *Arum* lily, induces flowering in a range of plants, controls ion uptake by roots and stomatal conductivity (Raskin, 1992). There are experimental data indicating participation of SA in signal regulation of gene expression in the course of leaf senescence in *Arabidopsis* (Morris *et al.*, 2000). Moreover SA might serve as a regulator of gravitropism (Medvedev and Markova, 1991), inhibition of fruit ripening (Srivastava and Dwivedi, 2000) and of other processes. A lot of data exist on the protective effect of SA against ultraviolet light (Yalpani *et al.*, 1994), salinity (Shakirova *et al.*, 2003), drought (Singh and Usha, 2003), heavy metal toxicity (Metawally *et al.*, 2003), high temperatures (Dat *et al.*, 1998) and paraquat (Ananieva *et al.*, 2002). Salicylic acid can also play a significant role in plant water relations

(Barkosky and Einhelling, 1993), photosynthesis, growth and stomatal regulation (Khan *et al.*, 2003; Arfan *et al.*, 2007) under abiotic stress conditions. Plants produce proteins in response to abiotic and biotic stress and many of these proteins are induced by phytohormones such as ABA (Jin *et al.* 2000) and salicylic acid (Hoyos and Zhang 2000). SA is synthesized by many plants (Raskin *et al.* 1990) and is accumulated in the plant tissues under the impact of unfavorable abiotic factors, contributing to the increase of plants resistance to salinization (Ding *et al.* 2002, Kang and Saltveit 2002). In addition, SA-induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova 1997). Thus the detrimental effects of high salts on the early growth of wheat seedlings may be alleviated by treating seeds with the proper concentration of a suitable hormone (Darra *et al.*, 1973). At the same time at present considerable interest has been aroused by the ability of SA to produce a protective effect on plants under the action of stress factors of different abiotic nature. Thus convincing data have been obtained concerning the SA-induced increase in the resistance of wheat seedlings to salinity (Shakirova and Bezrukova, 1997), and water deficit (Bezrukova *et al.*, 2001), of tomato and bean plants to low and high temperature (Senaratna *et al.*, 2000), as well as the injurious action of heavy metals on rice plants (Mishra and Choudhuri, 1999). The important role of SA in protecting is probably played by its ability to induce

expression of genes coding not only for PR-proteins but also for example the extension gene in Arabidopsis plants (Merkouropoulos *et al.*, 1999). There are data about SA induced synthesis of heat shock proteins in tobacco plants (Burkhanova *et al.*, 1999) and accumulation of wheat lectins (Shakirova and Bezrukova, 1997), fast activation of 48-kD protein kinase in suspension cell culture of tobacco at osmotic stress (Mikolajczyk *et al.*, 2000). This suggests the involvement of SA in realization of different anti stress programs. Water is one of the important factors affecting plant growth and yield. In addition, water resources need to be used efficiently because of the increasing competition of the limited water resources between domestic, industrial and agricultural consumptions. Biotic and abiotic stress conditions such as high temperature, drought, salinity and chemical toxicity have been a threat to agriculture and agricultural fields in many parts of the world (Wang, 2004; Vinocur and Altman, 2005; Berenguer, 2009). There is a need to limit the use of water resources in arid and semi-arid climates, particularly due to the threat of climate change. Decreased water reserves due to global warming accompanied by irregular seasonal and annual precipitations have increased the importance of irrigation scheduling, including deficit irrigation programs (Anonymous, 2011a). One of the most important stress factors that influences plant growth is stress occurring because of limited water application (Steele, 1997; Wanjura, 1990; Imtiyaz, 2000; Kaçar, 2006). Increasing plant production per unit of water is one of the greatest challenges facing the researchers especially in arid and semi-arid areas, which have limited water resources and in tropic and subtropics, characterized by hot dry weather. Efficient use of water by irrigation is becoming increasingly important, and alternative water application methods such as drip and sprinkler, may contribute substantially to the best use of water for agriculture and improving irrigation efficiency. The trend in recent years has been towards conversion of surface to drip irrigation which is considered to be a more efficient delivery system. Scheduling water application is very critical to make the most efficient use of drip irrigation system, as excessive irrigation reduces yield, while inadequate irrigation causes water stress and reduces production. On the other hand, the intensity of the operation requires that the soil water supply be kept at the optimal level to maximize returns to the farmer. High-frequency water management by drip irrigation minimizes soil as a storage reservoir for water, provides at least daily requirements of water to a portion of the root zone of each plant, and maintains a high soil metric potential in the rhizosphere to reduce plant water stress (Tiwari (1998) Singh and Rajput (2007) Al-Harbi (2008) Zotarelli (2009). Water deficit can destroy the chlorophyll and prevent making it (Lessani and Mojtahedi, 2002). Also some researchers have reported damage to leaf pigments as a result of water deficit (Montagu and WOO, 1990; Nilsen and Orcutt, 1996). Mensah *et al.* (2006) found that subjecting Sesames to drought stress caused leaf chlorophyll was increased and then remained unchanged. Beeflink *et al.* (1985) reported increase in chlorophyll in onion under drought stress. A reason for decrease in chlorophyll content as affected by water deficit is that drought or heat stress by producing reactive oxygen

