

Effects of drought stress and superabsorbent polymer on morpho-physiological and biochemical traits of Caper (*Capparis spinosa* L.)

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Abstract

Capparis spinosa, commonly known as caper bush, is native to certain hostile growing conditions including sandy or gravelly soils, rocky hillsides, cliffs, stone walls and rock crevices in Mediterranean coastal regions. Caper plant is used for the prevention of soil erosion in sloppy areas. Synthetic superabsorbent polymer was developed as a soil conditioner to heighten plant establishment and growth in drought-prone growing area. During growing seasons of 2016-2017, the effects of soil amendment with the superabsorbent Polymer A200 in four levels ($S_1=0$, $S_2=75$, $S_3=150$ and $S_4=225$ g) were investigated for each caper plant, considering three levels of irrigation ($I_1=0$, $I_2=$ One irrigation per month and $I_3=$ one irrigation every two months) on the physical properties of the soil as well as their physiological parameters (chlorophyll a, b and total chlorophyll, carotenoid, Tss, electrolyte leakage) and plant height, yield per hectare, WUE, Soil moisture, RWC and leaf area of an established caper plant under drying conditions. Analyses of variance showed that the interaction effects of treatments were significant ($p<0.01$) in all the studied traits. The results showed that water stress significantly decreased the height of a plant, yield per hectare, WUE, Soil moisture, RWC, leaf area, total Chlorophylla, Carotenoid and electrolyte leakage, whereas the application of superabsorbent polymer compensated for the negative effect of drought stress, especially in high rates of polymer application (150 g), where the maximum effect was attained for all the studied traits. These findings strongly suggested that the irrigation intervals of caper can lead to an increase in the application of the superabsorbent polymer.

Keywords: Biological yield; Caper; Water retention; WUE; leaf chlorophyll content; Polymer A200.

Introduction

Caper (*Capparis spinosa* L.) belongs to the Capparidaceae family, which is native to the Mediterranean region. Caper, as a perennial winter-deciduous shrub, is an aromatic plant that grows widely at various regions of the world along the roadside, on the slopes, rocky and stony areas and generally it is well adapted to basin of dry areas (Chalak and Elbitar, 2006; Inocencio et al., 2000; Sozzi., 2006). Immature flower bud, semi-mature fruits, young tender shoots and small leaves are pickled for pharmacological and cosmetic fields; however, they are especially used as condiments (Kara et al., 1996; Anonim, 1997; Alkire, 1998; Riviera et al., 2003). Caper favors dry, hot summer with intense sunlight where temperature exceeds 40°C and average rainfall is 350 mm during spring and winter (Barbera and Lorenzo, 1984). Moreover, Trewartha and Trewartha (2005) found a positive correlation between high temperatures and caper bud yields. Caper is also effective in controlling soil erosion with its thick, deep root system and dense canopy. These features make caper suitable for growing on arid, degraded, steep rocky areas, sloppy hills and on sandy, loamy soils with nutrient deficiency (Pugnaire and Esteban., 1991). Caper yields within the second year but economic production

starts at third year (Barbera and Lorenzo, 1984). Accordingly, water supply in soil was increased using superabsorbent polymer (SAP) that supplies water for crop roots (Pawlowski et al., 2009). The superabsorbent polymer application positively influences capacity of water storage in soil (Akhter et al., 2004; El-Hady and Wanas, 2006; Sarvas et al., 2007) which leads to the reduction of waste water and enhancement of nutrition materials of soil (Adams and Lockaby, 1987) which consequently supply better growth and increases the yield under normal irrigation and water stress condition. Mahalleh et al., (2011) found that SAP contributed to yield, yield components and water using efficiency of corn. Application of polymers is suitable for drought stress control and can protect plants in drought stress conditions (Dabhi et al., 2013). Using SAP in the production of Sweet Pepper significantly affects the morphological, physiological and biological parameters of sweet pepper. In addition, by increasing irrigation intervals, drought stress caused growth parameters, yield, chlorophyll and relative water content (RWC) of leaf decreased and total soluble solids (TSS) electrolytes leakage and proline content increased (Sayyari and Ghanbari., 2012). Moreover, the

