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Impact of Super Absorbent Polymer on Physiological Traits and Activity of Antioxidant Enzymes in Wheat (*Triticum aestivum* L. cv. Mihan) Affected Drought Stress Conditions

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RESEARCH ARTICLE

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ABSTRACT

BACKGROUND: Drought stress and climate changes cause damage led to reduction in agricultural production.

OBJECTIVES: Evaluation the impact of different levels of super absorbent polymer (SAP) on reducing the effects of drought stress on some physiological traits and activity of some antioxidant enzymes in wheat crop.

METHODS: Current research was conducted with using a split-plot arrangement based randomized complete block design with three replications in research field of Islamic Azad University of Kaleybar, Iran in crop year 2017. Main plots included different irrigation regime at three level (D_1: after 60mm, D_2: 90mm and D_3: 120mm evaporation pan class A) and different amounts of super absorbent polymer (S_1Zero or control:, 75 kg.ha^{-1}: S_2 and 150 kg.ha^{-1}: S_3 super absorbent polymer; SAP) belonged to sub plots.

RESULT: Analysis of variance showed the effects of super absorbent polymer, drought stress and interaction between super absorbent and drought stress in the probability levels of 1 and 5 percent were significant for most traits. The results also showed by exerting drought stress, the activity of antioxidant enzymes increased and the levels of chlorophyll a, chlorophyll b, carotenoids and relative water content decreased. Nevertheless, although using super absorbent significantly increased physiological traits but it decreased the activity of antioxidant enzymes. The irrigation level of 210 mm evaporation had the greatest impact on the activity of antioxidant enzymes and osmotic regulators.

CONCLUSION: The highest increase in most examined traits obtained when 75 kg.ha^{-1} of super absorbent was used. According to the results, super absorbent polymer resulted in mitigating the adverse effects of drought stress on wheat plants.

