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Exogenously spermidine alleviates damage from drought stress in the photosystem II of tall fescue

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Abstract: Drought stress is one of the major limiting factors to crop productivity around the globe. It has been well documented that spermidine (Spd) plays an important key role in plant growth and development, especially in the defense response to stress. The objective of this study was to explore the effect of Spd on protecting photosynthetic apparatus in tall fescue under drought stress. Spd application significantly improved the OJIP (fluorescence rise kinetics O-J-I-P) curve compared to non-Spd application during drought. Exogenous Spd exhibited higher F_J (fluorescence value at the J-step (2 ms) of OJIP) and F_P (maximal recorded fluorescence intensity, at the peak P of OJIP) than non-Spd application. Moreover, normalised total complementary area (S_m) and the number of Q_A (primary quinone acceptor of PS II) reduction events (N) significantly reduced after the application of Spd in tall fescue under drought stress. In terms of quantum yields and efficiencies and specific energy fluxes, exogenous Spd notably decreased the values of efficiency of electron transfer from Q_B (secondary quinone acceptor of PS II) to PSI acceptors (δR_0), absorption flux per RC (ABS/RC) and trapping flux per RC (TR_0/RC) compared to the non-Spd application without watering. All the above suggested that exogenous Spd facilitated the photosynthetic system of tall fescue in drought. These observations involved in the electron transport capacity of photosystem II assist in understanding better the protective role of exogenous Spd in tall fescue under drought stress.

Keywords: abiotic stresses; plant growth regulator; *Festuca arundinacea* Schreb.; chlorophyll *a* fluorescence transient

Drought is a major environmental problem in crop yield and agricultural productivity worldwide (Kaya et al. 2019, Cohen et al. 2021) and can significantly affect the morphology, physiology and biochemical processes of plants (Ahmad et al. 2018, Kaya et al. 2020, Kosar et al. 2021). With the number of drought episodes increasing with climate change across most regions worldwide (Williams and de Vries 2020), researching on alleviating drought-induced damage is critical to facilitate maintenance of the productivity of managed and natural ecosystems (Farooq et al. 2020).

Photosynthesis is the most basic life activity of plants and a central, primary response of plants to drought stress (Wang et al. 2020). Being the core element of photosynthesis, photosystem II (PSII) is more sensitive to stress than photosystem I (PSI) (Wang et al. 2017). The excess light energy produced by photosynthetic electron transport under stress can cause active oxygen burst and photooxidative damage of the PSII core complex D1 protein (D'Alessandro and Havaux 2019). If it can not be repaired in time, sustained photoinhibition leads to reduced PSII photochemical activity (Huang et

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al. 2018). In general, the photochemical activity of PSII was studied from the transport and utilisation of photosynthetic electrons included the aspect of absorption, capture, transmission and dissipation of light energy by rapid chlorophyll (*Chl*) fluorescence kinetics and *Chl* fluorescence quenching analysis (Živčák et al. 2008, Kalaji et al. 2014, Dąbrowski et al. 2019).

Spermidine (Spd) is one of the three major polyamines (PAs), which are new type of low-molecular-weight plant growth regulators. PAs are implicated in plant growth and developmental processes, as well as in defence responses to biotic and abiotic stresses (Patel et al. 2020). Recently, the application of exogenous Spd has attracted extensive attention. Exogenously applied Spd can alleviate growth inhibition and thylakoid membrane photodamage caused by salinity-alkalinity stress in tomato seedlings (Hu et al. 2014). Furthermore, under drought stress, exogenous Spd application protects maize seedlings from photoinhibition and photodamage (Li et al. 2018). Exogenously applied Spd also improves drought tolerance in creeping bentgrass (Li et al. 2015). It was reported that exogenous Spd application elicited higher *Chl a* content and PSII activity than spermine or putrescine application in *Physcia semipinnata* under the UV-A (352 nm) stress (Unal et al. 2008). Furthermore, Spd is the most efficient of the three main PAs in restoring maximum photochemical efficiency (F_v/F_m) of thylakoids under low salt stress (Ioannidis and Kotzabasis 2007).