species (ROS) such as O₂- and H₂O₂, can lead to lipid peroxidation and consequently, chlorophyll destruction (Mirnoff, 1993; Foyer *et al.*, 1994). Also, with decreasing chlorophyll content due to the changing green color of the leaf into yellow, the reflectance of the incident radiation is increased (Schelmmmer *et al.*, 2005). It seems that this mechanism can protect photosynthetic system against stress. According to the Lawlor and Cornic (2002) reduction of carbon assimilation confronting water deficit resulted in destruction of D1 protein of photosystem 2 (Xian-He *et al.*, 1995) but the reason have not been known, yet. The chlorophyll content was decreased with decreasing the irrigation water and this decrease was correlated with relative water content in leaves (Munne-Bosch and Alegre, 2000). Although studies in relation to cultivation techniques in rosemary have been realized, the agronomic and physiological responses to irrigation are scarce (Nicola's, 2008). There have been few studies performed. Chlorophyll loss is a negative consequence of water stress; however, it has been considered as an adaptive feature in plants grown under water deficit (Munne-Bosch and Alegre, 1999). In addition, the negative effect of deficit irrigation was reflected in decreasing the chlorophyll content of rosemary leaves. On the other hand, some authors found an opposite trend since chlorophyll increased by deficit irrigation. Khayatnezhad, (2011) and Alaei (2011) reported that drought stress condition increased the leaf chlorophyll content in wheat genotypes. This is because the exact effect of deficit irrigation may vary according to the intensity of the water stress imposed (Cameron, 1999). Seed protein content and baking quality highly depend on genetic background and environmental factors, especially influence of drought and heat stress, during the grain filling period and nitrogen availability (Luo *et al.*, 2000; Ottman *et al.*, 2000; Rharrabti *et al.*, 2001). In recent years, the applications of proteomic tools have become popular, and the tools are powerful methodologies for detecting and examining changes in protein composition accurately (Singh *et al.*, 1993). Storage protein is a method to investigate genetic variation and to classify plant varieties (Iqbal *et al.*, 2005). Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop. It has been widely suggested that such banding patterns could be important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivar or when cultivars are to be patented (Singh *et al.*, 1994). Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice (Singh *et al.*, 1994). For gave to highest seed yield in agriculture addition to both nitrogen and phosphate fertilizer is very important (Shaban, 2013a,b). Bideshki and Arvin (2010) reported that, drought stress decreased plant height, leaf area and yield of garlic but application of SA improved these traits in both drought and control conditions. In another research drought stress substantially reduced the rice stem height; nevertheless application of various compounds such as glycinebetaine (GB), SA, nitric oxide, brassinosteroid and spermine assuaged the damaging effects of drought. Foliar spray of all chemicals improved growth possibly because of the improved carbon

assimilation, enhanced synthesis of metabolites and maintenance of tissue water status (Farooq *et al.*, 2010). Alike to our results, Umebese *et al.*, (2009) found that water stress reduced tomato and amaranth stem height significantly at the vegetative stages and 3 mM application of SA was effective in keeping plant height similar to the control which was related to the ability of SA to induce antioxidant responses that protect them from damage. Role of SA in amelioration of plant height under water stress may be related to improve mitosis and cell elongation. Our findings are in agreement with the results of other researches (Gutiérrez-Coronado *et al.*, 1998; Hussein *et al.*, 2007).

