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Can Super Absorbent Polymer Improve the Water-Deficit Tolerance of Young Myrtle Plants?

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Abstract

Water scarcity is a serious problem affecting young plants' growth, development, and establishment. Superabsorbent polymers (SAPs) have revealed an excellent capacity to absorb and retain water, increase soil moisture, and improve the growth of plants under water shortage. A greenhouse study was performed with three irrigation regimes (50 %, 75 %, and 100 % FC) and three levels of SAP-A200 (0, 1, and 2 g kg^{-1} soil) in a factorial experiment based on a completely randomized design with four replications. The results showed that soil water deficit affected physiological and biochemical characteristics of young myrtle plants. Photosynthetic parameters, total chlorophyll, and relative water content (RWC) decreased by increasing water-deficit stress. In contrast, leaf electrolyte leakage (EL), malondialdehyde (MDA), total soluble sugars (TSSs), and starch, increased. However, the application of minimal amounts of SAP (1 and 2 g kg^{-1} soil) improved most of these characteristics in both well-watered and water-deficit conditions, it appears that a higher amount of SAP is needed in moderate and severe water stress conditions. Therefore, SAP-A200 can be utilized as an efficient and economical method for rapidly establishing woody young plants in low-water areas.

Keywords: A200 polymer, *Myrtus communis* L., Photosynthesis, Starch, Woody plants.

INTRODUCTION

The establishment of young plants can be adversely affected by limited water resources. In addition, water stress has many detrimental effects on plants, such as inhibiting photosynthesis and growth are affecting hormone metabolism and enzyme activity (Okunlola *et al*., 2017).

Plants respond to water stress in complicated ways that involve changes in their morphology, physiology, and metabolism. Water availability affects the relationship between tissue water and gas exchange. Stomata closure, osmotic regulation, changes in cell flexibility, and growth reduction of aerial parts can improve plant water status and its tolerance to water stress by limiting water loss (Sánchez-Blanco *et al*., 2004).

The management of soil moisture can increase plant yield and products in arid and semi-arid regions. A strategy to maintain soil moisture is the use of SAPs. These polymeric materials can absorb water hundreds or thousands of times their weight. In addition, SAPs have a high water-holding capacity and can absorb water frequently and release it when necessary. It can also regulate soil water content, reduce soil saturation conductivity, improve soil biological activity, and increase soil accumulation and water holding capacity. Plants produce reactive oxygen species (ROS) as a result of water stress, which limits plant growth and activates antioxidant enzymes (Liu *et al*., 2013).

SAPs are very hydrophilic due to their low crosslinking structure and a high potential for soil repair and water storage for plant growth. The positive effect of SAPs on water stress has been proven in some woody plants such as *Pinus halepensis* Mill. (Hüttermann *et al*., 1999), *Poncirus trifoliata* [L.] Raf. × *Citrus sinensis* [L.] Osb. (Arbona *et al*., 2005), *Cupressus arizonica* (Abedi Koupaei and Asad Kazemi, 2006), *Quercus rubra* L. (Apostol *et al*., 2009), *Populus* × *canescens* (Beniwal *et al*., 2010), *Fagus sylvatica* L. (Jamnicka *et al*., 2013), *Acacia victoriae* L. (Tongo *et al*., 2014), *Gossypium hirsutum* L. (Fallahi *et al*., 2015), *Eucalyptus saligna* (Khodadadi-Dehkordi, 2017).

Using SAPs significantly increased the biomass, chlorophyll content, and photosynthesis of *Areca catechu* L. under drought stress (Li *et al*., 2018). In another study, SAP (Stockosorb-660) increased the growth responses, RWC, plant pigments, and biomass of *Olea europaea* L. seedlings under water stress (M'barki *et al*., 2019). Lertsarawut *et al*. (2021) reported that Cassava starch-based super water-absorbent (SWA) enhanced the survival percentage of *Hevea brasiliensis* Muell. cultured in the arid region up to 40% after six months.