Tall fescue (*Festuca arundinacea* Schreb.) is a cool-season perennial species of forage and turf grass widely used in the temperate zones (Takamizo and Sato 2020). Owing to its well-developed root system and wide adaptability to different climates and environments, tall fescue is widely used as a model species (Zhu et al. 2018). Although tall fescue is more adaptable than other cold-season lawns, drought is still the main abiotic stress limiting worldwide use (Chen et al. 2018).

In recent years, researches on the response of tall fescue to stress have mainly focused on high temperature and salt stress (Wang et al. 2017). However, to the best of our knowledge, there is no report on the response of the photosynthetic characteristics of tall fescue with spermidine to drought stress. Therefore, the purpose of this study was to explore the protective function of Spd on the photosynthetic apparatus of tall fescue under drought stress and the regulation of its photosynthetic performance.

MATERIAL AND METHODS

Plant materials and growth conditions. Seeds of commercial tall fescue "Huntdog 5" were selected according to their uniformity and sown in plastic pots (13 cm in diameter and 11 cm in depth) filled with matrix (brown coal soil:sand = 1:1). The plants were stored for 50 days in a greenhouse (day/night temperature: 24/20 °C, and average relative humidity of 70%) with a photoperiod of 16 h (light intensity: 300 μmol/m^2/s) to allow root and shoot growth. The plants were then watered every day until drainage occurred from the bottom, fertilised with half-strength Hoagland nutrient solution twice a week, and mowed at 7 cm above the matrix surface every week.

Reagent and drought-stress treatments. The plants were divided into four treatment groups: (1) drought stress, i.e., no irrigation until the matrix moisture was lower than 30% at the optimum temperature (Huang and Gao 2000); (2) drought-stressed plants treated with 0.4 mmol/L Spd; (3) drought-stressed plants treated with 0.8 mmol/L Spd; (4) drought-stressed plants treated with 1.2 mmol/L Spd. These concentrations of Spd (0, 0.4, 0.8 and 1.2 mmol/L) were selected according to previous experimental reports of tall fescue (Zhang et al. 2017). All treatments were performed using completely randomised experimental plants, with three replicates per group.

***Chl a* fluorescence transient.** *Chl* fluorescence is a non-invasive index for rapid evaluation of photosynthesis *in vivo*. *Chl a* fluorescence transient was measured by pulse amplitude modulation (PAM) fluorometer (PAM 2500, Heinz Walz GmbH, Effeltrich, Germany). After 30 min of dark adaptation, fully expanded third leaves (from bottom) were triggered with a red light at 3 000 μmol/m^2/s. The measurements were made between 10 μs and 320 ms. Each measurement was replicated at least three times.

The JIP-test. Based on the energy flux theory in biomembranes, JIP testing was used to analyse the OJIP transient parameters developed by Strasser (Strasser 1997, Chen et al. 2013). The energy flow begins with light absorption by PSII antenna pigments (ABS) and ends at the terminal electron acceptor (RE) on the PSI electron-acceptor side driven by PSI (Stirbet and Govindjee 2011). It represents the conversion of raw data into useful information regarding biophysical parameters, which quantify the energy flow through PSII (Shao et al. 2010). A typical JIP-test includes the process of changes from point O to point P. Fluorescence parameters were calculated using three replicates.

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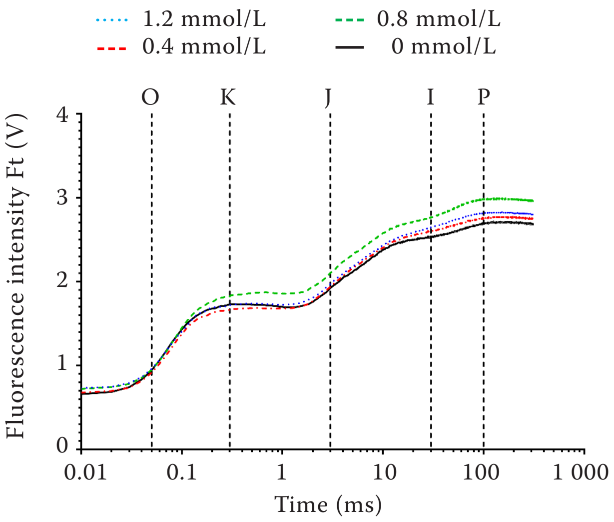

Figure 1. The effect of chlorophyll fluorescence transients (OJIP (fluorescence rise kinetics O-J-I-P) curve) in tall fescue upon the exogenous application of spera midine at different concentrations (0, 0.4, 0.8 and 1.2 mmol/L) under drought stress

Statistical analysis. The data were analysed using SPSS one-way analysis of variance for Windows® v. 18 (SPSS Inc., Chicago, USA). Statistically significant differences ($P < 0.05$) between the different treatments were calculated based on the Student's *t*-test. The data are presented as the mean ± standard error (SE) of at least three replicates.

