EFFECTS OF SUPER ABSORBENT POLYMER ON THE PHYSIOLOGICAL CHARACTERISTICS AND DROUGHT RESISTANCE OF BERMUDAGRASS [*CYNODON DACTYLON* (L.) PERS.] SEEDLINGS

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Abstract. *Cynodon dactylon* (L.) Pers. seedlings was used as test material and applied five SAP concentrations: 0.00%, 0.10%, 0.20%, 0.30%, and 0.40%. The effects of the five SAP treatments on the physiological characteristics of *C. dactylon* seedlings under two water supply conditions were studied and a comprehensive drought resistance evaluation method was used to analyze and evaluate the drought resistance of *C. dactylon*. Results: (1) the concentrations of photosynthetic pigments, including Chlorophyll a, Chlorophyll b, and Chlorophyll a+b were all higher than those in the CK group when the SAP concentration was 0.10%–0.30%; (2) The malondialdehyde, superoxide dismutase, peroxidase, and catalase activities in the leaves of *C. dactylon* treated with SAP were all altered, and the concentrations of MDA were higher than those in the CK group, indicating that applying SAP could minimize the environmental stress experienced by *C. dactylon*. (3) The addition of SAP could improve the drought resistance of *C. dactylon* seedlings. Under adequate water conditions, the order of drought resistance of *C. dactylon* under the five SAP concentrations would be 0.30% > 0.10% > 0.20% > 0.40% > 0.00%, and under limited water conditions, the order would be 0.20% > 0.30% > 0.40% > 0.10% > 0.00%.
Keywords: *super absorbent polymer concentrations, Cynodon dactylon, (L.) Pers, physiological ecological characteristics, evaluation of drought resistance, under adequate and limited water supply*

Abbreviations: SAP: super absorbent polymer; *C. dactylon*: *Cynodon dactylon*, (L.) Pers; SOD: superoxide dismutase ($U \cdot g^{-1}$); POD: peroxidase ($U \cdot (g \cdot min^{-1})$); CAT: catalase ($U \cdot g^{-1}$); MDA: malondialdehyde ($nmol \cdot g^{-1}$); Chl-a: chlorophyll a ($mg \cdot g^{-1}$); Chl-b: chlorophyll b ($mg \cdot g^{-1}$); Chl a+b: the total chlorophyll content ($mg \cdot g^{-1}$); Chla/b: chlorophyll a/b ($mg \cdot g^{-1}$); Car: carotenoid ($mg \cdot g^{-1}$)

Introduction

Cynodon dactylon (L.) Pers., which is widely distributed globally but occurs predominantly in tropical and warm temperate regions (Zeng and Li, 2019; Hameed et al., 2008), is a perennial herb in the genus *Eragrostoideae* Pilger (Jayanthi et al., 2016). Because of its low height, strong breeding capacity, and drought, salinity, and heavy metal resistance (Archer and Caldwell, 2004; Kim et al., 2009; Hameed et al., 2008; Xie et al., 2016), it is widely used in soil and water conservation (Srivastava and Singh, 2012), ecological restoration, and green building (Singh et al., 2013; Leung et al., 2015). In China, *C. dactylon* is distributed in areas with varying rainfall from the north to the south. Moisture conditions influence plant survival and growth, and excessive water or drought would affect plant morphology and physiology (Hu et al., 2014).

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Super absorbent polymer (SAP) is a safe and harmless polymer compound (Suresh et al., 2018). It can absorb and hold water weighing more than a hundred or even a thousand times its own weight (Zohuriaan-Mehr and Kabiri, 2008). Therefore, it could be used to improve soil structure, enhance soil water retention, reduce soil moisture loss, improve the emergence rates of plants and drought tolerance (Arbona et al., 2005; Yang et al., 2014), and promote aboveground and belowground growth (Islam, et al., 2011). Appropriate SAP concentration selection is very critical. A too low dosage would not achieve the desired effects, while a too high dosage would affect soil permeability severely (Li et al., 2019), which would, in turn, result in respiration challenges or even root rot.