incorporation of superabsorbent polymer leads to the enhancement of weight, number of leaves, proportion of root dry weight to aerial organs and chlorophyll. Peroxidase enzyme activity is also significant as it reduces the effect of drought stress and improves the growth characteristics and reduces the activity of catalase and peroxidase enzymes (Tongo et al., 2014). There is a scarcity of research on the function and the functional elements of Capper in tensed environment, especially in the Iranian context. However, the aim of this study was to evaluate the effect of four amounts of super absorptions (0, 75, 150 and 225 gr for 1 plant) and three levels of irrigation treatments (non-irrigation, one-month irrigation and tow month irrigation) on morpho-physiological traits in field conditions.

Results

Based on variance analysis, Interaction effects of irrigation and SAP levels were significant ($P < 0.01$) in all examined traits.

Plant height and yield ha^{-1}

As mean comparison date (Table 1) showed that no significant differences were obtained among treatments with using one irrigation every two months (I_3). The highest value for plant height (66.5 cm) was observed in 225 gr super absorption (SAP) \times one irrigation every two month (S_{4I_3}) treatment. The lowest value for plant height (26.5 cm) was observed in 75gr SAP \times one irrigation per month (S_{2I_2}) treatment. Mean comparison also revealed significant contribution of superabsorbent (150 g) with one irrigation per month (S_{3I_2}) on yield performance (Table 1).

Water Use Efficiency (WUE) and soil moisture

As shown in table 1, application of 150 g of superabsorbent along with one irrigation per month (S_{3I_2}) had significant effects on water use efficiency. Therefore, water use efficiency (WUE) increased from 0.0032 (S_{1I_1}) to 0.135 (S_{3I_2}). Water absorption rates had positive trend with SAP content under the irrigation from 0.046 to 0.56% (Table 1). By using of any amount of examined SAP \times one irrigation every two months, no significant differences were observed among soil moisture contents. The highest soil moisture (0.063%) was obtained in combination of 150 g SAP \times one irrigation every two months (S_{3I_3}).

Electrolyte leakage and Relative water content (RWC)

The mean comparison of SAP \times irrigation on the studied properties revealed significant effects on increasing electrolyte leakage (Table 1). The highest electrolyte leakage (55.27%) was observed using 225 g SAP in combination with one irrigation per month (S_{4I_2}). and the lowest value (24.18%) was achieved in treatment of 75 g SAP \times one irrigation per month (S_{2I_2}). Mean comparison revealed that increasing the drought intensity, Leads to a significant reduction on RWC (Table 1). Also, incensement in superabsorbent application caused more impartment of RWC. The highest soil moisture (71.22%) was obtained with 150 g SAP \times one irrigation per month (S_{3I_2}).

Leaf area (LA)

All SAP treatments significantly increased leaf area (LA) in comparison with the controls (Table 1). The maximum LA value was achieved at 150gr SAP \times one irrigation per month (S_{3I_2}). This value did not show significant difference with 75 g SAP \times one irrigation per month (S_{2I_2}). By increasing drought stress, leaf area revealed a significant reduction.

Chlorophyll a and b

Results indicated that various irrigation and superabsorbent treatments had significant effects on Chlorophyll a and Chlorophyll b. The highest values of chl a were observed at 150 g SAP \times one irrigation per month (S_{3I_2}), but no significant differences were revealed among S_{1I_2} , S_{2I_2} , S_{3I_2} , S_{4I_1} and S_{4I_3} combinations (Figure 1). Chlorophyll b had also the same significant value at S_{1I_2} , S_{2I_2} and S_{3I_2} treatments. The least amount of Chla and Chlb content was observed in S_{2I_3} .