KEYWORDS: *Carotenoid, Catalase, Chlorophyll, Glutathione peroxidase, Proline.*

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1. BACKGROUND

Drought is one of the most important problems in crop production across the world, especially in arid and semi-arid regions like Iran (Yang *et al.*, 2006). Drought more than other environmental factors restricts plant growth and reduces crop production (Huang, 2000). Cereals, directly and indirectly, have the greatest importance in human nutrition. Wheat, among others, plays the most important role in human nutrition. Drought stress in all stages of wheat growth can affect its growth and yield in different ways. The extent of these effects varies depending on the duration and severity of drought stress (Guttieri *et al.*, 2001). Therefore, taking advantage of proper management and advanced controlling methods through preserving soil moisture content and increasing water holding capacity of the soil, it will be possible to improve the use of limited water resources in rainfed conditions. Relative water content is an important characteristic of a plant which is directly related to soil water content, and indicates soil water condition (Ghooshchi, 2015). Short-term drought stress caused a complete termination of photosynthesis and increased chlorophyll a/b ratio, whereas did not affect leaf chlorophyll content in wheat (Ahmadi and Baker, 2000). It has been observed that using superabsorbent polymers increased leaf relative water content in corn plants (Sishuai *et al.*, 2011). Photosynthesis persistence and leaf chlorophyll maintenance during drought stress are of suitable physiological indices in drought tolerance studies (Pessarkli, 1999). Increasing of soluble carbohydrate concentration is one of the reactions of plants, including wheat, when encountering drought stress (Bohnert *et al.*, 1995). Accumulation of soluble sugars in a cell plays an important role in osmoregulation by conserving more water in cell, through reducing cell water potential, in order to maintain turgor pressure during water deficit stress (Sato *et al.*, 2004). Pessarkli (1999) suggested that accumulation of amino acids and soluble carbohydrates like glucose, fructose and sucrose is significantly positively correlated with stability of biomembranes and proteins and also plant resistance to drought and salinity. According to (Ashraf *et al.*, 1994), drought stress reduces the concentration of chlorophyll b more than chlorophyll a in wheat. Proline accumulation in dry conditions has many biological effects. It reduces the capacity of soil solution for water and increases production of proline that in turn leads to an increase in osmotic pressure of the cell sap (Naeimi *et al.*, 2018). In agriculture, polyacrylamide is the main synthetic polymer used, and it absorbs water through the formation of hydrogen bonds (Ahmed, 2015). SAP can absorb water up to 200-400 times its weight and increase its size by up to 100 times. The end products of SAP degradation in soil are carbon dioxide, water, and ammonia (Wallace, 1986). Polymers absorb and store water and nutrients in a gel form and undergo cycles of hydrating and dehydrating according to moisture demand, increasing both water and nutrient use efficiency in crop plants (Islam *et al.*, 2011, a). A superabsorbent polymer can hold 400 to 1500 times as much water as its dry hydrogel (Fazeli Rostampour *et al.*, 2013). Polymers are safe, nontoxic, and decompose to CO_2, water, NH_4, and K^+ without any residue (Keshavars *et al.*, 2012). Superabsorbent polymers can absorb large quantities of water or aqueous solutions and swell (Buchholz *et al.*, 1997). These water storage tanks, when placed inside the soil, absorb irrigation water and rainfall and prevent it from subsiding. After drying, the water inside the polymer is gradually evacuated and thus the soil remains wet for a longer period of time without the need for reirrigation (Kuchakzadeh *et al.*, 2000). Islam *et al.* (2011, b) evaluate the effectiveness of different rates of SAP (low, 10; medium, 20; high, 30 and very high, 40 kg.ha^{-1}) for winter wheat production under drought-affected field and reported the optimum application rate of SAP would be 30 kg.ha^{-1} as it increases both wheat yield and soil fertility. Lower rates (10 and 20 kg.ha^{-1}) are not sufficient and higher rate (40 kg.ha^{-1}) is not economic. They suggested that the application of SAP at 30 kg.ha^{-1} could be an efficient soil management practice for winter wheat production in the drought-affected regions. In order to save soil moisture, some materials such as crop residue, mulch plants, waste, litter, straw and stubble, and other synthetic materials like Hydroplus. Super absorbent polymers are compounds that absorb water and swell into many times their original size and weight. They are lightly cross-linked networks of hydrophilic polymer chains. Super absorbents, depending on their source and structure, are divided in two main groups of natural and synthesis. They are applied in gardens, landscapes and agriculture to protect and store humidity in soils and release water slowly through soil (Orzeszyna *et al.*, 2006). Fallahi *et al.* (2015) by investigate the effect of water deficit, irrigation after 120 (control), 155 (moderate water stress) and 190 mm (sever water stress) pan evaporation and super absorbent polymer rates (SAP) (0, 30, 60 and 90 kg.ha^{-1}) on growth, yield and water use efficiency of cotton reported that moderate water stress (irrigation intervals of aprox. 15 days) along with 60 kg.ha^{-1} SAP application was the best treatment in terms of growth and yield indices of cotton, also water deficit and SAP application improved the water use efficiency (WUE) of cotton, the amount of WUE in moderate water stress treatment along with consumption of 60 or 90 kg.ha^{-1} SAP was 26% higher than for control treatment. These substances are odorless and colorless and do not contaminate soil, water and plant tissue (Roshan, 2002). Superabsorbent polymers increase soil water holding capacity and reduce irrigation times by 50% (Nazarli *et al.*, 2010). In another study, the relationship between superabsorbent polymers consumption and increase in plant available water was investigated. The results of the study showed using superabsorbent leads to a 10.68% increase in soil water holding capacity (Wu *et al.*, 2008). Impact of superabsorbent leads to a significance increase in grain yield per unit area. Non-enzymatic antioxidants include beta-carotene, ascorbic acid, alpha-tocopherol, glutathione and enzymatic antioxidants include superoxide dismutase, guaciole peroxidase, ascorbate peroxidase, catalase, polyphenol oxidase, and glutathione reductase (Xu *et al.,* 2006). Catalase, which is found mainly in proxies, is the major enzyme that directly converts H_2O_2 into water molecules and O_2 (Mittler, 2002). Plants are equipped with an antioxidant defense system like superoxide dismutase enzymes and catalase to protect themselves against reactive oxygen species (Chandlee and Scandalios, 1984). An increase in activity of glutathione peroxidase in cotton and wheat exposed to drought stress suggests that H_2O_2 production simultaneously can lead to absorption of frodoxin electrons by NADP, which results in a decrease in amount of superoxide production (Taarit *et al.*, 2009).