In recent years, numerous studies have explored the effects of SAP on agricultural applications, soil properties, plant growth and physiology, and its influence on plant production (Fernando et al., 2017; Tongo et al., 2014; Karimi et al., 2009; Wu et al., 2018). However, studies on the physiological characteristics of *C. dactylon* under different SAP concentrations and moisture conditions have not been conducted. And whether the application of SAP to *C. dactylon* can also promote the development of aboveground part and root system is less studied. Therefore, the present study, five SAP concentrations (0.00%, 0.10%, 0.20%, 0.30%, and 0.40%) were applied to soil under two moisture conditions (adequate and limited water supply) to investigated the effects of SAP on the physiological characteristics and drought resistance of *C. dactylon* seedlings under different water availability. In addition, we explore the optimal SAP concentrations for the growth of *C. dactylon*, which could offer a theoretical basis for the application of SAP in the production and exploitation of *C. dactylon*.

Materials and methods

Experimental design

The experiments used SAP manufactured by Henan God biological science and technology, which exhibits the highest water holding capacity, at 300–500 times the SAP weight. The experimental matrices were mixed at a 1:1:1 volume ratio, and the soil, sand, and straw ash in their natural states were mixed in an experimental basin with a height of 13 cm, a length of 35 cm, and a width of 26 cm, and the weight of each substrate was 8 ± 0.5 kg. The concentration of SAP was mixed with the test substrate based on mass ratios of 0.00% (the CK group), 0.10%, 0.20%, 0.30%, and 0.40%. Before planting *C. dactylon* stolons, they were processed into 1-3 cm (2 buds) segments, placed in a basin filled with distilled water, soaked for 0.5 h, seeded by layering every pot planting 30 segment, covering 1 cm, dealing with repeated 3 times in each group. The experiments were carried out from March to May 2016 in a sun culture room at the Key Laboratory of Poyang Lake Wetland and Watershed Research, Ministry of Education, Nanchang, Jiangxi, China, lasted for 60 days. Water treatments were divided into a limited water supply group and an adequate water supply group. In the limited water supply group, the plants were watered with 150 ml every 20 days, while in the adequate water supply group, the plants were watered with 150 ml every 4 days. The soil moisture concentrations in the two treatment groups were measured using a HH2 soil moisture meter (Delta-T, Burwell, Cambridge, UK) every 3 days. The average soil moisture concentrations in the two groups were 28.5 ± 3% and 10.5 ± 5%.

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Determination of physiological indices of seedlings

At the end of the experiments, the seedlings were harvested from the pots, washed with distilled water, and dried. The fresh plant leaves were collected to measure the physiological indices of the seedlings. The physiological indices were determined according to Wang (2006). Chlorophyll concentrations and Carotenoid (Car) concentrations were determined following extraction with 95% ethanol and spectrophotometry. Superoxide dismutase (SOD) concentrations were determined using the nitrogen blue tetrazolium method, while peroxidase (POD) concentrations was determined by measuring guaiacol peroxidase activity using guaiacol. Catalase (CAT) concentrations were determined using the potassium permanganate titration method, and malondialdehyde (MDA) concentrations were measured using the thiobarbituric acid method (Wang., 2006).

Comprehensive evaluation of drought resistance

Analysis of drought resistance in *C. dactylon* was based on the measured physiological indicators. Principal component analysis was adopted for performing dimensionality reductions on the original indices, and *Equation 3* was used to determine the percentages of each factor. Subsequently, *Equations 1, 2,* and *4* were used for the evaluation of each synthetic index of drought resistance, in addition to SOD, POD, and MDA in the anti-subordinate function analysis, the remaining indexes using the method of subordinate function. Eventually let every comprehensive index can represent the difference basically between the original.

(1) Membership function analysis (Wang et al., 2013):
Membership function values:

$$R(X_i) = (X_i - X_{min})/(X_{max} - X_{min}) \quad \text{(Eq.1)}$$

Anti-membership function values:

$$R(X_i) = 1 - (X_i - X_{min})/(X_{max} - X_{min}) \quad \text{(Eq.2)}$$

Where X_i is the index value, and X_{min} and X_{max} are the minimum and maximum values of any indices of the test materials.