Total chlorophyll

The total content of chlorophyll was significantly influenced by irrigation treatments and superabsorbent application. Mean comparison revealed that increasing the drought tension owing to significant reduction in chlorophyll content (Figure 2). The lowest value for chlorophyll content was obtained in 75g SAP \times one irrigation per month (S_{2I_2}) while the highest value was obtained at 150gr SAP \times one irrigation per month (S_{2I_2}).

Carotenoid and Total Soluble Solids (TSS)

The highest value of Carotenoid (0.201 mg/g FW) was obtained in treatment with 150 g SAP \times one irrigation per month (S_{3I_2}). Severe drought stress (S_{2I_2}) led to achievement of the lowest carotenoid value (0.0156 mg/g FW) (Figure 3). The mean comparison of total soluble solid indicated that, excepts S_{1I_2} , S_{1I_3} and S_{3I_3} , there were no differences among other combinations. The interaction effects of using 225 g SAP \times non irrigation (S_{4I_1}) treatment leads the highest leaf TSS (Figure 4).

Discussion

The results of this study strongly confirmed (Table 1) that increasing drought stress can lead to the enhancement of the plant height, yield in hectare, and Water Use Efficiency (WUE), while electrolyte Leakage, RWC and leaf area decreased significantly. The effects of water stress on reducing the growth of various parts of plant and yield in pepper were reported in Sayyari and Ghanbari (2012) and Woodhouse and Johnson (1991), whose findings were in strong agreement with those of the present study. Studies such as Arji et al. (2002) on olive trees confirmed the effect of drought stress on reducing the number and weight of leaf. It seems that drought would affect generating primary cells of leaf and their distinction, which causes a decrease in the leaf number (Lobato et al., 2008), while positive effects of superabsorbent on stem elongation are reported by Brar et al. (2001). This effect of superabsorbent polymers is of great importance for the increase of water absorption and reduction of water evaporation from the surface of soil and

Table 1. Mean comparison of using different amount of superabsorbent (SAP) on morpho-physiological parameters of *Capparis spinosa* under different levels of drought stress.

Treatment	Plant height (cm)	Yield in hectare (kg)	Water use efficiency (WUE)	Soil moisture (%)	Electrolyte leakage (%)	RWC (%)	Leaf area (cm ²)
S ₁ I ₁	31.16 bc	8.19 de	0.0032 de	0.045 b	27.58 e	57.35 e	0.738 e
S ₁ I ₂	54 a	21.85 c	0.046 c	0.039 bc	30.59 d	60.20 cd	3.22 b
S ₁ I ₃	0 d	0 f	0 e	0.06 a	0 g	0 f	0 e
S ₂ I ₁	39.5 b	14.65 d	0.0058 de	0.037 c	43.00 c	63.69 bc	1.97 cd
S ₂ I ₂	61.33 a	35.83 b	0.078 b	0.043 b	41.66 c	58.92 d	3.82 ab
S ₂ I ₃	26.5 c	5.20 f	0.0061 de	0.056 ab	24.18 f	64.09 b	2.61 c
S ₃ I ₁	38.66 b	35.98 b	0.0143 d	0.043 b	51.64 b	65.43 b	2.82 bc
S ₃ I ₂	64.66 a	63.32 a	0.135 a	0.048 b	29.91 d	71.22 a	5.09 a
S ₃ I ₃	0 d	0 f	0 e	0.063 a	0 g	0 f	0 e
S ₄ I ₁	36 bc	27.61 c	0.011 de	0.043 b	51 b	66.46 b	3.22 b
S ₄ I ₂	66.5 a	35.90 b	0.076 b	0.038 bc	55.27 a	57.85 de	3.39 b
S ₄ I ₃	29.87 bc	11.18 de	0.013 d	0.049 ab	27.37 e	54.53 e	1.57 d

Values in each column with different superscripts are significantly different at 0.01 probability level. S₁ = 0, S₂ = 75g SAP, S₃ = 150 g SAP and S₄ = 225 g SAP. I₁ = non irrigation, I₂ = one irrigation per month and I₃ = one irrigation every two months