RESULTS

Effects of Spd on tall fescue OJIP transients against drought stress. To study the role of Spd in tall fescue, the responses of *Chl a* fluorescence transients to drought at different concentrations of Spd were evaluated (Figure 1). Significantly different changes were observed in *Chl* fluorescence transients at different concentrations of Spd under drought stress. 0.4 mmol/L of Spd exhibited the best performance against drought stress by improving F_0 (fluorescence at time 20 µs after the onset of actinic illumination), F_K (fluorescence value at 300 µs), F_J (fluorescence value at the J-step (2 ms) of OJIP), F_I (fluorescence value at the I-step (30 ms) of OJIP) and F_P (maximal recorded fluorescence intensity, at the peak P of OJIP). The results show that Spd application significantly increased the OJIP curve compared with non-Spd application under drought stress. This indicates that under drought stress, Spd plays a positive role in the photosynthetic performance of tall fescue.

Effects of Spd on *Chl a* fluorescence basic parameters under drought stress. To investigate the effect of Spd on the photosynthetic system under drought stress, the parameters of OJIP transient curves were analysed using the JIP-test. The basic parameters of F_0, F_K, F_J, F_I, and F_P are plotted in Table 1. Data revealed that the three groups (F_0, F_K, and F_I) did not show remarkable differences among the treatments. However, the application of Spd significantly increased the F_J and F_P basic parameters under drought stress.

Effects of Spd on structural and functional parameters under drought stress. To further infer the structural and functional parameters of photosynthesis intall fescue, photosynthetic parameters were analysed (Figures 2 and 7). There were no significant differences in the values of F_v, V_k, V_j, V_i, M_0, and S_s between the control and treatment regimes under drought stress. It is noteworthy that S_m and N values were reduced after the application of Spd under drought stress. Compared to those observed in the non-Spd application, the values of S_m and N decreased by up to 12.37% and 16.91%, respectively, upon Spd application. S_m that is the normalised

Table 1. Basic parameters of OJIP (fluorescence rise kinetics O-J-I-P) transient curves extracted by the JIP-test (the analysis of strong actinic light-induced O-J-I-P transients)

Treatment	F_0	F_K	F_J	F_I	F_P
0 mmol/L	0.69 ± 0.02[a]	1.73 ± 0.05[a]	1.76 ± 0.06[b]	2.54 ± 0.14[a]	2.74 ± 0.11[b]
0.4 mmol/L	0.74 ± 0.01[a]	1.83 ± 0.04[a]	1.93 ± 0.01[a]	2.77 ± 0.04[a]	3.02 ± 0.03[a]
0.8 mmol/L	0.70 ± 0.03[a]	1.67 ± 0.07[a]	1.78 ± 0.05[b]	2.61 ± 0.08[a]	2.80 ± 0.08[ab]
1.2 mmol/L	0.75 ± 0.02[a]	1.72 ± 0.05[a]	1.82 ± 0.03[ab]	2.65 ± 0.04[a]	2.86 ± 0.04[ab]

The values in the table are means ± standard error of three independent biological replicates, and the different letters represent the significance of statistical analysis ($P < 0.05$; Duncan's test). F_0 – fluorescence at time 20 µs after the onset of actinic illumination; F_K – fluorescence value at 300 µs; F_J – fluorescence value at the J-step (2 ms) of OJIP; F_I – fluorescence value at the I-step (30 ms) of OJIP; F_P – maximal recorded fluorescence intensity, at the peak P of OJIP

