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Evaluation of Relative Membrane Permeability of Sorghum (*Sorghum bicolor*) Affected Super Absorbent Polymer and Water Deficit Conditions

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ABSTRACT

Sorghum is among the most important forages used in arid and semi-arid regions of south-eastern Iran. Application of some materials such as super absorbent polymer (SAP) in soil can increase soil water storage capacity and increase water use efficiency. The aim of the study was to estimate the relative membrane permeability of sorghum under irrigation regimes and super absorbent polymer application to find the relationship between antioxidant enzymes, leaf rolling index, compatible solutes with relative membrane permeability. This experiment was conducted in Dashtak region of Zahedan during 2016 and 2017 growing seasons. The research was arranged as split plot experiment based on randomized complete blocks design with three replications. The main factor included four levels of irrigation regime (40, 60, 80, and 100% of ET_c or crop evapotranspiration) and four level of SAP application (0, 75, 150 and 225 $kg.ha^{-1}$) belonged to subplots. Analysis of regression indicates ascorbate peroxidase, catalase, superoxide dismutase, glutathione peroxidase, proline, glycine betaine, soluble sugars, leaf rolling index and relative membrane permeability showed that by increasing amount of irrigation and super absorbent polymer, decreased. The results showed that application of SAP reduced RMP by 4.98% under 100% ET_c, but it increased the RMP (Relative Membrane Permeability) significantly by 14.14%, 12.18% and 11.48% under 80, 60 and 100% ET_c.