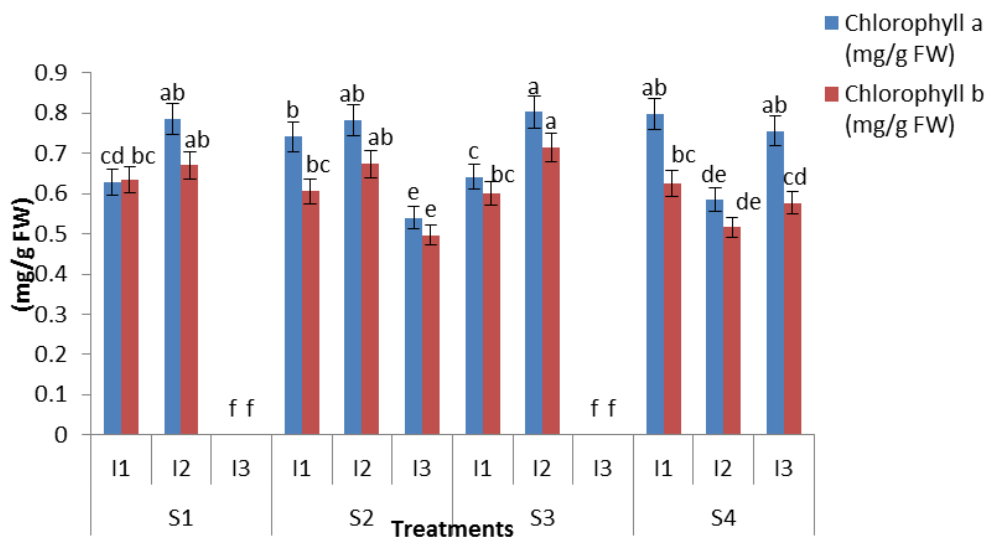


Fig 1. Effect of SAP on chlorophyll a and b contents of *Capparis spinosa* under different levels of drought stress. Values in each column with different superscripts are significantly different at 0.01 probability level. S₁ = 0, S₂ = 75g SAP, S₃ = 150 g SAP and S₄ = 225 g SAP. I₁ = non irrigation, I₂ = one irrigation per month and I₃ = one irrigation every two months

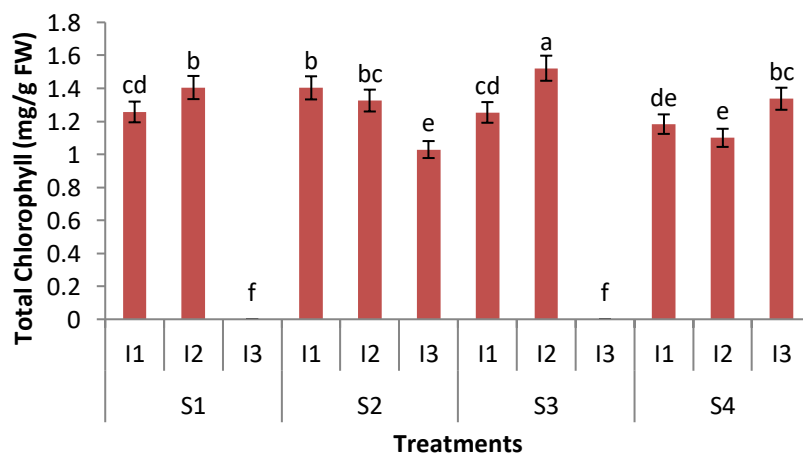


Fig 2. Effect of SAP levels on total chlorophyll of *Capparis spinosa* under different levels of drought stress. Values in each column with different superscripts are significantly different at 0.01 probability level. S₁ = 0, S₂ = 75g SAP, S₃ = 150 g SAP and S₄ = 225 g SAP. I₁ = non irrigation, I₂ = one irrigation per month and I₃ = one irrigation every two months.