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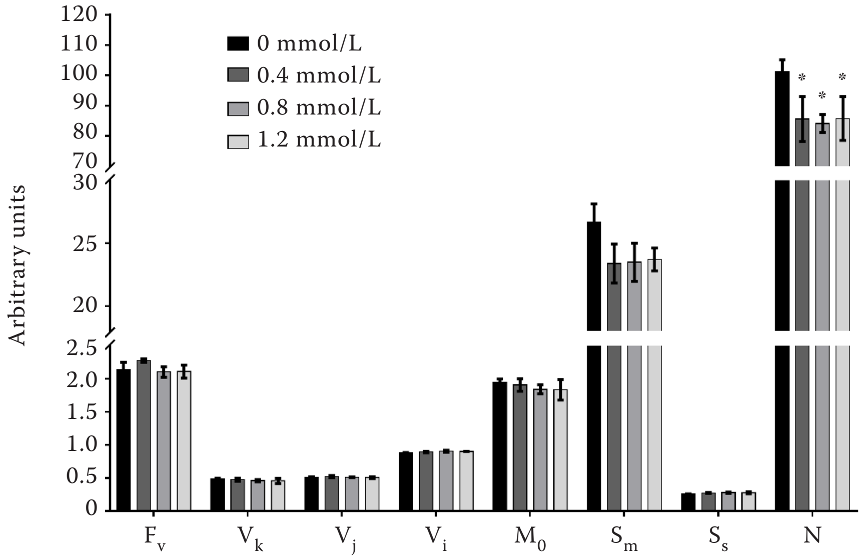

Figure 2. The effect of photosynthetic parameters analysed by the JIP-test (the analysis of strong actinic light-induced O-J-I-P transients) in tall fescue leaves upon the exogenous application of spermidine at different concentrations (0, 0.4, 0.8 and 1.2 mmol/L) under drought stress. F_v – aximal variable fluorescence; V_k – relative variable fluorescence at K step; V_j – relative variable fluorescence at J step; V_i – relative variable fluorescence at I step; M_0 – approximated initial slope (in/ms) of the fluorescence transient; S_m – normalised total complementary area above the OJIP (fluorescence rise kinetics O-J-I-P) transient; S_s – normalised total complementary area corresponding only to the O-J phase; N – number of redox cycles of Q_A (primary quinone acceptor of PS II). The values are means ± standard error of three independent biological replicates. *$P < 0.05$ among the treatments by Student's *t*-test

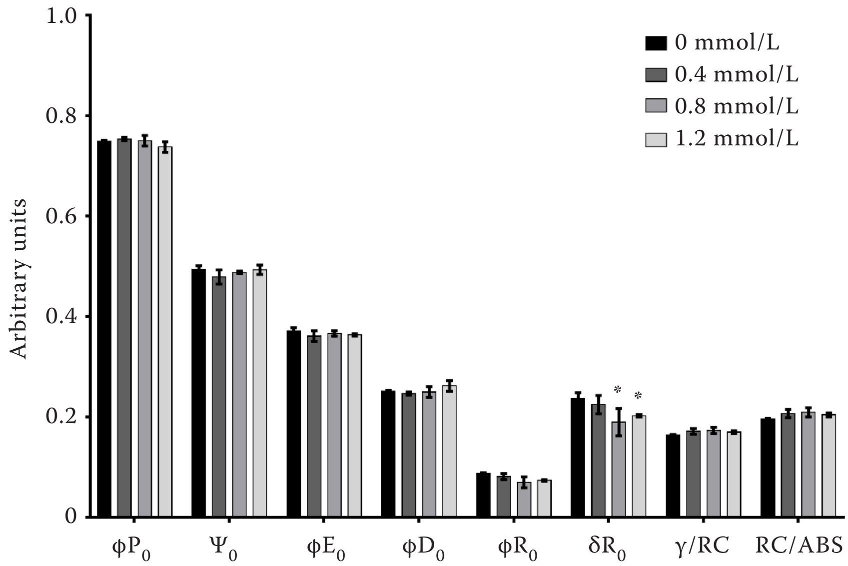

Figure 3. The effect of quantum yield and efficiency were analysed by the JIP-test (the analysis of strong actinic light-induced O-J-I-P transients) in tall fescue upon different concentrations of exogenous spermidine (0, 0.4, 0.8 and 1.2 mmol/L) under drought stress. ϕP_0 – maximum quantum yield of primary photochemistry (at $t = 0$); Ψ_0 – efficiency/probability that an electron moves further than Q_A (primary quinone acceptor of PS II); ϕE_0 – quantum yield of electron transport (at $t = 0$); ϕD_0 – quantum yield (at $t = 0$) of energy dissipation (at $t = 0$); ϕR_0 – quantum yield for reduction of end electron acceptors at the PSI acceptor side; δR_0 – efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE); γ/RC – probability that a PSII *Chl* molecule functions as RC; RC/ABS – Q_A-reducing RCs per PSII antenna *Chl* (reciprocal of ABS/RC). The values are means ± standard error of three independent biological replicates. *$P < 0.05$ among the treatments by Student's *t*-test