Keywords*:* *Antioxidant enzymes, Forage, Leaf relative water content.*

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INTRODUCTION

Drought is one of the most important abiotic stresses that adversely affect agricultural productivity and causes significant crop loss. Sorghum, which is grown in Sistan and Baluchistan, are exposed with drought stress often occurring during the growth season (Fazeli Rostampour *et al*., 2013). Sorghum is the fifth most important cereal crop grown for human consumption in the world being surpassed only by rice, wheat, barley and corn. Most of sorghum grown in Asia and the African tropics is used for human food and also fed to livestock or poultry (Gul *et al*., 2005). Sorghum is a drought resistant summer annual crop (Aishah *et al*., 2011). Sorghum speedfeed is a crop of world-wide importance and is unique in its ability to produce under a wide array of harsh environmental condition (Sadeghzade *et al*., 2012). Sorghum is grown as fodder crop due to the poor pollination and seed set during the extremely hot dry season (April-August) in the southeastern provinces of Iran-Sistan and Baluchistan. Applying superabsorbent polymers can increase the water-holding capacity of soils and reduce the harmful effects of short-term drought in drought-prone arable areas (Karimi and Naderi, 2007). 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*., 2011a). 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). Similar results have also been reported for improving soil physical properties and reducing soil erosion, runoff, and nutrient loss (Ekebafe *et al*., 2011). The problem of inefficient use of rain and irrigation water by crops is the most important in semiarid and arid regions, where shortage of water is frequently experienced and water is the main limiting factor for growth and yield of crops. In arid and semiarid regions of the world, intensive research on water management is being carried out and use of superabsorbent polymers (SAP) may effectively increase water and fertilizer use efficiency in crops. The application of SAP for stabilizing soil structure resulted in increased infiltration and reduced water use and soil erosion in a furrow irrigated field (Lentz and Sojka, 1994; Lentz *et al*., 1998). When polymers are incorporated in soil, it is presumed that they retain large quantities of water and nutrients, which are released as required by the plant. Thus, plant growth could be improved with limited water supply (Gehring and Lewis, 1980). Johnson (1984) reported an increase of 171 to 402% in water retention capacity when polymers were incorporated in coarse sand. Addition of a polymer to peat decreased water stress and increased the time to wilt (Karimi *et al*., 2009). The incorporation of superabsorbent polymer with soil improved soil physical properties (El-Amir *et al*., 1993), enhanced seed germination and emergence (Azzam, 1983), crop growth and yield (Yazdani *et al*., 2007) and reduced the irrigation requirement of the plants (Blodgett *et al*., 1993). The use of hydrophilic polymer materials as carrier and regulator of nutrient release was helpful in reducing undesired fertilizer losses, while sustaining vigorous plant growth (Mikkelsen, 1994). Drought stress, like other abiotic stresses, could also lead to oxidative stress through increasing reactive oxygen species (ROS), such as O_2^- (superoxide radical), H_2O_2 (Hydrogen peroxide) and OH^- (Hydroxyl radical) are formed by a successive reduction of molecular O_2 during the aerobic metabolism in mitochondria (Mittler *et al*., 2004). Since ROS are highly reactive, their excess accumulation would lead to the oxidative damage of various cellular components such as lipids, proteins, nucleic acids, chlorophyll and cell membrane properties. Numerous studies have demonstrated that drought conditions could induce membrane damage, increase membrane permeability and the accumulation of free radicals in plants (Blokhina *et al*., 2003). Peroxidation of lipids, commonly taken as indicators of oxidative stress, disrupt the membrane integrity of a plant cell (Valentovic *et al*., 2006). This means that essential solutes leak out from organelles and cells and could cause damage to membrane function and metabolic imbalances (Blokhina *et al*., 2003). In plants, there are many potential places for the generation of ROS, such as in chloroplasts (Foyer and Noctor, 2003) as well as in mitochondria and peroxisomes (Del Rio *et al*., 2002). It is well documented that a critical component of the dehydration tolerance for grasses is cell membrane stability (Volaire and Lelievre, 2001). One of the most common stress responses in plants is the production of different types of antioxidant enzymes (Doulati Baneh *et al*., 2013). The enzymes activity could cause the detoxification of reactive oxygen species and the protection of the membrane integrity (Kiffin *et al*., 2006). These mechanisms employed ROS-scavenging enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (ASP), glutathione peroxidase (GPX), and catalase (CAT) (Morsya *et al*., 2003). SOD belongs to a class of metallo proteins and SOD catalyzes the dismutation of superoxide (O_2^-) into molecular oxygen (O_2) and H_2O_2. CAT enzyme is ubiquitous in the peroxisome, where it dismutates H_2O_2 into water and oxygen. Together with SOD and hydroxyl peroxidases, CAT is part of a defense system for scavenging superoxide radicals (Mittler, 2002). ASP catalyzes the reduction of hydrogen peroxide to water by using ascorbate as the electron donor. APX plays a similar role to that of CAT. While APX and CAT are predominant in H_2O_2 detoxification, GPX could play a role in other parts of antioxidant metabolism, including the removal of lipid peroxides. GPX generally increases in plants subject to environmental stresses (Navrot *et al*., 2006). Cereals leaves roll as a defense mechanism to reduce net radiation load on the leaf (Samarah, 2005). Rolling reduces transpiration and leaf water use and it is found to protect PSII functionality against damage (Nar *et al*., 2009).

Osmotic adjustment is also mechanism to avoid drought. Proline (PRL), glycinebetaine (GLB) and the soluble sugars (SLS) are the key osmolytes which help plants to maintain cell turgor (Hessini *et al*., 2009). A large number of plant species accumulate PRL, GLB and SLS in response to drought stress and the accumulation could play a role in defense against drought stress (Iqbal *et al*., 2008). Leaf relative water content (LRWC) is an appropriate measure of plant water status in terms of the physiological consequences of cellular water deficit (Keyvan, 2010). Vyn and Hooker (2002) proposed that LRWC was a better indicator of water status than