2. OBJECTIVES

Present study conducted to examine the impact of different levels of superabsorbent polymer on reducing the effects of drought stress on some physiological traits and activity of some antioxidant enzymes in wheat crop (*Triticum aestivum* cv. Mihan).

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

In order to investigate the effects of superabsorbent polymer application on reduction of drought stress impacts on some physiological traits and activity of some antioxidant enzymes in wheat (*cv.* Mihan), an experiment was conducted by using a split-plot arrangement based randomized complete block design with three replications in research field of Islamic Azad University of Kaleibar, Iran in crop year 2017. Main plots included different irrigation regime at three level (after 60mm: D_1, 90mm: D_2 and 120mm: D_3 evaporation from class A pan) and different amounts of superabsorbent polymer (Zero: S_1 as control, 75 kg.ha^{-1}: S_2 and 150 kg.ha^{-1}: S_3 superabsorbent polymer; SAP) belonged to sub plots.

3.2. Farm Management

The experimental field was prepared by a deep fall tillage followed by double passes of disc plow at right angles to each other. Required amounts of NPK fertilizers were calculated according to the results of the soil test. Nitrogen (urea), phosphorus (triple superphosphate) and potassium fertilizers were applied at 25, 60 and 60 Kg.ha^{-1} rate, respectively at the time of planting. An additional 25 kg.ha^{-1} N was added to the soil as top dressing in spring. According to the recommended conditions for wheat plantation in the climate of Kaleibar, seeds were planted on 12th October 2017. The field was irrigated evenly immediately afterwards and then with seven-day intervals prior to the drought stress. Physical and chemical characteristics of the soil are presented in table 1.

Table 1. Physical and chemical characteristics of the soil

Depth of sampling (cm)	**Soil class**	**pH**	**EC (ds.m^{-1})**	**P (mg.kg^{-1})**	**K (mg.kg^{-1})**	**N (%)**	**Clay (%)**	**Silt (%)**	**Sand (%)**
0-30	Sand-loam	1.7	1.8	12	185	0.06	32	27	41

Characteristics of the applied SAP are presented in table 2. Applied super absorbent polymer was SAP A200, produced by Rahab Resin Co. Ltd., under license of Iran Polymer and Petrochemical Institute (IPPI). At the time of planting, SAP was incorporated into the soil 15 cm under the seeds, which were then planted at the depth of 5 cm in order to access the SAP easily. As mentioned above, irrigation was done immediately afterwards.

Table 2. Characteristics of applied SAP

Components	Potassium poly acrylate poly acrylamide
Dry matter	85-90%
Specific weight	1.1 gr cm^{-3}
pH	8.1
Maximum absorbance	150-400 fold
CEC	4.6 mEq.gr^{-1}
Shelf lifetime	5 years

3.3. Measured Traits

3.3.1. Measuring photosynthetic pigments

To measure content of chlorophyll pigments, 200 mg of fresh leaves with 10 ml of 80% acetone were grinded in China mortar and the resulting solution was centrifuged for five minutes at 3000 rpm. Then, absorbance of the supernatant was read by spectrophotometer at 647, 664, and 470 nm wavelengths. The levels of chlorophyll a, b and carotenoids were calculated in µg.g^{-1} of fresh weight, using the following relationships (Dere *et al.*, 1998): **Equ. 1.** $\text{Chlorophyll}_a = 15.65A_{666} - 7.340A_{653}$

Equ. 2. $\text{Chlorophyll}_b = 27.05A_{653} - 11.21A_{666}$

Equ. 3. $\text{Carotenoid} = [(.785A470 + 3.657A_{663.2} - 12.76A_{646.8}) \times 8.1] / FW$