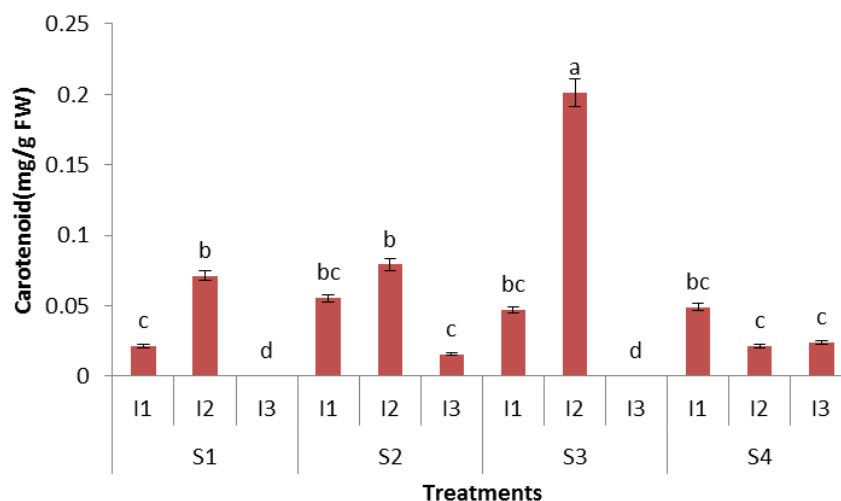


Fig 3. Effect of SAP levels on carotenoid content of *Capparid spinosa* under different levels of drought stress. Values in each column with different superscripts are significantly different at 0.01 probability level. S₁ = 0, S₂ = 75g SAP, S₃ = 150 g SAP and S₄ = 225 g SAP. I₁ = non irrigation, I₂ = one irrigation per month and I₃ = one irrigation every two months.

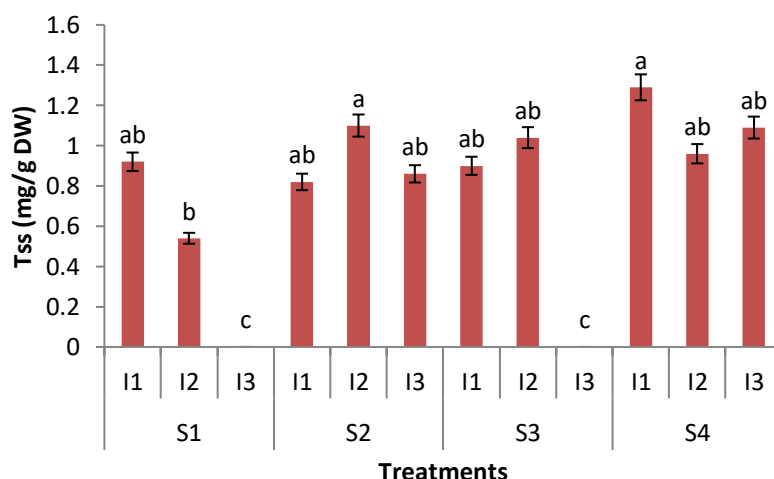


Fig 4. Effect of SAP levels on TSS of *Capparid spinosa* under different levels of drought stress. Values in each column with different superscripts are significantly different at 0.01 probability level. S₁ = 0, S₂ = 75g SAP, S₃ = 150 g SAP and S₄ = 225 g SAP. I₁ = non irrigation, I₂ = one irrigation per month and I₃ = one irrigation every two months.

the decrease of bad effects of drought stress (Sarvas et al., 2007). Reduction in water supply is associated with diminishing cell elongation (Yang et al., 2006), owing to high potential of superabsorbent for absorption and conservation of water in the soil (Boman and Evans, 1991).

Physical properties of soil were improved by superabsorbent polymer (Boman and Evans, 1991; Poormohammad Kiani et al., 2007).