The myrtle plant (*Myrtus communis* L.) is distributed in Southern Europe, North Africa, and Iran. It is an evergreen ornamental shrub widely cultivated as a hedge plant or a single small tree in green spaces due to its leathery leaves, great flowering, and pleasant odour. The essential oils compounds, phenols, flavonoids, and antioxidant activity make it valuable for pharmaceutical, food, and cosmetic applications, as well (Anwar *et al*., 2016).

Few studies have been performed on the response of young myrtle plants to water-deficit stresses, limited to the effects of mycorrhizal fungi, plant growth regulators (PGRs), and recycled water. There were no reports of the effects of SAP application on water-deficit tolerance of young myrtle plants and its association with physiological or biochemical responses.

The purpose of the experiment was to examine the physiological and biochemical responses of young myrtle plants to different irrigation levels and A200 (SAP) treatments.

MATERIALS AND METHODS

Plant materials and experimental conditions

This study was done at the research greenhouse of the Department of Horticultural Science, School of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran, from March to July 2021. The annual *Myrtus communis* L. plants derived from stem cuttings were used in this experiment. Plants are cultured in a soil mixture consisting of a 4: 2: 1 ratio of soil, completely rotted cow manure, and sand in pots with a top diameter of 28 cm and height of 24 cm. A200-SAP (Iranian Nano Arian Company) was used according to the manufacturer's instructions, mixed with potting soil, and then placed around the roots of plants. A200-SAP is a tripolymer of acrylamide, acrylic acid and acrylate potassium. Some physico-chemical properties of A200-SAP are granular particles with a size of 0.5-1.5 mm, a density of 1.4-1.5 g cm^{-3}, a maximum stability in the soil of 7 years, and a practical water absorption capacity of 220 g g^{-1}. Immediately after culturing, the plants were irrigated once every two days until completely established. Plants of uniform size were selected and subjected to different treatments. Irrigation treatments were determined using field capacity (FC) and permanent wilting point (PWP), which were 32.8% and 20.4%, respectively. The experiment treatments included three levels of irrigation (100%, 75% and 50% FC) and three levels of SAP-A200 (0, 1, and 2 g kg^{-1} dry soil) with four replications. Soil mixture properties, including soil texture (Silty loam), organic matter percentage (3.8%), bulk density (1.16 g cm^{-3}), EC (4 dS m^{-1}), and pH (7.3) measured before the start of the test. In the experiment, the mean temperature and light intensity were 28± 2 °C and 5700 lux, respectively.

Net photosynthesis (Pn) and transpiration (E)

Pn and E rates were measured using a photosynthetic meter (Lci Console model, UK). These parameters were measured in each replication by selecting leaves from nodes 3 to 6 in the upper part of the plant (Hnilickova *et al*., 2021).

Plant pigments, RWC and EL

Total chlorophyll (Chl) and carotenoids were measured according to the method of Lichtenthaler (1987). Leaf RWC and EL were calculated by the methods Ghoulam *et al*. (2002) and EL by Lutts *et al*. (1996), respectively.

MDA, TSSs, and starch content

MDA levels was assayed using the method of Heath and Parker (1968), respectively. Leaf TSSs and starch contents were measured with the methods described by Irigoyen *et al*. (1992) and Marshall (1986).

Experimental design and data analysis

Treatments were arranged in a complete randomized design with four replications. Data were analyzed using SAS 9.4 Software, and means were compared using the Duncan test at $P < 0.05$.

RESULTS

Effects of SAP and irrigation levels on photosynthetic parameters

Water-deficit stress significantly reduced photosynthesis and transpiration rates compared to the control treatment. In non-SAP treatment, there was a decrease of about 58.79% at a photosynthesis rate under 50% FC compared to the control treatment (100% FC). Adding 2 g kg^{-1} soil of SAP significantly increased the photosynthesis rate by about 75.63% at 50% FC compared to non-SAP treatment (Table 1).