Equ. 4. $A_{663.2}$= absorbance level at wavelength of 632.2 nm
Equ. 5. $A_{646.8}$= absorbance level at wavelength of 646.8 nm
Equ. 6. A_{470}= absorbance level at wavelength of 470 nm
FW= fresh weight

3.3.2. Soluble carbohydrates

0.2 gr of leaf tissue along with 10 cc ethanol 95% (or 5 cc ethanol 96%) were heated in closed cap test tubes in bain-marie at 80 C^0 for one hour. Then, 1cc phenol 5% and 5cc sulfuric acid 98% were added to 1cc of the cooled sample. The absorbance at 483 nm was determined in a spectrophotometer, and the amount of soluble carbohydrate, expressed as µg glucose g^{-1} fresh weight, was elicited from the table of standard values (Irigoyen *et al*., 1992).

3.3.3. Leaf proline content

0.5 gr of fresh leaves was crushed using mortar and pestle, and homogenized with 10 ml sulfosalicylic acid 3%. The extract was filtered, and 2 ml acetic acid along with 2 ml ninhydrin was added to 2 ml of the filtered extract. Then, solution was heated in water bath at 100 C^0 for one hour. Concentration of proline in toluene was determined using a spectrophotometer at 520 nm, and calculated as $mg.g^{-1}$ FW based on the standard curve (Bates *et al.,* 1973).

3.3.4. Leaf relative water content

15 and 25 days after applying SAP, samples were taken from young fully expanded leaves to measure leaf RWC. First, fresh weights of the leaves were measured by a sensitive scale. After that, samples were soaked in DW at 25 C^0 for 24 hours. Turgid samples were weighed using the sensitive scale, and then dried in oven at 80 C^0 for 24 hours after which their dry weights were measured. Leaf WRC of each sample was calculated according to equation (Mortazavi *et al*., 2015): **Equ. 7.**
RWC= (Fw-Dw)/(Sw-Dw) ×100.
Where FW is leaf fresh weight measured immediately after harvest, DW is leaf dry weight after heating leaves in oven, and SW indicates leaf saturation weight after soaking the leaves in DW.

3.3.5. Enzyme activity measurement method

The activity of catalase enzyme was measured using Chandlee and Scandalios (1984) method. First, 100 µl of enzyme extract was poured into 2.8 ml of potassium phosphate buffer and then 30 µl of oxygenated water was added. In a wavelength of 240 nm, the potassium buffer become zero and 30 seconds after reading the first optical absorption, the second optical absorption was read. To measure the glutathione peroxidase enzyme, the leaves transferred to the laboratory were washed with distilled water and immediately inserted into tris buffer 0.16 M, pH= 5.7 and then were chopped and distributed evenly. Finally, 0.5 ml of homogenous solution was taken to measure the protein and the protein content was determined in milligrams per milliliter. Then, the amount of glutathione enzyme was measured in the residual extraction solution by Holy method (1972).

3.4. Statistical Analysis

Analysis of variance and mean comparisons were done via SAS (Ver.9.3) software and Duncan multiple range test at 5% probability level.

4. RESULTS AND DISCUSSION

4. 1. Chlorophyll a and b, Cartenoid

According result of analysis of variance effect of drought stress, super absorbent and interaction effect of treatments on chlorophyll a was significant at 1% probability level (Table 3).