(2) Weight calculation:

$$W_i = \frac{P_i}{\sum_{i=1}^{n} P_i} \quad i = 1, 2, \ldots n \quad \text{(Eq.3)}$$

Where W_i is the important degree of the ith comprehensive index in all indices, and P_i is the contribution rate of the ith comprehensive index.

(3) Evaluation of drought resistance:

$$D = \sum_{i=1}^{n} \left[R(X_i) \times W_i \right] \quad i = 1, 2, \ldots n \quad \text{(Eq.4)}$$

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Where D value is the comprehensive evaluation value of plant drought resistance calculated at the concentration of different super absorbent polymer concentrations.

Statistics analysis

Data analysis was completed by MS Excel 2010 (Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics 21.0 (IBM Corp., Armonk, NY, USA). The effects of different SAP treatments on physiological ecological characteristics of *C. dactylon* seedlings were analyzed using one-way analysis of variance, and the differences between indices in different groups were tested using the least significant difference test.

Results

Effects of different SAP concentrations on chlorophyll concentration in C. dactylon leaves

The concentrations of chlorophyll a (Chl-a), chlorophyll b (Chl-b), chlorophyll a+b (Chl a+b), and carotenoid (Car) increased significantly after the application of reasonable concentrations of SAP under the two water conditions. Under the adequate water supply condition, the concentrations of Chl-a (10.646 ~11.704 mg·g^{-1}) and Chl-b (4.192 ~ 4.811 mg·g^{-1}) were significantly lower in the leaves of *C. dactylon* treated with different SAP concentrations, which were higher than the concentrations in the CK group and were the highest at the 0.30% SAP concentration. Chl-a, Chl-b, and Chla+b concentrations increased by 22.41%, 22.09%, and 23.74%, respectively. In addition, the Car concentration at the 0.40% SAP concentration (1.767 mg·g^{-1}) was the highest increased compared to the CK group (0.990 mg·g^{-1}), which represented a 43.95% increase. Under limited water conditions, the concentrations of Chl-a, Chl-b, Chl a+b, and Car first increased and then decreased with an increase in SAP concentration. When the SAP concentrations were 0.20%–0.30%, the concentrations of all the indices were higher than in the CK group. The concentrations of Chl-a, Chl-b, and Chl a+b were the highest at the 0.20% SAP concentration, which represented 15.53%, 10.31%, and 14.08% increases, respectively. However, the Car concentrations in all the treatment groups were the highest at the 0.30% SAP concentration (2.001 mg·g^{-1}), compared with the CK group (1.567 mg·g^{-1}), which represented a 21.71% increase compared to the CK group.

Under the adequate water supply and the limited water supply conditions, the chlorophyll a/b concentrations between the treatment groups treated with SAP varied minimally. However, with an increase in SAP concentration, chlorophyll a/b content of sufficient water supply (2.449 ~ 2.593 mg·g^{-1}) and limited water supply (2.497 ~ 2.649 mg·g^{-1}) are both higher than CK group (2.360 and 2.451 mg·g^{-1}). Compared to the CK group, the chlorophyll a/b concentrations in the adequate water supply treatment group increased by 3.63%, 7.06%, 2.98%, and 8.96%, under the 0.10%, 0.20%, 0.30%, and 0.40% SAP concentrations, respectively. The greatest increase was an increase of 8.96%, under the 0.4% SAP concentration. In the limited water supply treatment groups, chlorophyll a/b concentrations increased by 1.81%, 5.82%, 7.44% and 5.25%, under the 0.10%, 0.20%, 0.30%, and 0.40% SAP concentrations, respectively, with the maximum increase observed under the 0.30% SAP concentration, which was 7.44%. According to the results, the increase of the chlorophyll a/b concentrations in the leaves of the seedlings was not obvious by applying SAP under the two moisture conditions (*Table 1*).