than water potential. Barros *et al*. (2017) reported the use of SAP application in sorghum seeds, and likely in other annual crops, improves seedling vegetative development and survival rate under water deficit. Zohuriaan-Mehr and Kabiri (2008) reported superabsorbent polymer materials are hydrophilic networks that can absorb and retain huge amounts of water or aqueous solutions. They can uptake water as high as 100%. Islam *et al*. (2011b) 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 moderate water stress (irrigation intervals of aprox. 15 days) along 60 $kg.ha^{-1}$ SAP application was best treatment in terms of growth, yield indices of cotton, also water deficit and SAP application improved water use efficiency (WUE) of cotton, amount of WUE in moderate water stress treatment along consumption of 60 or 90 $kg.ha^{-1}$ SAP was 26% higher than for control treatment. Main aim of this research is to improve knowledge about relative permeability of cell membranes, compatible solutes, antioxidant enzymes responses in sorghum to irrigation regimes and super absorbent A200 polymer.

MATERIALS AND METHODS

Field and Treatment Information

This research was conducted in Dashtak region of Zahedan at south-eastern of Iran (30°18' N and 60°86' E), with annual rainfall (mean of 68 mm), 584 m above sea level with arid and tropical climate, sandy loam soil (a sandy skeletal, mixed, hyper thermic Typic Torriorthent) during 2016 and 2017. Some physical and chemical properties of soil were inserted in Table 1. The research was arranged via split plot experiment based on randomized complete blocks design with three replications. Main factor included four levels of irrigation regime (40, 60, 80, and 100% of ET_c or crop evapotranspiration) and four level of SAP (super absorbent polymer) application (0, 75, 150 and 225 $kg.ha^{-1}$) belonged to subplots.

Field Management

Seeds of Speedfeed cultivar were planted on 4th April. Before sowing seeds were treated with an insecticide (Thiodicarb, dimethyl N, N'-[thiobis [(methylimino) carbonyloxy]] bis [ethanimidothioate]).

Table 1. Some physical and chemical properties of a representative soil samples from the experimental site before sowing (0-30 cm depth) in 2016 and 2017 (n = 3).

Soil properties	2014	2015
Silt, %	24.9	24.8
Sand, %	65.5	65.8
Clay, %	9.70	9.40
Texture	sandy loam	sandy loam
Organic matter, %	0.08	0.09
Electrical conductivity (1:1 extract), $ds.m^{-1}$	6.90	6.80
pH (1:1 suspension)	7.80	7.70
Total N, % (w/w)	0.17	0.18
Total $CaCo_3$, % (w/w)	0.90	1.10
$NaHCO_3$-extractable P, $mg.L^{-1}$	3.70	3.90
NaOAC-extractable K, $mg.L^{-1}$	91.0	93.3

The seeds were planted by hand. Two weeks after planting, the seedlings were thinned to a population of 300,000 $plants.ha^{-1}$. The operations for seedbed preparation consisted of plowing, disking, and leveling, which were performed early in March. A fertilizer with 10–15–20; N–P_2O_5–K_2O formulation was used at 200 $kg.ha^{-1}$, which was followed by seeding at an approximate depth of 2 for sorghum. Pre plant weed control was achieved by incorporation into the soil of trifluralin [2, 6-dinitro-N, N-dipropyl-4-(trifluoromethyl) benzenamine] at 0.5 kg a.i. ha^{-1}, followed by hand weeding as needed. The soil amendment material (SAP) used was a hydrophilic polymer, produced by Rahab Resin Co. Ltd., under license to Iran Polymer and Petrochemical Institute. Its chemical properties are shown in Table 2 (Abedi-Koupai and Asadkazemi, 2006). Before planting the seeds, SAP was placed into the middle of rows by hand, where roots were expected to have the greatest density (45–50-cm depth) for sorghum (Singh and Singh, 1995).

Table 2. The properties of super absorbent A200 polymer.