Result of analysis of variance revealed effect of drought stress and super absorbent on chlorophyll b was significant at 1% probability level, but interaction effect of treatments was not significant (Table 3). According result of analysis of variance effect of drought stress, super absorbent and interaction effect of treatments on Cartenoid was significant at 1% probability level (Table 3). The mean comparison results of different irrigation regime showed that the highest amount of chlorophyll b (2.62 mg.g^{-1} FW) obtained from D_1 treatment (60 mm evaporation) and the lowest one (1.8 mg.g^{-1} FW) belonged to D_3 treatment (120 mm evaporation) (Table 4). On the other hand, the highest amount of chlorophyll b (2.46 mg.g^{-1} FW) obtained from S_3 treatment (150 kg.ha^{-1} superabsorbent) and the lowest one (1.72 mg.g^{-1} FW) belonged to S_1 treatment (nonuse superabsorbent) (Table 4). The results of the study also showed using superabsorbent polymer significantly increases chlorophyll a and carotenoids compared to the control group. Mean Comparison interactions effect of treatments showed that the highest amounts of chlorophyll a and carotenoids, with the means of 6.57 and 2.05 mg.g^{-1} FW, respectively, belonged to D_1S_3 treatment (60 mm evaporation and 150 kg.ha^{-1} superabsorbent) and the lowest amounts of chlorophyll a and carotenoids with the means of 4.43 and 0.85 mg.g^{-1} FW, respectively, was for D_3S_1 treatment (120 mm evaporation and non-use superabsorbent), (Table 5). Various ROSs are produced in response to stress, and chlorophyll loss is an indicator of the intensity of oxidative damage. Reduction of important photosynthetic pigments can be due to disorders in absorption of nutrients which are essential for photosynthetic pigment synthesis.

Table 3. ANOVA result of measured traits

S.O.V	df	Chl.a	Chl.b	Carotenoid	RWC
Replication	2	0.009^{ns}	0.078^{ns}	0.00061^{ns}	231.03^{ns}
Drought stress (D)	2	3.07**	0.67**	0.38**	982.27**
Error I	6	0.412	0.107	0.0128	7.835
Super absorbent (S)	3	2.89**	1.19**	0.054**	68.582^{ns}
D×S	4	0.47**	0.38^{ns}	0.0076**	7.08^{ns}
Error II	12	0.208	0.029	0.0065	6.921
CV (%)		7.83	11.92	13.01	8.92

^{ns}, *and ** are non-significant and significant at 5% and 1% probability levels, respectively.

Continue Table 3.

S.O.V	df	Proline	Carbohydrate	Catalase	Glutathione peroxidase
Replication	2	0.171^{ns}	4529.04^{ns}	4.84^{ns}	8.09^{ns}
Drought stress (D)	2	4.09**	15127.01*	39.68*	59.23**
Error I	6	0.39	2692.274	1.93	3.04
Super absorbent (S)	3	4.11**	1139.41**	4.105**	6.08**
D×S	4	0.83**	947.9^{ns}	6.21^{ns}	3.79*
Error II	12	0.18	698.16	1.57	2.85
CV (%)		12.07	9.27	12.89	8.13

^{ns}, *and ** are non-significant and significant at 5% and 1% probability levels, respectively.

Furthermore, this reduction can be attributed to the inhibition of different stages of chlorophyll biosynthesis, or increase of chlorophyllase activity and consequently the chlorophyll degradation (Goldani, 2012). It was reported that in corn plants the highest amount of chlorophyll a was obtained from full irrigation treatment, whereas the lowest amount of chlorophylls a and b belonged to the treatment of fixed furrow irrigation (also called every-other fixed furrow irrigation) (Lotfi Agha *et al.*, 2017). Ratio of chlorophyll a to chlorophyll b changes under drought stress as the new plastids of both types of chlorophylls are synthesized (Vaziri and Nader, 2014). A study on German chamomile (*Matricaria chamomilla*) revealed that chlorophyll content declined considerably under severe drought stress, compared with a suitable irrigation condition (Razban and Pirzad, 2011). Mild drought stress raised the amount of chlorophylls a and b as well as total chlorophyll content, which reached their minimum levels as the severe drought stress continued. The most chlorophyll content was obtained by applying the highest amount of SAP. This is in agreement with results of the present study in which SAP application led to increase Chl. leaf. The results of the study also showed that an increase in drought stress leads to a decrease in chlorophyll a, chlorophyll b and carotenoids. Carotenoids, as biological antioxidants, play a key role in protecting plant tissue. Lack of carotenoids can cause severe phytoxidation in plant tissue (Kaboosi and Novdehi, 2016).