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total complementary area above the OJIP transient represents the size of the plastoquinone pool or the ability of the electron transmitter to accept electrons after Q_A. N represents the number of redox cycles of Q_A. The reduction in both S_m and N values indicates that the subsequent electron transfer is restricted. These results suggested that Spd application under drought stress might lead to a photosynthetic electron transport traffic jam.

Effects of Spd on quantum yields and efficiencies parameters under drought stress. Some parameters of quantum yields and efficiencies were displayed (Figures 3 and 7). There was no significant difference in these parameters except for the value of δR_0 (efficiency of electron transfer from Q_B to PSI acceptors) after the application of Spd under drought stress. Moreover, a significant decrease of 20.05% was observed in the value of δR_0 following the application of 0.8 mmol/L of Spd under drought stress in comparison to that observed with the non-Spd application.

Effects of Spd on specific energy fluxes parameters under drought stress. The values of the fluorescence parameters with respect to specific energy fluxes were shown (Figures 4 and 7). In terms of specific energy fluxes, the values of ABS/RC and TR_0/RC following application of 0.8 mmol/L of Spd decreased by 6.10% and 6.38% compared with that observed in no Spd application under drought stress. The application of Spd tended to reduce the values of ET_0/RC, DI_0/RC and RE_0/RC under drought stress.

There were no significant differences in phenomenological energy fluxes (Van Heerden et al. 2004) parameters of RC/Cs_0, ABS/Cs_0, TR_0/Cs_0, ET_0/Cs_0, and DI_0/Cs_0 after the application of Spd under drought stress (Figures 5–7). Nevertheless, 0.8 mmol/L of Spd had a positive influence on PI_{ABS} and PIcs, which were notably higher than those observed with the non-Spd application under drought stress (Figures 6 and 7).

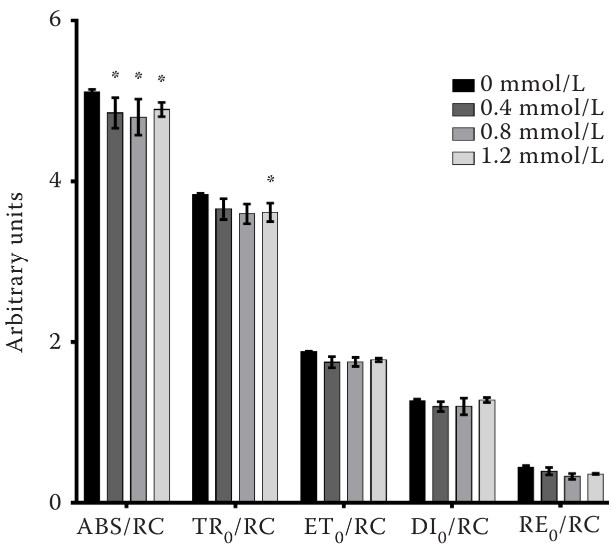

Figure 4. The effect of specific energy fluxes was analysed by the JlP-test (the analysis of strong actinic light-induced O-J-I-P transients) in tall fescue upon different concentrations of exogenous spermidine (0, 0.4, 0.8 and 1.2 mmol/L) under drought stress. ABS/RC – absorption flux (of antenna *Chls*) per RC (at $t = 0$); TR_0/RC – trapping flux (leading to Q_A reduction) per RC (at $t = 0$); ET_0/RC – electron transport flux (further than Q_{A-}) per RC (at $t = 0$); DI_0/RC – dissipated energy flux per RC (at $t = 0$); RE_0/RC – electron flux reducing end electron acceptors at the PSI acceptor side, per RC. The values are means ± standard error of three independent biological replicates. *$P < 0.05$ among the treatments by Student's t-test