Property	Value
Appearance	White granule
Grain size, mm	0.5-1.5
Water content, %	3-5
Density, $g.cm^{-3}$	1.4-1.5
pH	6-7
Actual capacity for absorbing a 0.9 % NaCl solution, $g.g^{-1}$	45
Actual capacity for absorbing tap water, $g.g^{-1}$	190
Actual capacity for absorbing distilled water, $g.g^{-1}$	220
Maximum life span, yr	7

Measured Traits

Water requirements were determined according to the FAO method (Howell *et al*., 2008). The crop water requirement was calculated using the method described by Allen *et al.* (1998):

Equ. 1. $ET_c = K_c \times ET_0$

Where ET_c is the crop water requirement ($mm.d^{-1}$), ET_0 is the evapotranspiration of a reference plant under specified conditions and calculated by the pan evaporation method, and K_c is the crop water requirement coefficient for sorghum, which varies with genotype and growth stage (Giovanni *et al*., 2009). The K_c values were extracted from Allen *et al*. (1998) and ET_0 was calculated as:

Equ. 2. $ET_0 = K_{pan} \times E_p$

Where K_{pan} and E_p are the pan coefficient (the K_{pan} was 0.66 according to Alizadeh (2002) and pan evaporation, respectively. The LRWC (leaf relative water content) was determined in the fully expanded topmost leaf 1 day before irrigation. This was accomplished by excising three 1 cm disks of each leaf sample. The results were then averaged, and the resulting single value was used to represent that plot.

The fresh weight of the samples was recorded and the samples were immersed in distilled water in a Petridis. After 24 h, the leaves were removed, surface water was blotted off, and their turgid weights were recorded. The samples were then dried in an oven at 70°C to constant weight (Munne-Bosch *et al*., 2007), and LRWC was calculated as: **Equ. 3.**

$$\text{LRWC}_{(\%)} = \frac{\text{Fresh Weight - Dry Weight}}{\text{Turgid Weight - Dry Weight}} \times 100$$

The relative membrane permeability of the leaf cells was determined as the extent of ion leakage following Bajji *et al.* (2001). The third fully expanded youngest leaf from each plant was cut into three discs with 1.0 cm diameter. These freshly prepared discs were placed into the test tubes containing 10.0 ml deionized distilled water. After vortex the samples for 3 s, initial electrical conductivity of each sample was measured (EC_0). The samples were then incubated at 4°C for 24 h and electrical conductivity measured again (EC_1). The samples were then autoclaved at 120°C for 15 min and then cooled to room temperature and electrical conductivity measured for the third time (EC_2). RMP (Relative Membrane Permeability) was calculated by following formula:

Equ. 4. $$\text{RMP}_{(\%)} = \frac{(Ec_1 - Ec_0)}{(Ec_2 - Ec_0)} \times 100$$

In three fully expanded topmost leaves, maximum width of leaf blade in rolling position and the maximum width of leaf blade in rolling position at the midpoint of each leaf were measured to determine leaf rolling index (LRI). The expression used for the calculation of LRI was (Pande and Singh, 1981):

Equ. 5. LRI (%): Maximum width of leaf blade in rolling position (mm)/Maximum width of same leaf blade in non rolling position (mm)

Extraction and assay of enzyme activities

Fully expanded upper most leaves were taken from three plant samples were taken from each plot prior to forage harvest. The leave samples were ground with mortar and pestle in liquid nitrogen and homogenized in 100 mM potassium phosphate buffer (pH 7.0), containing 1 mM EDTA and 0.5 % insoluble Polyvynil pyrrilidone (the tissue/buffer ratio was 1:5, w/v). The homogenate was centrifuged at 12000 g for 20 min and the supernatant was used for analyzing the activities of enzymes. Superoxide dismutase was measured according to Tatari (2012) method. Catalase (CAT, EC 1.11.1.6) activity was determined spectro photo metrically following H_2O_2 treatment at 240 nm (Bailly *et al*., 1996). Glutathione Peroxidase and ascorbate Peroxidase activity were determined according to the procedures of Nakano and Asada (1981) methods, respectively. Extraction and determination of the free proline, glycinebetaine and soluble sugars of fresh leaves were performed using the method of Bates *et al*. (1973); Grieve and Grattan (1983) and Irigoyen *et al*. (1992), respectively.