Table 4. Mean comparison relative water content (RWC), chlorophyll b, carbohydrate and catalase affected drought stress and SAP treatments

Treatments	Chlorophyll b (mg.g^{-1} FW)	RWC (%)	Carbohydrate (µg glucose g^{-1} FW)	Catalase (u.mg^{-1}.Pr)
Drought stress (Mm evaporation)				
60	2.62^{a}	81.38^{a}	127.31^{a}	37.43^{c}
90	2.12^{b}	75.73^{b}	119.01^{b}	64.12^{b}
120	1.81c	63.29^{c}	89.11^{c}	129.08^{a}
Super absorbent polymer (Kg.ha^{-1})				
0	1.72^{b}	76.08^{a}	120.33^{c}	144.31^{a}
75	2.28^{ab}	75.82^{a}	124.56^{b}	141.27^{a}
150	2.46^{a}	76.46^{a}	131.70^{a}	96.36^{ab}

4.2. Leaf relative water content (RWC)

Irrigation and superabsorbent treatments had a significant impact on leaf relative water content at 1% probability level, but interaction effect of treatments was not significant (Table 3). The mean comparison results of different irrigation regime showed that the highest leaf relative water content (81.38%) is related to D_1 treatment (60 mm evaporation) and the lowest one (63.29%) is related to D_3 treatment (120 mm evaporation) (Table 4). The results of the study also showed that to use superabsorbent polymer significantly increases leaf relative water content. The highest leaf relative water content (76.46%) obtained from S_3 treatment (150 kg.ha^{-1} superabsorbent) and the lowest one (75.82%) was for S_1 treatment (no superabsorbent use) (Table 4). Differences among RWC values may be due to the differences among the effects of treatments on the ability of plants in absorbing water from soil, controlling water loss through stomata or regulating osmotic pressure in order to maintain turgor pressure of tissues and increase physiological activities. In addition, more leaf RWC probably results from less osmo regulatory ability or ability of roots to absorb water resulting in more leaf water retention capacity under drought stress. Leaf RWC at the time of severe stress has a close relationship with plant water potential. Water deficit stress leads to stomatal closure and reduction of leaf expanding both of which reduce Co_2 availability to the plant and eventually reduce photosynthesis rate (Mojadam *et al*., 2016). Significant reduction of leaf RWC following increase of drought stress was reported in other studies, too (Vaziri and Naderi, 2014). Decreasing leaf RWC which causes to stomatal closure is on the one hand because of less water absorption through roots and on the other hand due to more transpiration through leaves (Valizadeh Ghalebeig *et al*., 2015). During drought stress, SAP provides tissues, especially the leaf tissue, with water and consequently causes them to maintain more water. This inhibits the reduction of leaf water potential and turgor pressure, and increases leaf area (Harvy, 2000). Results of a study on corn plants revealed that SAP application, with a positive impact on leaf RWC, led to more accumulation of photosynthetic assimilates in vegetative parts resulting in less amount and proportion of photosynthetic assimilate reallocation in grain yield (Fazeli Rostampour and Mohebian, 2011).

Table 5. Interaction effect of drought stress and SAP on Cartenoid, Proline, Chlorophyll. a and Glutathione peroxidase

Treatments		**Chlorophyll a ($mg.g^{-1}$ FW)**	**Cartenoid ($mg.g^{-1}$ FW)**	**Proline ($MolCO_2.m^{-2}.s^{-1}$)**	**Glutathione peroxidase ($u.mg^{-1}Pr$)**
Drought stress (Mm evaporation)	**SAP ($kg.ha^{-1}$)**				
60	0	5.77^{*b}	1.72^{a}	3.5^{c}	98.34^{e}
	75	6.57^{a}	2.55^{a}	3.62^{bc}	74.01^{ef}
	150	6.30^{a}	1.87^{a}	4.00^{b}	46.19^{f}
90	0	5.13^{bc}	1.04^{c}	3.91^{bc}	163.82^{c}
	75	5.66^{b}	1.48^{ab}	4.02^{b}	153.28^{c}
	150	5.86^{b}	1.52^{ab}	4.41^{b}	91.08^{e}
120	0	4.43^{cd}	0.983^{cd}	4.43^{b}	249.973^{a}
	75	4.95^{c}	1.09^{c}	4.54^{ab}	201.27^{b}
	150	5.33^{bc}	1.31^{b}	4.93^{a}	147.82^{d}

*Similar letters in each column show non-significant difference at 5% probability level, via Duncan test.