In non-SAP treatment, the transpiration rate showed a significant decrease of about 44.83% at 75% FC compared to the control treatment (100% FC). Appling 2 g kg^{-1} soil of SAP significantly increased the transpiration rate in all irrigation levels (Table 1).

Table 1. Effect of different irrigation levels and SAP, and their interaction on some physiological parameters of *M. communis*.

Variable	SAP (g kg^{-1} of soil)	Irrigation levels			Mean
		100% FC	75% FC	50% FC	
Photosynthesis ($\mu mol\ m^{-2}\ s^{-1}$)	0	4.78a±0.10	2.76bc±0.47	1.97c±0.18	3.17B±0.38
	1	2.95b±0.12	2.77bc±0.23	2.65bc±0.34	2.79B±0.13
	2	4.43a±0.34	3.30b±0.23	3.46b±0.30	3.73A±0.21
	Mean	4.05A±0.26	2.94B±0.19	2.69B±0.23	
Transpiration ($mmol\ m^{-2}\ s^{-1}$)	0	1.45bc±0.21	0.80d±0.02	0.92d±0.03	1.06B±0.10
	1	1.64b±0.29	1.09cd±0.07	1.05cd±0.06	1.26B±0.12
	2	2.07a±0.02	1.40bc±0.08	1.46bc±0.06	1.64A±0.09
	Mean	1.72A±0.13	1.14B±0.07	1.10B±0.08	
Electrolyte leakage (%)	0	38.48cde±2.65	51.53b±1.75	60.38a±0.69	50.13A±2.88
	1	34.33e±2.69	39.98cd±2.19	39.77cd±1.01	38.02B±1.34
	2	35.52de±1.74	40.46cd±1.23	43.09c±1.05	39.69B±1.18
	Mean	36.11C±1.36	43.99B±1.85	47.75A±2.76	
Relative water content (%)	0	76.13a±2.53	59.97b±1.57	61.70b±1.58	65.93B±2.41
	1	74.61a±3.44	75.34a±3.57	78.03a±3.81	75.99A±1.93
	2	81.69a±3.83	77.00a±1.19	73.81a±1.23	77.50A±1.59
	Mean	77.48A±1.96	70.77B±2.61	71.18AB±2.45	
Total chlorophyll ($mg\ g^{-1}$ F.W.)	0	0.84cd±0.04	0.81d±0.07	0.63e±0.04	0.76C±0.04
	1	1.04a±0.01	0.89bcd±0.03	0.80d±0.03	0.91B±0.03
	2	0.96abc±0.02	1.01ab±0.03	0.96abc±0.01	0.98A±0.01
	Mean	0.95A±0.02	0.91A±0.03	0.80B±0.04	
Total carotenoid ($mg\ g^{-1}$ F.W.)	0	0.17d±9.01	0.28a±0.02	0.23b±0.01	0.23A±0.01
	1	0.20bcd±8.49	0.31a±0.01	0.21bc±7.95	0.24A±0.01
	2	0.18cd±0.01	0.28a±0.01	0.19cd±5.99	0.22A±0.01
	Mean	0.18C±6.60	0.29A±8.98	0.21B±7.13	

*In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the Duncan test. SAP (Super Absorbent Polymer), SAP0: non-SAP, SAP1: 1 g kg^{-1} SAP, and SAP2: 2 g kg^{-1} SAP.

Effects of SAP and irrigation levels on plant pigments, EL and RWC

Based on data analysis, the interaction of irrigation and SAP levels significantly affected total chlorophyll in treated plants. A 25% reduction in total chlorophyll content was found at 50% FC compared to the control treatment (100% FC). SAP levels could raise total chlorophyll content in all irrigation regimes compared to non-SAP treatment (Table 1).