The results of comparing the average of simple effects of SAP revealed the efficiency of this substance in keeping WUE. Effect of water stress on WUE depends on plant species, phenological stage of plant in drought period and stress intensity (Kumari 1988). In majority of crops, improvement of WUE does not lead to improvement in biomass, surprisingly because of improvement in harvest index. In drought stress, Nazarli et al. (2010) reported reduction of RWC. In addition, changes in leaf temperature seem to be an important factor in controlling the leaf water status under drought stress (Yang and Miao, 2010).

RWC decreased as evapotranspiration increased in plant society and as reported by Santos et al., (2002) in drought stress in comparison with non-stress conditions which led to reduction in RWC. These results were in correspondence with the reports of Poormohammad Kiani et al. (2007). Therefore, osmotic regulation would help cell development and plant growth in water stress. It is said that decrease in RWC would reduce the photosynthesis rate (Cornic, 2000). It is reported that high RWC is a preferential mechanism to drought tolerance (Merah, 2001) and incensement in superabsorbent application cause improvement in RWC (Table 1). The highest content for RWC observed in application of 150g SAP × one irrigation every two months (Table 1). The lowest content of RWC was related to (S₄I₃) (Table 1) and no significant difference was observed between the control samples. By applying SAP, humidity fluctuations were reduced and irrigation intervals and plant growth increased. It is obvious that continuing plant growth and reduction of drought stress cause reduction in the plant yield. One of the indices that expresses the status of plant

water is RWC, which causes the reduction of RWC of leaf by increasing irrigation interval (Table 1), Reduction of RWC of the leaf due to the notion that drought stress is related to the reduction of soil humidity. In such conditions, one must close the stomata to avoid more water waste. In the previous studies (Saneoka et al., 2004), applying SAP caused reduction in RWC in lenti by drought stress in comparison to non-stress conditions. Moreover, the reduction of RWC of the leaf had direct relation with water reduction in soil (Nautiyal et al., 2002). Using absorbent substances as SAP via storage of considerable water cause storage of humidity in the soil and the amount of water is increased in the plant. Results obtained in this study are similar with those reported by Tongo et al. (2014) about leaf area. Using absorbent substances such as SAP with considerable storage capacity of water can keep humidity in the soil and the amount of water absorption is increased in the plant. This result is in accordance with the results of Ghasemi and Khoshkholi (2007) in *Chrysanthemum* plant. In this study, a change of leaf area was related to leaf falling, twisting and reducing of leaf pressure turgor. By increasing drought stress, leaf area indicated significant reduction, while the most leaf area was obtained in the conditions with available moisture (150g SAP × one irrigation per month) and the lowest was in control sample treatment (drought stress condition) (Table 1).

In the present study, applying SAP caused more water retention in soil. The hydraulic conductivity of soil was influenced by the particle size distribution of soil (Arya et al., 1999), which depended on soil texture (Hultine et al., 2006). The positive effect of SAP under drought stress decreased hydraulic conductivity of soils (Bhardwaj et al., 2007; Agaba et al., 2010). Furthermore, soil is proportional with more potential ability of water adsorption and it slowly releases absorbed water in soil, which leads to the reduction of moisture (Ni et al., 2010). One of the signs of environmental stress in plants is the reduction of chlorophyll and this reduction depends on the plant genotype (Colom and Vazzana, 2001). In the present study, increasing drought stress leads to increasing chlorophyll content. Antolin et al. (1995) reported that drought stress decreased total chlorophyll content. So, this phenomenon caused an increase in chlorophyll a/chlorophyll b ratio and chlorophyll-meter data. The dehydration stress is assaulted with oxidative process, which subsequently disturbed the chloroplast structure and diminished photosynthesis (Antolin et al., 1995). Similarly, Ashraf et al. (1994) reported that decreasing chlorophyll b occurred more than chlorophyll a under drought stress. The least content of Chla and Chlb was observed in S₂l₃ and S₃l₁, respectively (Figure 1). Chlorophyll content is a distinct index for evaluation of stress intensity (Ommen et al., 1999) and its reduction is an indication of stomata limiting factor in drought stress conditions (Kuroda et al., 1990; Zlatev and Yordanov, 2004). In drought stress, tolerance genotypes reveal high content of chlorophyll (Sairam and Siravastava, 2002). Severe drought stress refuse photosynthesis as a consequence of chlorophyll content reduction and damaging the photosynthetic apparatus (Iturbe Ormaetxe et al., 1998). The results of comparing the average of simple effects of SAP revealed that this substance keeps chlorophyll. The positive effect of SAP under drought stress on the reduction