Statistical Analysis

Statistical analysis was done via SAS software (Ver.8). Year was considered as a random effect in the statistical analysis. Irrigation regime and SAP were considered as the fixed effects. The analysis of variance for each physiological variable was performed with the PROC MIXED procedure in SAS (Littell *et al*., 2006). The Linear, quadratic, and cubic models were tested for irrigation regime and SAP level in sorghum. The regression equations were fitted by PROC REG. All the estimated parameters in the regression models were significant at $P \leq 0.01$.

The multiple linear regression analysis was based on the following statistical model (Hoshmand, 2006):

$$y = b_0 + b_1 x_1 + b_2 x_2 + k + b_k x_k + e$$

Where y is the dependent variable, b is the independent variables 1 through k, and e is the error or residual.

RESULTS

Leaf Relative Water Content (LRWC)

Irrigation regime, SAP level, and their interactions were significant at $P < 0.01$ (data not shown). Means of LRWC for 40, 60, 80, and 100% ET_c were 58.73, 67.61, 77.8, and 83.9%, respectively. Responses of LRWC to SAP levels for 60, and 80% ET_c were linear (Table 3). The highest and the lowest LRWC value for sorghum were obtained from 40 and 100% ET_c treatments. The LRWC was significantly lower in water-stressed plants than in plants grown under normal conditions (Farouk, 2011). The LRWC increased with increasing volume of irrigation water. Application of SAP improved LRWC under all irrigation regimes except 100% ET_c. Means of LRWC for 0, 75, 150, and 225 kg $SAP.ha^{-1}$ were 69.29, 71.84, 72.93, and 73.38%, respectively. The results were in agreement with those of Fazeli Rostampour *et al*. (2013).

Antioxidant Enzymes

The antioxidant enzymes were also significantly affected by the irrigation regime, SAP level, and their interactions at $P < 0.01$. The effects of water deficit on ASP, CAT, GPX, and SOD were determined for sorghum. The values of antioxidant enzymes increased with decreasing amount of irrigation water. The highest and the lowest antioxidant enzymes values for sorghum were obtained from 40 and 100% ET_c treatments.

Responses of ASP and CAT to SAP levels for 40, 60, and 80% ET_c were linear. In addition, responses of GPX to SAP levels for 40, 60, and 80% ET_c were linear (Table 3). Responses of SOD to SAP levels for 40, 60, and 80% ET_c were linear (Table 3). In all of crops studied, antioxidant enzymes were significantly higher in water stressed plants than in plants grown under normal conditions (Smirnoff, 1998).

Compatible Solutes

The relationship between ETc and PRL, SLS and GLB were evaluated. Interaction effect of irrigation regime and SAP levels on PRL, SLS, and GLB was significant. Responses of PRL to SAP levels for 60 and 80% ETc were linear and quadratic. In addition, responses of PRL to SAP levels for 40, 60, and 80% ET_c was quadratic, linear and linear (Table 3). Responses of SLS to SAP levels for 40, 60, and 80% ET_c were linear (Table 3). Responses of GLB to SAP levels for 40, 60, and 80% ETc were quadratic, linear, and linear, respectively. PRL, SLS, and GLB values increased with decreasing amount of irrigation water. The highest and lowest PRL, SLS, and GLB value was obtained from 40 and 100 % ET_c treatments. Increasing number of reports provide evidence that there is negative relationship between water use and compatible solutes (Caballero *et al*., 2005). The PRL, SLS, and GLB values significantly decreased via application of SAP. Responses of PRL and GLB to SAP levels were quadratic except for SLS was linear. Highest and lowest values of compatible solutes were obtained from 0 and 225 $kg.ha^{-1}$ treatments.