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***Table 1.** Effects of SAP on chlorophyll concentration in C. dactylon leaves (mg•g^{-1})*

Treatment (%)	Sufficient water supply					Limited water supply				
	Chl-a	**Chl-b**	**Chl a+b**	**Car**	**Chl a/b**	**Chl-a**	**Chl-b**	**Chl a+b**	**Car**	**Chl a/b**
CK	8.847a	3.748a	12.595a	0.990a	2.360a	11.767a	4.799a	16.566a	1.567a	2.451a
0.10	11.487b	4.690b	16.177b	1.376b	2.449a	13.153b	5.268b	18.421b	1.920b	2.497a
0.20	10.646c	4.192c	14.838c	1.526b	2.539a	13.930c	5.351b	19.281c	1.923b	2.603a
0.30	11.704b	4.811b	16.515d	1.415b	2.433a	12.991b	4.904c	17.895d	2.001b	2.649a
0.40	10.904c	4.206c	15.110e	1.767c	2.593a	11.388d	4.401d	15.788e	1.749ab	2.588a

The letters represent the differences within the group. The same letters represent no differences. The different letters represent differences ($p < 0.05$)

Effects of different SAP concentrations on C. dactylon leaf enzyme activity

The concentrations of SOD and POD in the leaves of *C. dactylon* were significantly lower than those in the CK group, and CAT concentrations were higher than those in the CK group under the two moisture conditions. There were differences in the change range of each enzyme activity index. Under the adequate water supply condition, the concentrations of SOD in each group were relatively low, ranging from 146.48 to 156.72 U·g^{-1}, and the lowest value was observed at the 0.30% SAP concentration treatment, which was a 16.67% decrease compared to the CK. In addition, the concentrations of POD in the leaves of *C. dactylon* varied considerably, and the values in each group ranged between 20.67 and 53.34 U·(g·min^{-1}). In the treatment group with the 0.30% SAP concentration, the POD concentration was 75.60% lower than the concentration in the CK group. In addition, CAT activity was higher than in the CK group, with the highest value observed under the 0.30% SAP concentration, which was a 67.42% increase compared to the value in the CK group.

Under the limited water supply condition, the concentrations of SOD and POD decreased with an increase of the concentration of SAP, and the lowest values were observed under the 0.40% SAP concentration, which were 21.36% and 46.61% lower, respectively, than the concentration in the CK group. CAT activity was higher than in the CK group, with the highest value observed under the 0.40% SAP concentration, which a 78.55% increase compared to the value was observed in the CK group (*Fig. 1*).

Effects of different SAP concentrations on MDA content in C. dactylon leaves

Under water stress conditions, plants will increase MDA concentrations, which could have toxic effects on cell membranes. Under both moisture conditions, the changes in MDA concentrations in each group were lower than the changes in the CK group under the different SAP concentrations. Under adequate water supply, with an increase in the concentrations of the water-absorbing agent, MDA concentrations decreased by 25.77%, 29.36%, 11.67%, and 8.69%, under 0.10%, 0.20%, 0.30%, and 0.40% SAP concentrations, respectively, compared to those in the CK group. The lowest value was observed under the 0.20% SAP concentration.

Under the limited water supply conditions, the MDA concentrations in the 0.10%, 0.20%, 0.30%, and 0.40% treatments decreased by 8.84%, 12.34%, 9.05% and 27.96%, respectively, compared to the CK group. In addition, the lowest MDA concentration was observed under the 0.40% SAP concentration. According to the results, the application of SAP could reduce the degree of stress on *C. dactylon* to minimize the accumulation of MDA, and, in turn, minimize potential cell membrane damage (*Fig. 2*).