MATERIALS AND METHODS

Location of experiment

The experiment was conducted at the zahedan which is situated between 29° North latitude and 60° East longitude.

Composite soil sampling

Composite soil sampling was made in the experimental area before the imposition of treatments and was analyzed for physical and chemical characteristics.

Field experiment

The field experiment was laid out split plot with randomized complete block design with three replications.

Treatments

Treatments included drought stress (I₀: control, I₁: Irrigation cut in pod stage, I₂: Irrigation cut in flowering) as main plot and sub plot consisted of foliar application of salicylic acid (S₁: control, S₂: 4%Mm, S₃: 7%Mm and S₄: 1 Mm).

Data collect

Data collected were subjected to statistical analysis by using a computer program SAS. Least Significant Difference test (LSD) at 5 % probability level was applied to compare the differences among treatments' means.

RESULTS AND DISCUSSION

Plant height

Analysis of variance showed that the effect of water stress on Plant height was significant (Table 1). The maximum of Plant height of treatments control was obtained (Table 2). The minimum of Plant height of treatments podding stage was obtained (Table 2). Analysis of variance showed that the effect of foliar application of salicylic acid on Plant height was significant (Table 1). The maximum of Plant height of treatments 0.7 was obtained (Table 2). The minimum of biological yield of treatments 0.4 was obtained (Table 2). Bideshki and Arvin (2010) reported that, drought stress decreased plant height, leaf area and yield of garlic but application of SA improved these traits in both drought and control conditions. In another research drought stress substantially reduced the rice stem height; nevertheless application of various compounds such as glycinebetaine (GB), SA, nitric oxide, brassinosteroid and spermine assuaged the

damaging effects of drought. Foliar spray of all chemicals improved growth possibly because of the improved carbon assimilation, enhanced synthesis of metabolites and maintenance of tissue water status (Farooq *et al.*, 2010). Alike to our results, Umebese *et al.*, (2009) found that water stress reduced tomato and amaranth stem height significantly at the vegetative stages and 3 mM application of SA was effective in keeping plant height similar to the control which was related to the ability of SA to induce antioxidant responses that protect them from damage. Role of SA in amelioration of plant height under water stress may be related to improve mitosis and cell elongation. Our findings are in agreement with the results of other researches (Gutiérrez-Coronado *et al.*, 1998; Hussein *et al.*, 2007).

Protein (%)

Analysis of variance showed that the effect of water stress on protein was significant (Table 1). The maximum of protein of treatments control was obtained (Table 2). The minimum of protein of treatments podding stage was obtained (Table 2). Analysis of variance showed that the effect of foliar application of salicylic acid on protein was significant (Table 1). The maximum of protein of treatments 0.7 was obtained (Table 2). The minimum of protein of treatments 1 was obtained (Table 2). Seed protein content and baking quality highly depend on genetic background and environmental factors, especially influence of drought and heat stress, during the grain filling period and nitrogen availability (Luo *et al.* 2000; Ottman *et al.* 2000; Rharrabti *et al.* 2001). In recent years, the applications of proteomic tools have become popular, and the tools are powerful methodologies for detecting and examining changes in protein composition accurately (Singh *et al.*, 1993). Storage protein is a method to investigate genetic variation and to classify plant varieties (Iqbal *et al.*, 2005). Seed protein is not sensitive to environmental fluctuations; its banding pattern is very stable which advocated for cultivars identification purpose in crop. It has been widely suggested that such banding patterns could be important supplemental method for cultivars identification, particularly when there are legal disputes over the identity of a cultivar or when cultivars are to be patented (Singh *et al.*, 1994). Seed storage protein is useful tool for studying genetic diversity of wild and cultivated rice (Singh *et al.*, 1994). For gave to highest seed yield in agriculture addition to both nitrogen and phosphate fertilizer is very important (Shaban, 2013a,b).