Leaf Rolling Index (LRI)

The LRI was also significantly affected by the irrigation regime, SAP level, and their interactions at $P< 0.01$.

Responses of LRI to SAP levels for 40, 60, and 80% ET_c were linear (Table 3). Means of LRI for the 40, 60, 80, and 100% ET_c treatments were 33.99, 23.11, 13.84, and 7.13%. The lowest LRI values were observed from 100% ET_c treatment. Most research have shown that an increase of LRI in response to drought stress condition (Kadioglua *et al*., 2012). Regression analysis showed that there was a negative linear relationship between the use of SAP and the LRI. Responses of LRI for sorghum to SAP levels were quadratic. The highest and lowest LRI values were obtained from 0 and 225 $kg.ha^{-1}$ treatments.

Relative Membrane Permeability (RMP)

The relationship between the seasonal water use and RMP of sorghum was evaluated, there was a significant interaction between irrigation regime and SAP levels on RMP (data not shown). Means of RMP for the 40, 60, 80, and 100% ET_c treatments were 60.83, 56, 45.04, and 38.01%, respectively. Ahmadizadeh *et al*. (2011) reported that RMP increased with water stress. According to the regression equations, the response of RMP to SAP levels in the 40, 60, and 80% ET_c treatments were Linear (Table 3). Response of RMP to SAP levels for the 100% ET_c was not significant. Means of RMP for the 0, 75, 150, and 225 kg $SAP.ha^{-1}$ treatments were 53.63, 49.79, 48.78, and 47.68%, respectively. There was a negative linear relationship between the use of SAP and the RMP and RMP decreased with increasing SAP. The multiple linear regression analysis indicated that ASP, LRI, and GLB were the most important traits affecting RMP in sorghum. The model used to estimate RMP is: RMP = 47.88 + 0.54 ASP + 0.6 LRI - 0.2 GLB.

DISCUSSION

This experiment was done to compare relative water content, antioxidant enzymes, leaf rolling index, compatible solutes, and the relative membrane permeability under polymer and water deficit conditions. The results showed that LRWC, ASP, CAT, GPX, ROS, PRL, GLB, SLS, LRI, and RMP were all significantly affected by the interaction of irrigation regime and SAP levels during the two growing seasons of 2014 and 2015. The values of ASP, CAT, GPX, ROS, PRL, LRI, GLB, SLS, and RMP decreased by increasing of irrigation volume water and SAP. It seems that osmotic adjustment through accumulating compatible solutes, which facilitate extracting water from dry soils and maintaining cell turgor in dry environments, can maintain higher LRWC. Saneoka and Agata (1996) reported that when different cereal genotypes are compared under drought stress, genotypes expressing relatively less leaf rolling might have relatively better access to soil water or better osmotic adjustment. Consequently, in this condition, the lower leaf rolling is the preferred phenotype. It seems that the higher leaves roll as a defense mechanism could reduce the net radiation load on the leaf under water deficit conditions, reducing transpiration and leaf water use as well as protecting PSII function against damage. The activities of antioxidant enzymes (ASP, CAT, and GPX) were altered when both crops were exposed to water stress. It is well documented that antioxidant enzymes could cause a tolerance to dehydration in grasses (Nar *et al*., 2009). Also the activity of antioxidant enzymes can cause the detoxification of reactive oxygen species and the protection of membrane integrity (Volaire and Lelievre, 2001).

Table 3. Parameter estimates for regression models, including the intercept (X_0) and the goodness-of-fit of the model (R^2), leaf relative water content (LRWC, %), ascorbate peroxidase (ASP), catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), proline (PRL), soluble sugars (SLS), glycinebetaine (GLB), leaf rolling index (LRI) and relative permeability of cell membranes (RMP) in sorghum under different irrigation regimes (I), determined as percentage of crop evapotranspiration, application of super absorbent polymer (SAP, $kg.ha^{-1}$).