of activity of antioxidant enzymes was reported in *Acacia Victoriae* seedlings under drought stress (Tongo et al., 2014). Avoiding the stress of humidity fluctuations in arid regions, the polymer minimizes the stress levels of humidity fluctuations by gradual keeping of water for the plant, and it is one of the most important applications of these substances in agriculture (Nazarli et al., 2010) and this increase cause a better growth for plants in stress conditions.

One of the efficient mechanisms of the plant in drought conditions is osmosis control. Osmosis control is a physiological phenomenon, during which reduction in osmosis potential occurs following stressed tissues. This occurs due to the accumulation of osmosis substances including mineral elements (e.g. potassium, sodium and calcium) and some of the metabolites, such as sugar, amino acids (proline) and organic acids. These metabolites do not have any contradiction with normal biochemical reactions of the cells. Water shortages, as other bad environmental conditions, create oxidative stress and cause blockage (preservation) of photosynthesis via closure of stomata and shortage of CO₂. They lead to Reactive Oxygen Species (ROS) in chloroplast, which damage membrane due to lipid peroxidation (Mascher et al., 2005). In this study, increasing electrolyte leakage was observed by increasing irrigation interval (Table 1), which increased damage to cell membrane, make electrolyte leakage of the membrane, and also damage the plant. It seems that reactive radicals produced because of drought stress, can increase peroxidation reaction and increase electrolyte leakage in pepper under drought stress. The results of this study revealed that superabsorbent can reduce electrolyte leakage by reducing humidity fluctuations. In other words, the drought stress severity is reduced by applying SAP.

The main reason for the concentration of TSS in the cell due to the shortage of irrigation water was by compensated with reduction of osmosis potential and reduction of stored water and increasing TSS (Mishell et al., 1991). In the current study, based on the above scientific justification, the maximum TSS (1.29%) in 225g SAP and minimum (0.54 %) were observed in one irrigation every two months. Using polymer substances led to the storing of humidity in the soil and thus plants can have gradual access to this moisture. This could help avoiding the drought effects including the increase of sugar and it was in agreement with the results of Sayyari and Ghanbari's (2012) study about peppers.

Materials and Methods

Sowing and planting

Caper seedlings were produced from seeds of the population of *Capparis spinosa* in the city of Gachsaran, Kohgiluyeh and Boyer-Ahmad Province, Iran, in June 2015. Observations were carried out in 2015 and 2017 in two growing seasons. Seeds were sown into round plastic pots (of 13 cm diameter and 17 cm height). The pots were filled with a mixture of sand, compost and soil ratio (2, 1 and 2). After 4 months, the plants were reached to the four-leaf stage. Adaptation was done outside the greenhouse and then plants were transplanted to the field. This research was carried out in the research field of the Researchers Center of Emamzadeh Jaafar in the city of Gachsaran (longitude 59

and 50° of east, latitude 30 and 28° of north with height of 668 m above the sea level). In this area, the yearly average air temperature is 22.5 °C, with warm and dry summer. Yearly rainfall average is 458 mm. The field soil texture was Silt with organic matter 1.3%, acidity 7.8 and 1.9 dS.m⁻¹ electrical conductivity. N, P, K content in soil were 0.13, 18 and 759 ppm, respectively. This study was carried out as 4×3 factorial experiment in completely random blocks design (RCBD) with four levels of Superabsorbent Polymer A₂₀₀ (0, 75, 150 and 225 gr for each plant) and three regimes of irrigation during growing seasons (I₁=non-irrigation, I₂=one irrigation per month and I₃=one irrigation every two months). Each treatment was done in three replicates and each replicate consisted of five cavities. By considering factors level (treatment 4×3=12) and replication number (3) and the number of observations (5), 180 plants were examined. Before planting, superabsorbent polymers was applied for each cavity. Observations were carried out in 2016 and 2017 in two growing seasons.