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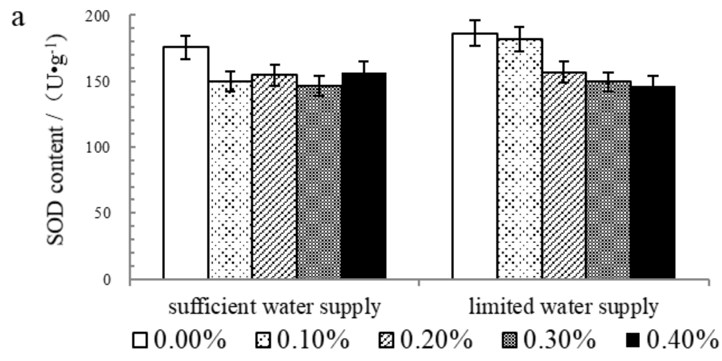
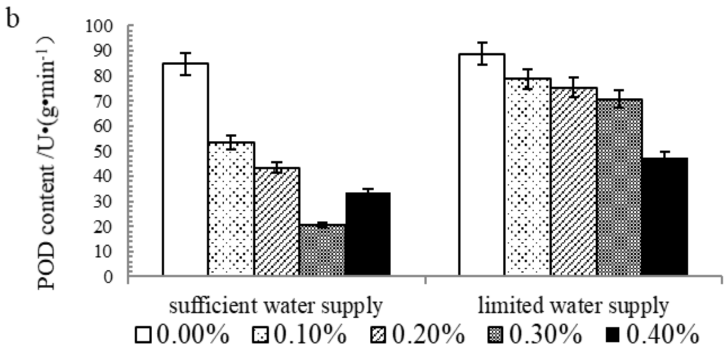
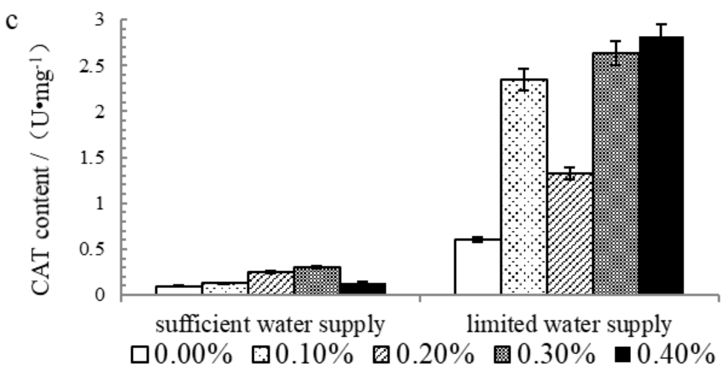


a: Effect of SAP on SOD in *C. dactylon* leaves



b: Effect of SAP on POD in *C. dactylon* leaves



c: Effect of SAP on CAT in *C. dactylon* leaves

Figure 1. *Effects of different SAP concentrations on C. dactylon leaf enzyme activity*

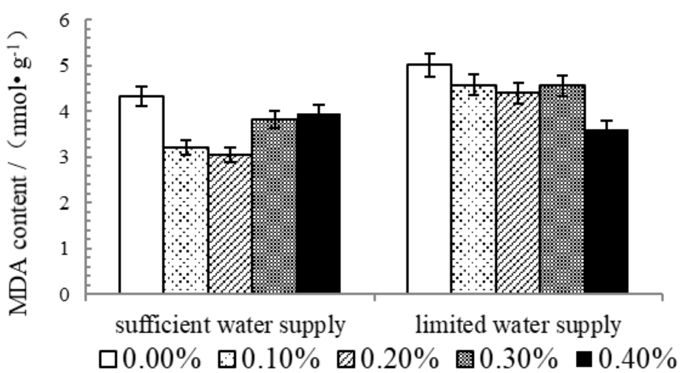


Figure 2. *Effect of different SAP concentrations on MDA in C. dactylon leaves*

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Effects of different SAP concentrations on drought resistance of C. dactylon seedlings