Total carotenoids significantly increased by reducing irrigation levels. As shown in table 1, an increase of about 64.71% in carotenoid content was found at 75% FC compared to control plants (100% FC) in non- SAP treatment. Whereas, added SAP could increase or maintain carotenoid content at 100% FC and 75% FC treatments compared with non-SAP treatments. Total carotenoids decreased to 17.39% by adding SAP (2 g kg^{-1} soil) compared to non-SAP treatment at 75% FC (Table 1).

Data analysis showed that the interaction of irrigation and SAP levels had significantly affected EL and RWC (Table 1). EL of leaf control plants (100% FC) was not significantly different among non-SAP and SAP treatments. However, water-deficit stresses significantly increased EL in plants compared to control plants (100% FC). There was an increase of about

56.91% at 50% FC compared to control plants (100% FC) under non-SAP treatment (Table 1).

Leaf RWC decreased with increasing water stress levels. Results of the main effects of SAP on RWC showed a significant increase of about 18.29% compared to non-SAP treatment. The highest leaf RWC was obtained by adding 2 g kg^{-1} SAP at 100% FC. In addition, 2 g kg^{-1} SAP had a significant increase of about 28.40% compared to non- SAP treatment at 75% FC (Table 1).

Effects of SAP and irrigation levels on MDA, TSSs and starch content

As shown in Fig. 1A, MDA content significantly increased with decreasing irrigation levels. There was a significant effect between with and without SAP treatments in all irrigation levels. However, there was no significant difference between 1 and 2 g kg^{-1} SAP. The highest and lowest MDA contents were obtained in 50% FC without SAP and 100% FC with 1 g kg^{-1} soil of SAP treatment, respectively.

Data analysis showed that adding SAP had no significant effect on the TSSs content of plants exposed to water-deficit stress. However, TSSs content increased with progressing water stress at 50% FC level in all plants regardless of SAP treatments. A notable increase of about 115.82% was found in TSSs content at 50% FC compared to 100% FC (Fig. 1B). According to Fig. 1C, water-deficit stress (50% FC) significantly increased leaf starch compared to 100% FC. The highest starch content was found at 50% FC and 2 g kg^{-1} soil of SAP treatment. A significant decline of about 23.66% was obtained in starch content by adding 2 g kg^{-1} SAP compared to 1 g kg^{-1} SAP under 100% FC.