More leaf RWC is probably obtained via the ability of osmoregulation or water absorption through roots which leads to the ability of leaves to maintain more water under drought stress. Super absorbent polymers provide plant roots with sufficient amount of water leading to more water absorption rate and water use efficiency by plants and consequently more leaf RWC. Increasing leaf RWC in response to more SAP application is in accordance with findings of other studies (Janson and Leah, 1990). It can be suggested that rising leaf RWC probably increases stomatal opening and consequently photosynthetic rate and production of dry matter. More leaf RWC following SAP application can be attributed to the positive effects of SAPs on water absorption (Yang *et al*., 2011).

4.3. Proline content

According result of analysis of variance effect of drought stress, super absorbent and interaction effect of treatments on Proline content was significant at 1%

probability level (Table 3). It was witnessed that proline content was affected by DS, and had a direct relationship with drought stress intensity. According to results of interaction effect of treatments, the highest amount of leaf proline content was produced in the treatment of 120 mm evaporation with no SAP, while the lowest one was observed was for the treatment of 60 mm evaporation with 150 $kg.ha^{-1}$ SAP (Table 5). It was suggested that proline accumulation in coconut plants (*Cocos nuciera*) under drought stress just indicates the stress in these plants (Gomes *et al.*, 2010). Proline accumulation in response to drought stress can be a result of stimulating its synthesis or inhibiting the degradation of proline or proteins (Amini *et al.*, 2014). Under water deficit conditions, accumulation of proline and soluble carbohydrate content in tolerant varieties increased. By increasing stress duration from three to nine days, a significant improvement was recorded for the amount of proline, glucose, fructose and total soluble carbohydrate content. In another study, drought stress in various stages of rice growth enhanced amount of proline (Pirdashti *et al.*, 2009). A rise in proline content has also reported in pea (Sanchez *et al.*, 1998).

4.4. Soluble carbohydrate content

According result of analysis of variance effect of drought stress, super absorbent on Soluble carbohydrate content was significant at 5% and 1% probability level, respectively, but interaction effect of treatments was not significant (Table 3). Under drought stress, raising the amount of sugars is important, because they are osmotic compounds and protect proteins against oxidative damage induced by free radicals under water deficit conditions (Chehelgerdi *et al.*, 2014). Decreasing the amount of carbohydrates under DS in some reports has called into doubt the ability of soluble carbohydrate content to participate in osmo regulation (Thakur and Rai, 1980). According to mean comparison results increasing the level of drought stress from 60 to 120 mm evaporation raised the carbohydrate accumulation in leaf tissue. So, the most amount of soluble carbohydrate content (121.31 μg $glucose.g^{-1}$ FW) was obtained from the treatment of 120 mm evaporation (severe stress). In contrast, the least amount of soluble carbohydrate content (89.11 μg $glucose.g^{-1}$ FW) was related to the treatment of normal irrigation without DS (Table 4). Findings of the present study revealed that applying SAP raised the amount of SCs significantly, compared with the control treatment. The most and the least amounts of SCs were observed in the treatments of 75 $kg.ha^{-1}$ SAPA and control (no SAPA), respectively (Table 4). Intracellular accumulation of SCs plays an important role in osmoregulation. It reduces cell water potential and preserves more water in cell to maintain suitable turgor pressure under drought stress (Arduini *et al.*, 2006). In another study on wheat plants, it was observed that in control and drought stress treatments, the amount of SCs in two upper internodes of *cv.* Zagros (a tolerant variety) was significantly more than that of *cv.* Marvdasht (Sheikhmoradi *et al.*, 2011).