Measurement of traits

Finally, at the end of growth period (16 months later), the measurements were done in relation to the height plant, yield per hectare, WUE, Soil moisture, Cell membrane stability, RWC, leaf area, Chlorophyll a,b, total, Carotenoid and total soluble solid (TSS). Before the initiation of irrigation intervals, the value of applied water in each treatment combination was measured with graduated cylinder and total performance was computed. Relative water content (RWC) was measured on leaf samples and after cutting the base of lamina, leaves were sealed in plastic bags and quickly transferred to the laboratory where fresh weight (FW) was determined after excision. Turgid weights (TW) were obtained after soaking leaves with distilled water in test tubes for 4 (h) at room temperature (25°C) and low light condition. After soaking, determination of turgid weight was estimated by blotting the leaves with blotting papers and dry weights (DW) were obtained after oven drying for 72 (h) at 70°C. Relative water content (RWC) was calculated according to the following:

$$RWC (\%) = [(FW - DW) / (TW - DW)] \times 100$$

FW = fresh weight

DW = dry weight

TW = total weight

Water Use Efficiency (WUE) was determined through the following formula:

$$WUE (g/l) = (\text{The general performance} / \text{the used water}) \times 100.$$

Chlorophyll a, b and carotenoids were estimated by extracting the leaf material in 80% acetone according to Strain and Svec (1966). Content of chlorophyll was calculated according to the following formulae:

$$Chla = (12.25A_{663} - 2.79A_{647})$$

$$Chlb = (21.50A_{646} / 8 - 5.10A_{663} / 2)$$

$$Car = ((1000 A_{470} - 1.8 Chl. a - 85.02 Chl. b) / 198)$$

Electrolyte leakage of leaf

In order to assess the membrane permeability, electrolyte leakage was determined according to Korkmaz et al (2010). Leaf discs (1cm in diameter) from randomly chosen plants

per replicate were taken from the middle portion of the fully developed leaf and were washed with distilled water to remove surface contamination. The discs were placed in individual vials containing 10 ml of distilled water. After incubating the samples at room temperature on a shaker (150 rpm) for 24 h, the electrical conductivity (EC) of the bathing solution (EC1) was determined. The same samples were then placed in an autoclave at 121 °C for 20 min and again EC was recorded (EC2) after cooling the solution to room temperature. The electrolyte leakage was calculated as EC1/EC2 and expressed in percentages.

Statistical analysis

Statistical analysis of data was done with SPSS 21 and Microsoft Excel 2010. Additionally, means were compared through Duncan's method.

Conclusion

The results indicated that water stress significantly ($P < 0.05$) decreased yield, growth parameters, RWC, photosynthetic pigments and electrolyte leakage and increased TSS and proline content. Furthermore, the application of SAP moderated the negative effect of deficit irrigation on plant growth and productivity. This effect can be due to the considerable absorption of water in superabsorbent structure and putting gradual absorbed water to the surrounding soil and plant root. Based on the results of this study and the according to durability of superabsorbent polymer in soil, it can be claimed that not only can this matter be used under drought stress conditions, but it also can be utilized under adequate irrigation conditions as it can increase the yield along with compensating for its purchase costs and benefits. Furthermore, soil with SAP could absorb more water than soil without SAP and allow the absorbed water to be released slowly when the soil moisture decreased, and fertilizer nutrients could also be released slowly as water decreased. Finally, nutrient retention in hydrogel amended substrate could be increased.

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