Under water stress conditions, different concentrations of SAP will influence plant morphology and physiology variably, in addition to the effects of interactions and influence of various indices. The fuzzy membership function method could simplify the interrelationships among various indices, reveal the linkages among the indices of drought resistance, avoid the one-sidedness associated with the single index, and evaluate the drought resistance of plants more comprehensively (Wang et al., 2013). The analysis of fuzzy membership function of drought resistance of *C. dactylon* was based on the analysis of 9 indices, including Chl-a, Chl-b, Chl a+b, Car, Chl a/b, SOD, POD, CAT, and MDA. To evaluate the drought resistance of *C. dactylon* under the different SAP concentrations, the comprehensive evaluation value was calculated. According to the results, adding SAP could improve the drought resistance of *C. dactylon* seedlings. In addition, under adequate water conditions, the optimal SAP concentrations for the growth of *C. dactylon* based on their impacts on drought resistance in the species would be in the order of 0.30% > 0.10% > 0.20% > 0.40% > 0.00%. Conversely, under limited water conditions, the order would be 0.20% > 0.30% > 0.40% > 0.10% > 0.00% (*Table 2*& *3*).

Table 2. *The membership function and comprehensive evaluation of drought tolerance index for sufficient water supply of C. dactylon seedlings*

Determination index	**Treatment**					**Weight**
	CK	**0.10%**	**0.20%**	**0.30%**	**0.40%**	
Chl-a	0.000	0.924	0.630	1.000	0.720	0.356
Chl-b	0.000	0.886	0.418	1.000	0.431	0.356
Chl a+b	0.000	0.914	0.572	1.000	0.642	0.356
SOD	0.000	0.877	0.726	1.000	0.651	0.356
POD	0.000	0.49	0.646	1.000	0.802	0.356
Car	0.000	0.496	0.690	0.547	1.000	0.291
Chl a/b	0.000	0.382	0.771	0.312	1.000	0.291
CAT	0.000	0.146	0.790	1.000	0.185	0.199
MDA	0.024	0.462	1.000	0.209	0.000	0.154
Comprehensive evaluation value	0.004	1.812	1.802	2.261	1.774	
Order	5	2	3	1	4	

Discussion

Response of photosynthetic pigments of C. dactylon seedlings to different SAP concentrations

When plants are under water stress, the cell membrane system will be impaired, which would lead to the damage of the chloroplast ultrastructure and chlorophyll degradation, in addition to a decrease in chlorophyll concentration (Manuchehri and Salehi, 2014). According to the results of the present study, the concentrations of photosynthetic pigments in *C. dactylon* seedlings, including Chl-a, Chl-b, and Chl a+b, were all higher than the concentrations in the CK when the SAPs with 0.10%–0.30% concentrations were applied under the two moisture conditions. The results indicated that in the process of SAP regulating soil water, and in turn plant growth, soil water content was not lower than

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the plant stress threshold, so that the chlorophyll concentration was not affected, and over time, SAP could enhance plant chlorophyll concentration indirectly to ensure that plants exploit the available light energy efficiently (Khadem et al., 2010). Similarly, the present study revealed that applying SAP could improve the concentrations of Car and chlorophyll a/b in *C. dactylon*. The increase in Car concentrations in could facilitate *C. dactylon* to tolerate stress induced by the production of lipid peroxides by membrane lipids (Jia et al., 2009). Zhang and Tan (2001) also suggested that drought resistance in species could be assessed based on a decrease in the Chl a/b ratio (Zhang and Tan, 2001). In the present study, the Chl a/b ratio in each SAP group was higher than in the CK group, and the variations were relatively low, which indicated that SAP application could promote *C. dactylon* seedling growth.

Table 3. *The membership function and comprehensive evaluation of drought tolerance index for limited water supply of C. dactylon seedlings*

Determination index	**Treatment**					**Weight**
	CK	**0.10%**	**0.20%**	**0.30%**	**0.40%**	
Chl a/b	0.000	0.23	0.768	1.000	0.69	0.364
MDA	0.000	0.316	0.441	0.324	1.000	0.364
SOD	0.000	0.110	0.746	0.929	1.000	0.364
POD	0.000	0.242	0.323	0.435	1.000	0.364
Chl-a	0.149	0.695	1.000	0.631	0.000	0.282
Chl-b	0.42	0.912	1.000	0.53	0.000	0.282
Chl a+b	0.223	0.754	1.000	0.603	0.000	0.282
Car	0.000	0.812	0.819	1.000	0.419	0.282
CAT	0.000	0.787	0.328	0.918	1.000	0.163
Comprehensive evaluation value	0.022	0.151	0.205	0.195	0.191	
Order	5	4	1	2	3	