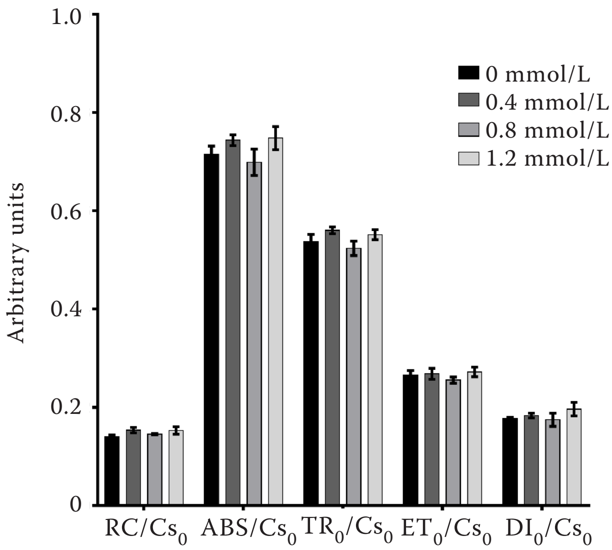

Figure 5. The effect of phenomenological energy fluxe were analysed by the JlP-test (the analysis of strong actinic light-induced O-J-I-P transients) in tall fescue upon different concentrations of exogenous spermidine (0, 0.4, 0.8 and 1.2 mmol/L) under drought stress. RC/Cs_0 – density of RCs (Q_A-reducing PSII reaction centers) (at $t = 0$); ABS/Cs_0 – absorption flux per CS, approximated by F_0 (at $t = 0$); TR_0/Cs_0 – trapped energy flux per CS (at $t = 0$); ET_0/Cs_0 – electron transport flux per CS (at $t = 0$); DI_0/Cs_0 – dissipated energy flux per CS (at $t = 0$). The values are means ± standard error of three independent biological replicates, and *$P < 0.05$ among the treatments by Student's t-test

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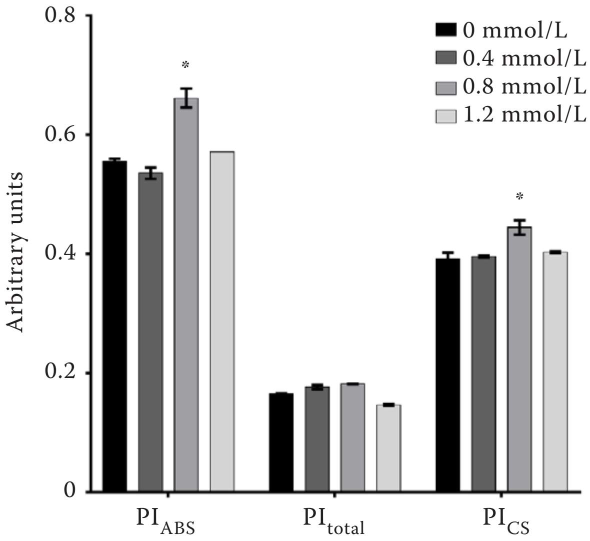

Figure 6. The effect of performance indexes was analysed by the JlP-test (the analysis of strong actinic light-induced O-J-I-P transients) in tall fescue upon different concentrations of exogenous spermidine (0, 0.4, 0.8 and 1.2 mmol/L) under drought stress. PI_{ABS} – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PI_{total} – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors; PI_{CS} – performance index on cross-section basis. The values are means ± standard error of three independent biological replicates. *$P < 0.05$ among the treatments by Student's *t*-test

DISCUSSIONS

Drought stress has emerged as a global concern, and effective strategies need to be formulated to prevent excessive losses in agricultural production (Begum et al. 2019). Drought stress causes various physiological interruptions and leads to senescence, which plays an important role in plant survival (Jan et al. 2019). In this study, we characterised the PSII photochemistry response to drought stress with different concentrations of Spd in tall fescue. We found that the exogenous application of Spd resulted in higher values of F_J and F_P and lower values of S_m, N, δR_0, ABS/RC and TR_0/RC than a non-Spd application under drought stress. These results indicated that exogenous Spd promoted the photosynthetic system under drought stress in tall fescue.