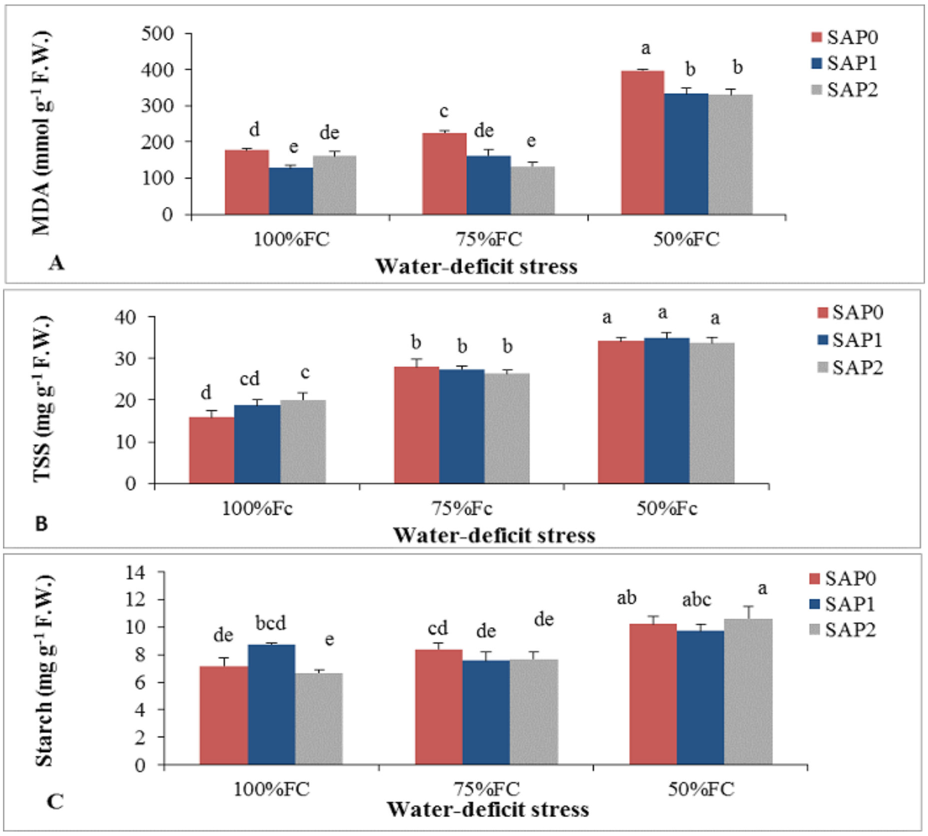

Fig. 1. Interaction of irrigation and SAP (super absorbent polymer) levels on, MDA (malonaldehyde) (A), TSSs (total soluble sugars) (B), and starch content (C). SAP0: non-SAP, SAP1: 1 g kg^{-1}, and SAP2: 2 g kg^{-1} of SAP. Data are the mean ± standard error (SE). Distinct letters represent statistically significant differences by the Duncan test ($P < 0.05$).

DISCUSSION

Water stress significantly reduced gas exchanges, including photosynthesis and transpiration rates. SAP treatments increased the photosynthesis rate under water-deficit stresses. Based on the results, a small amount of SAP (2 g kg^{-1} soil) could significantly increase the photosynthesis rate at 50% FC compared to control plants. However, the photosynthesis rate in all plants was almost low. One of the possible reasons for the lower photosynthesis of all plants is the presence of yellow spots on leaves caused by whiteflies during the experiment in the greenhouse.

Many studies have shown the reduction in photosynthetic activity under water stress associated with stomatal or non-stomatal mechanisms. The first reaction to water-deficit stress is stomatal closure, which reduces the absorption of CO_2 and ultimately reduces photosynthesis, and increases light respiration and ROS accumulation. Stomatal conductivity interacts with leaf water status, and there is a good correlation between stomatal conductance and RWC. Non-stomatal mechanisms include the change in chlorophyll synthesis, functional and structural changes in chloroplasts, and disruption in the accumulation process, transport, and distribution of adsorbed materials (Anjum *et al*., 2011). Similarly, adding a type of SAP (Stockosorb polymer) to soil improved the photosynthetic rate, plant growth, survival percentage, and biomass accumulation of *Fagus sylvatica* L. under water stress (Jamnická *et al*., 2013).

Plant pigments, particularly carotenoids, are an important non-enzymatic defense system against oxidative damage. Results showed that reducing irrigation levels up to 50% FC led to a notable reduction in total chlorophyll and carotenoids. A possible reason for the reduction of plant pigments under water stress is ROS production in thylakoids (Czyczyło-Mysza and Myśków, 2017). It also improves plant water relations and increases nutrient uptake, which leads to higher chlorophyll synthesis, and thus increases gas exchange under water stress conditions (Yang *et al*., 2020). Previous studies have also shown similar results. Recently, Tomášková *et al*. (2020) evaluated the effect of SAP treatments with sawdust, organic fertilizer, compost, wheat straw, subsoil, and subsoil with a cobble cover on the survival, growth, and physiological traits of 20 tree species. They suggested that all treatments significantly improved the performance of water-sensitive species. It was also stated that SAP efficacy depends on tree species' susceptibility to environmental stresses.

EL is a valuable indicator of plant tolerance to biotic and abiotic stress. According to the results, water stress significantly increased ion leakage in plants compared to control plants (100% FC). However, SAP levels mitigated EL of leaves in most treated plants. A minimal amount of SAP (1 g kg^{-1} soil) remarkedly reduced leaf EL at 50% FC of about 28.64% compared to non-SAP treatments. Several studies have indicated that SAP could maintain membrane integrity and prevent oxidative stress by reducing moisture fluctuations (Su *et al*., 2017; Kenway *et al*., 2018; Li *et al*., 2018).