Responses of C. dactylon antioxidant enzymes to different SAP concentrations

Under normal conditions, plants can eliminate the generated free radicals (O^{2-}) through their free radical-clearing systems, so that the production and elimination of reactive oxygen species in tissues are maintained at equilibrium states (Peng et al., 2002). When the equilibrium is lost, excess free radicals could trigger membrane lipid peroxidation and cause cell membrane system damage (Porcel et al., 2003). In the present study, the activities of membrane lipid peroxidation products, MDA, and protective enzymes, SOD, POD, and CAT, in *C. dactylon*, all changed following treatment with SAP under the two moisture conditions. The results showed that the SOD and POD activities in *C. dactylon* leaves treated with the SAP were lower than those in the CK, while CAT activity was higher than that in the CK. In addition, the MDA concentrations were also lower than in the CK. The reasons for the changes above could be as follows. Under the limited water supply condition, the SAP could reduce soil drought by regulating soil moisture availability, which is beneficial to the synergistic effects of SOD, POD, and CAT in *C. dactylon* leaves, to ensure that the free radicals are maintained at low levels to minimize the damage to the plant leaf membrane systems, similar to the production of lipid peroxidation product (MDA) to minimize the damage caused by drought on plants (Dacosta and Huang, 2007; Reddy et al., 2008). However, under adequate water conditions, sufficient water also led to shifts in the protective enzyme activities of

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seedlings, potentially due to the ability of SAP to absorb water and decrease the rate of evaporation of soil moisture, in turn, increasing the soil moisture concentrations under the adequate water supply condition, leading to a reduction in soil permeability, a decrease in nutrient-supply capacity, an increase in osmotic adjustment in leaves of seedlings, and eventually an increase in the concentrations of osmoregulation substances, and changes in protective enzyme activity and the lipid peroxidation products of leaves of seedlings leaves, as reported by Yang (2017).

C. dactylon drought resistance under different SAP concentrations

Under water stress, different SAP concentrations will influence plant morphology and physiology differently. This is consistent with the research on Wheat (*Triticum aestivum* L.) seedling conducted by Guan and Wu (2010). In addition, the indicators would interact and influence each other. A single drought resistance index may not comprehensively reflect the capacity of a plant to adapt to drought conditions (Wang et al., 2011). However, the values obtained from membership function analysis are average values, which could address the shortcomings of evaluations based on a few indicators. In addition, since the averages are whole numbers, the drought resistance indices become comparable. In the present study, under adequate water conditions, the five SAP concentrations were appropriate for *C. dactylon* seedlings in the order of 0.30% > 0.10% > 0.20% > 0.40% > 0.00%. Conversely, under limited water availability conditions, the order was 0.20% > 0.30% > 0.40% > 0.10% > 0.00%. The findings demonstrate that adding SAP could improve *C. dactylon* seedling drought resistance and are consistent with the results of previous studies where the addition of SAP to soil enhanced plant drought resistance (Qados, 2015; Yang et al., 2017).

Conclusion

In summary, the addition of SAP under different water conditions could improve the drought resistance of *C. dactylon* seedlings. The optimal SAP concentration was 0.30% under the adequate water conditions, and 0.20% under the limited water conditions. Therefore, in different areas where *C. dactylon* is adopted for greening, water conservation efforts should consider the 0.20-0.30% SAP concentration range for SAPs, which is conducive for the establishment of lawn and would extend the lawn irrigation time. In the present study, we investigated the effects of SAP on the physiological characteristics and drought resistance of *C. dactylon* seedlings under different water availability status. The effect of SAP on the entire life cycle of *C. dactylon* requires further investigation.

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