Photosynthesis is the process by which living plants absorb, capture, transfer, and store energy from the sun (Hniličková et al. 2017). In this process, the light energy is absorbed by a dense array of *Chl* molecules to the reaction center and then converted into chemical energy (Zhang et al. 2019). Photosystem II is one of the most sensitive processes to stress (Huang et al. 2020), which causes a series of changes due to drought. *Chl* fluorescence transients mainly reflect the changes in the structure and mechanism of photosynthesis in the original photochemical reaction (Zhang et al. 2017). Previous studies reported that the *Chl* fluorescence transient curve represented the sequential reduction of PSII electron acceptors (Najafpour and Allakhverdiev 2015). The O-J phase represents the reduction of Q_A on the PSII receptor side, which is driven by the original photochemical reaction and involves a single flow of Q_A; the J-P phase involves multiple circulations of Q_A. In the current study, no significant change in the O-J phase emerged. This may be due to the minor effect of Spd on the PSII donor side under drought stress. A significantly strengthened J-P phase was observed with Spd application under drought stress compared to that with the non-Spd application. Therefore, it is inferred that the main influence site of Spd under drought stress was located on the PSII acceptor side.

To verify these speculations, several parameters were analysed using the JIP test. As shown in Table 1, exogenous Spd significantly improved the F_J and F_P of tall fescue leaves under drought stress. Moreover, there was no obvious increase in F_0, F_K, and F_I compared to the non-Spd application under drought stress. These results indicated that exogenous Spd facilitated the photosynthetic system of tall fescue under drought stress. Similar results were also obtained for citrus seedlings (Khoshbakht et al. 2018), maize (Li et al. 2018), and tomato seedlings (Hu et al. 2014).

Plants are constantly exposed to drought stress leading to oxidative damage because of reactive oxygen species (ROS) production and accumulation (Raja et al. 2020). ROS accumulation in plants can trigger the loss of integrity of organelles, oxidation of cell components, and even lead to cell death (Zhang et al. 2021). In the present study, exogenous Spd decreased S_m and N values under drought stress. Research has shown that the decreased values of S_m and N suggested that the reduction times of Q_A also decreased (Ni et al. 2012). The reduction of both indicated that exogenous Spd inhibited photosynthetic

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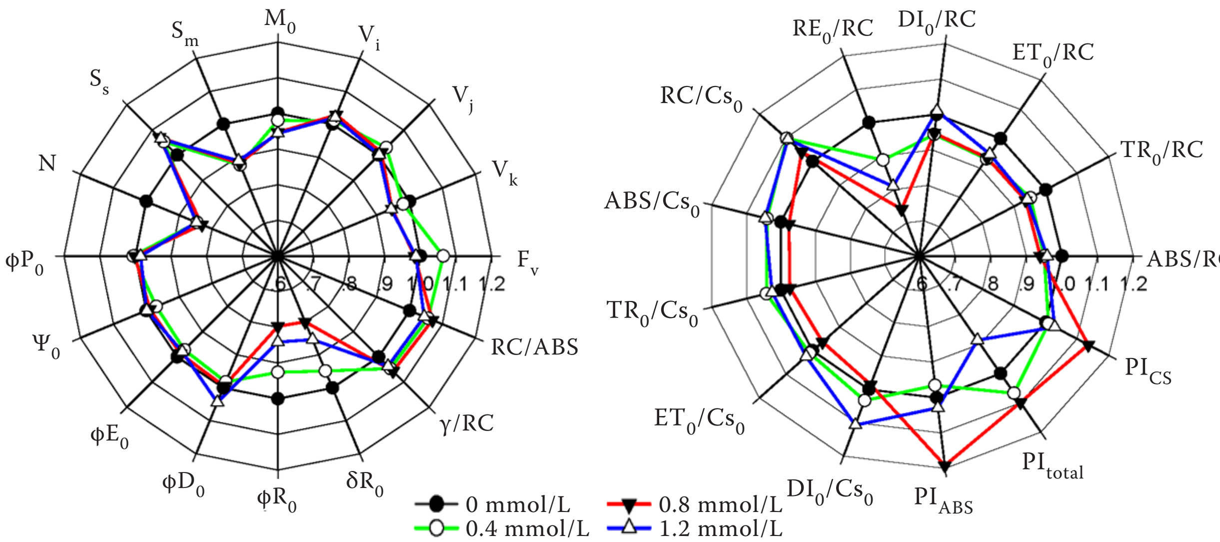