4.5. Examining antioxidant enzymes activity

According result of analysis of variance effect of drought stress, super absorbent on catalase (CAT) content was significant at 5% and 1% probability level, respectively, but interaction effect of treatments was not significant (Table 3). Result of analysis of variance revealed effect of drought stress and super absorbent on glutathione peroxidase (GPX) was significant at 1% probability level, also interaction effect of treatments was significant at 5% probability level (Table 3). An increase in drought stress increased the activity of antioxidant enzymes. The reason of increased activity of antioxidant enzymes in wheat was reduction in severity of damage to biomolecules and lack of oxidative stress. The mean comparison results of different irrigation regime showed that the highest activity of catalase enzyme (12.089 $u.mg^{-1}.Pr$) was related to 120 mm evaporation (Table 4). The results of the study showed that the use of superabsorbent polymer significantly reduces the activity of antioxidant enzymes. So the highest activity of catalase enzyme (144.31 $u.mg^{-1}.Pr$) was related to S_1 treatment (nonuse superabsorbent) and the lowest one (96.36 unit per milligram protein) was related to S_3 treatment (150 $kg.ha^{-1}$ superabsorbent) (Table 4). Drought stress in many plants has increased the activity of catalase enzymes. Same results was observed in present study regarding catalase activity. The catalase enzyme is also categorized as iron-containing proteins and operates in plant and animal cells when the amount of hydrogen peroxide is high in the environment. Accordingly, an increase in drought stress leads to an increase in activity of antioxidant enzymes. The catalase enzyme helps plants to survive by eliminating active oxygen species and preventing cell wall destruction (El-harris *et al*., 2007; Jiang and Zhang, 2001). The results of the research show that an increase in drought stress leads to an increase in the activity of chlorophylls enzyme in wheat leaves and a decrease in chlorophyll levels (Naeimi *et al*., 2018) which is consistent with the results of current study. The mean comparing results of interactions effect of treatments showed that the highest activity of glutathione peroxidase enzyme (249.76 $u.mg^{-1}.Pr$) was related to D_3S_1 treatment (120 mm evaporation and nonuse superabsorbent) and the lowest one (46.19 $u.mg^{-1}.Pr$) was related to D_1S_3 treatment (60 mm evaporation and 150 $kg.ha^{-1}$ superabsorbent) (Table 5). The glutathione enzyme, using phenolic materials as an electron donor, decomposes hydrogen peroxide (Appel and Hirt, 2004; Baily, 2004). Given the increased activity of the enzyme under drought and salinity stress and its role in glutathione reduction, it is likely to be one of the important enzymes of the plant that an increase in its activity will lead to an increase in the plant tolerance against oxidative stress. It is reported that increased activity of glutathione reductase is associated with salinity tolerance (Bor *et al*., 2003). The results of the study are consistent with the results of previous studies (Moharramnezhad and Valizadeh, 2015).

5. CONCLUSION

According to the results, it can be concluded that drought stress has different negative effects on physiological and biochemical processes of the plant. Therefore, an increase in drought stress leads to an increase in activity of glutathione peroxidase and catalase enzymes and a decrease in activity of photosynthetic pigments. In general, it can be concluded that by increasing the use of superabsorbent polymer, the activity of antioxidant enzymes decreases, but the amounts of chlorophyll a, chlorophyll b, carotenoids and leaf relative water content increases. The results of comparing the mean interactions between drought stress and superabsorbent showed that the maximum values of chlorophyll a and carotenoid obtained from “60 mm evaporation” and “75 $kg.ha^{-1}$ superabsorbent” treatments. Superabsorbent can cause normal growth of the plant in stress conditions by reducing negative effects of drought stress and increasing the features like soil and root water holding capacity, and also retaining the required elements of the plant, which in turn leads to retaining plant chlorophyll and continuity of photosynthesis. Therefore, a treatment of 75 kg.ha^{-1} superabsorbent can be used in areas where there is a shortage of water or unequal distribution of rainfall but the cultivation yet should be done under stress conditions. Economically, given the price of consumed superabsorbent and the resulting increase in production, the surplus costs paid to purchase superabsorbent will be compensated by the increase in production.

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FOOTNOTES

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