Chlorophyll

Analysis of variance showed that the effect of water stress on chlorophyll was significant (Table 1). The maximum of chlorophyll of treatments control was obtained (Table 2). The minimum of chlorophyll of treatments flowering stage was obtained (Table 2). Analysis of variance showed that the effect of foliar application of salicylic acid on chlorophyll was significant (Table 1). The maximum of chlorophyll of treatments 0.7 was obtained (Table 2). The minimum of chlorophyll of treatments control was obtained (Table 2). Water deficit can destroy the chlorophyll and prevent making it (Lessani and Mojtahedi, 2002). Also some researchers

Table 1: Anova analysis of the wheat affected by salicylic acid and drought stress

S.O.V	Plant height	Protein (%)	chlorophyll
R	3.25	0.36	5.36
Drought stress (a)	18.08*	20.02**	322.11**
Error a	3.70	2.23	1.36
salicylic acid (b)	21.21*	13.65**	63.44**
A*B	10.49 ^{ns}	6.10**	4.55 ^{ns}
Error b	4.37	0.981	2.21
CV (%)	2.19	5.08	2.97

*, **, ns: significant at $p < 0.05$ and $p < 0.01$ and non-significant, respectively.

Table 2: Comparison interaction of different traits affected by salicylic acid and drought stress

Treatments	Plant height	Protein (%)	chlorophyll
Drought stress			
control	96.41a	20.75a	56.00a
Podding stage	94.00b	19.16ab	47.66b
flowering	94.83ab	19.50b	46.50b
salicylic acid			
control	94.44b	19.11b	47.00c
0.4	93.88b	19.22b	49.22b
0.7	97.33a	21.22a	53.33a
1	94.66b	18.33b	50.66b

have reported damage to leaf pigments as a result of water deficit (Montagu and WOO, 1990; Nilsen and Orcutt, 1996). Mensah *et al.* (2006) found that subjecting Sesames to drought stress caused leaf chlorophyll was increased and then remained unchanged. Beeflink *et al.* (1985) reported increase in chlorophyll in onion under drought stress. A reason for decrease in chlorophyll content as affected by water deficit is that drought or heat stress by producing reactive oxygen species (ROS) such as O₂- and H₂O₂, can lead to lipid peroxidation and consequently, chlorophyll destruction (Mirnoff, 1993; Foyer *et al.*, 1994). Also, with decreasing chlorophyll content due to the changing green color of the leaf into yellow, the reflectance of the incident radiation is increased (Schelmmmer *et al.*, 2005). It seems that this mechanism can protect photosynthetic system against stress. According to the Lawlor and Cornic (2002) reduction of carbon assimilation confronting water deficit resulted in destruction of D1 protein of photosystem 2 (Xian-He *et al.*, 1995) but the reason have not been known, yet. The chlorophyll content was decreased with decreasing the irrigation water and this decrease was correlated with relative water content in leaves (Munne-Bosch and Alegre, 2000). Although studies in relation to cultivation techniques in rosemary have been realized, the agronomic and physiological responses to irrigation are scarce (Nicola's, 2008). There have been few studies performed. Chlorophyll loss is a negative consequence of water stress; however, it has been considered as an adaptive feature in plants grown under water deficit (Munne-Bosch and Alegre, 1999). In addition, the negative effect of deficit irrigation was reflected in decreasing the chlorophyll content of rosemary leaves. On the other hand, some authors found an opposite trend since chlorophyll increased by deficit irrigation. Khayatnezhad, (2011) and Alaei (2011) reported that drought stress condition increased the leaf chlorophyll content in wheat genotypes. This is because the exact

effect of deficit irrigation may vary according to the intensity of the water stress imposed (Cameron, 1999).

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