Variable			Parameter estimates for regression models					
Dependent variable	Treatments I	Treatments SAP	Model	$\hat{\beta}_0$	$\hat{\beta}_1$	X_0	R^2	Model significance
LRWC	40	all levels	-	-	-	-	-	ns
	60		L	0.026	-	64.66	0.97	<0.01
	80		L	0.04	-	71.77	0.95	<0.01
	100		-	-	-	-	-	ns
ASP	40	all levels	L	-0.04	-	74	0.98	<0.01
	60		L	-0.06	-	63.7	0.93	<0.01
	80		L	-0.0001	-	46.01	0.96	<0.01
	100		-	-	-	-	-	ns
CAT	40	all levels	L	-0.05	-	108.54	0.92	<0.01
	60		L	-0.07	-	92.75	0.95	<0.01
	80		L	-0.1	-	77.6	0.96	<0.01
	100		-	-	-	-	-	ns
GPX	40	all levels	L	-0.03	-	78.27	0.89	<0.01
	60		L	-0.02	-	68.76	0.96	<0.01
	80		L	-0.03	-	66.57	0.94	<0.01
	100		-	-	-	-	-	ns
SOD	40	all levels	L	-0.56	-	1698.67	-	<0.01
	60		L	-0.42	-	1468.42	0.92	<0.01
	80		L	-0.57	-	1192.85	0.90	<0.01
	100		-	-	-	-	-	ns
PRL	40	all levels	-	-	-	-	-	ns
	60		L	-0.009	-	10.37	0.96	<0.01
	80		Q	-0.02	0.00006	8.87	0.99	<0.01
	100		-	-	-	-	-	ns
SLS	40	all levels	L	-0.031	-	73.29	0.93	<0.01
	60		L	-0.033	-	60.15	0.96	<0.01
	80		L	-0.029	-	38.85	0.99	<0.01
	100		-	-	-	-	-	ns
GLB	40	all levels	Q	-0.17	0.0006	188	0.99	<0.01
	60		L	-0.02	-	174	0.95	<0.01
	80		L	-0.05	-	166.2	0.98	<0.01
	100		-	-	-	-	-	ns
LRI	40	all levels	L	-0.03	-	37.42	0.98	<0.01
	60		L	-0.036	-	27.19	0.99	<0.01
	80		L	-0.026	-	20.58	0.95	<0.01
	100		-	-	-	-	-	ns
RMP	40	all levels	L	-0.032	-	64.46	0.97	<0.01
	60		L	-0.03	-	59.59	0.92	<0.01
	80		L	-0.029	-	48.38	0.91	<0.01
	100		-	-	-	-	-	ns

The units ASP, CAT, GPX and SOD were "Enzyme activity per $min^{-1}.mg^{-1}$ protein". The units PRL and GLB were "$\mu g.g^{-1}$ Fw". The unit of SLS was "$mg.g^{-1}$ Fw". **Ns**. Not significant at the 0.05 level. **L**. linear regression model; Q. quadratic regression model.

Finally, a higher decline in LRWC could increase antioxidant enzymes activities, LRI value. On the other hand, drought stress increased PRL, GLB, and SLS, causing an increase in LRWC. Therefore, drought stress induced higher oxidative injury and increase in relative membrane permeability. It seems that a higher accumulation of PRL, GLB, and SLS, in other words osmotic adjustment had a more important role in declining RMP. Responses of most traits to SAP levels were quadratic and linear.

CONCLUSION

Analysis of regression indicates ascorbate peroxidase, catalase, superoxide dismutase, glutathione peroxidase, proline, glycine betaine, soluble sugars, leaf rolling index and relative membrane permeability showed that by increasing amount of irrigation and superabsorbent polymer, decreased. The results showed that application of SAP reduced RMP by 4.98% under 100% ET_c, but it increased the RMP significantly by 14.14%, 12.18% and 11.48% under 80, 60 and 100% ET_c.

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