Decreased leaf RWC due to water-deficit stress is associated with decreased soil moisture. These conditions cause the closure of stomata to prevent water wastage. Closure of the stomata is due to abscisic acid, produced in the roots under water stress and accumulating in the stomata cells, causing the stomata to close to prevent water wastage. Applying SAPs can significantly retain moisture surrounding roots and increase the tissue RWC (Kenway *et al*., 2018). Similarly, many studies demonstrated that the addition of SAPs to the rhizosphere of plants could prolong the interval between irrigations by retaining more water (Hüttermann *et al*., 1999; Abedi-Koupai *et al*., 2006; Fallahi *et al*., 2015; Khodadadi-Dehkordi, 2017; Lertsarawut *et al*., 2021).

Regardless of SAP treatment, MDA content significantly increased by reducing irrigation levels. MDA content seems to be more affected by water-deficit stresses. A notable increase of about 123.18% by reducing the irrigation level from 100% FC to 50% FC was found. However, SAP addition had a more pronounced effect on decreased MDA content in plants exposed to low and no stress than mild stress. A significant reduction of about 28.11% (1 g kg^{-1} SAP) and 41.35% (2 g kg^{-1} SAP) was found compared to non-SAP treatment under 100% and 75% FC, respectively (Fig. 1B). Therefore, higher levels of SAP may be more efficient in improving young myrtle plants exposed to mild and severe water-deficit stresses. These results agree with Basak (2020), who reported that applying SAP did not provide any oxidative stress and reduced the production of free radicals, which ultimately led to a reduction in MDA and proline contents. MDA is a product of cell membrane lipid oxidation, which is a good indicator of the degree of oxidative damage to lipids under stress. Increased MDA content indicates that water stress can lead to lipid oxidation of cell membranes by producing ROSs. SAP declined MDA accumulation due to its positive effect on soil moisture retention and available water supply. Thus, low MDA in the plants indicates that appropriate conditions have been provided, and oxidative stress has been adjusted to applying SAP treatments (Kenway *et al*., 2018).

Starch is a critical molecule in mediating plant responses to abiotic stresses, such as water stress, high salinity, or extreme temperatures. In these conditions, photosynthesis may be potentially impaired, so plants remobilize starch as a source of energy and carbon to continue growth and development. The released sugars and other derived metabolites support plant growth under stress, and function as osmoprotectant and compatible solutes to mitigate the negative effect of the stress (Krasensky and Jonak, 2012).

Most previous studies have shown the depletion of starch and the increase of TSSs under abiotic stresses, especially water stress. There were different responses to starch accumulation in plants under various stresses. In the present study, TSSs and starch content increased by progressing water-deficit stress. Sugars are also derived from photosynthetic carbon assimilation or accumulate due to decreased demand resulting from limited growth (Thalmann and Santelia, 2017). Some studies also reported an increase in starch accumulation under stress, including *Arabidopsis* sp. (Skirycz *et al*., 2010), *Chlamydomonas reinhardtii* (Siaut *et al*., 2011). The reason for this discrepancy is unclear. However, it has been proposed that plant non-structural carbohydrates (NSC) or depletion under water stress depends on species-specific strategies (Nardini *et al*., 2016; Thalmann and Santelia, 2017).

CONCLUSION

In summary, low levels of SAP could improve the physiological and biochemical characteristics of young myrtle plants at all irrigation levels. Results showed that SAP (1g kg^{-1} soil) improved physiological and biochemical characteristics such as RWC, total carotenoid, EL, MDA, TSSs, and starch content in all irrigation levels. While, SAP (2 g kg^{-1} soil) had an improved effect on other characteristics, including Pn, E, and total Chl. This method is a cost-effective, easy-to-apply technique that protects young plants during harsh conditions. Therefore, SAPs can develop green space in arid and semi-arid regions as well as with restoration projects of young myrtle and other same plants.

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