Figure 7. "Radar plots" of picked parameters characterising different behavior of photosystem II of tall fescue leaves upon different concentrations of exogenous spermidine (0, 0.4, 0.8 and 1.2 mmol/L) under drought stress. All values are shown as percent of control (control plants = 1). F_v – maximal variable fluorescence; V_k – relative variable fluorescence at K step; V_j – relative variable fluorescence at J step; V_i – relative variable fluorescence at I step; M_0 – approximated initial slope (in/ms) of the fluorescence transient; S_m – normalised total complementary area above the OJIP (fluorescence rise kinetics O-J-I-P) transient; S_s – normalised total complementary area corresponding only to the O-J phase; N – number of redox cycles of Q_A; ϕP_0 – maximum quantum yield of primary photochemistry (at $t = 0$); Ψ_0 – efficiency/probability that an electron moves further than Q_{A-}; ϕE_0 – quantum yield of electron transport (at $t = 0$); ϕD_0 – quantum yield (at $t = 0$) of energy dissipation (at $t = 0$); ϕR_0 – quantum yield for reduction of end electron acceptors at the PSI acceptor side; δR_0 – efficiency/probability with which an electron from the intersystem electron carriers moves to reduce end electron acceptors at the PSI acceptor side (RE); γ/RC – probability that a PSII *Chl* molecule functions as RC; RC/ABS – Q_A-reducing RCs per PSII antenna *Chl* (reciprocal of ABS/RC); ABS/RC – absorption flux (of antenna *Chls*) per RC (at $t = 0$); TR_0/RC – trapping flux (leading to Q_A reduction) per RC (at $t = 0$); ET_0/RC – electron transport flux (further than Q_{A-}) per RC (at $t = 0$); DI_0/RC – dissipated energy flux per RC (at $t = 0$); RE_0/RC – electron flux reducing end electron acceptors at the PSI acceptor side, per RC; RC/Cs_0 – density of RCs (Q_A-reducing PSII reaction centers) (at $t = 0$); ABS/Cs_0 – absorption flux per CS, approximated by F_0 (at $t = 0$); TR_0/Cs_0 – trapped energy flux per CS (at $t = 0$); ET_0/Cs_0 – electron transport flux per CS (at $t = 0$); DI_0/Cs_0 – dissipated energy flux per CS (at $t = 0$); PI_{ABS} – performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors; PI_{total} – performance index (potential) for energy conservation from exciton to the reduction of PSI end acceptors; PI_{CS} – performance index on cross-section basis

electron transport on the PSII acceptor side under drought stress. Studies have shown that a decrease in electron transfer can reduce ROS production (Asada 2006). Thus, we proposed that the Spd application weakened photosynthetic electron transfer, thereby reducing the risk of ROS generation. Using the JIP test, we evaluated the values of intersystem electron carrier moves to reduce the end electron acceptors at the PSI acceptor side (δR_0), absorption flux per RC (ABS/RC), and trapping flux per RC (TR_0/RC). As shown in Figures 3 and 7, exogenous Spd notably decreased the values of δR_0, which indicated that there was lower PSII activity in the photosystem. It has been reported that, compared with the heat-sensitive genotype, the value of δR_0 was relatively low in the heat-tolerant tall fescue genotype under heat stress (Chen et al. 2014), and after Asc (ascorbic acid) application, the value of δR_0 obviously decreased under heat stress in tall fescue (Chen et al. 2017), which was consistent with the results of this study. In terms of specific energy fluxes, the values of ABS/RC and TR_0/RC after Spd application were significantly lower than those of non-sprayed Spd under drought stress. These results indicated

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that Spd application notably decreased electronic transmission efficiency and PSII function under drought stress. Therefore, the reduced activity of PSII may help limit the energy input into the light system and the production of ROS to resist drought stress in tall fescue. These data are in line with our findings that exogenous Spd remarkably decreases the values of S_m and N under drought stress. In the present study, we ascertained that 0.8 mmol/L of Spd had a positive effect on PI_{ABS} and PI_{CS} compared to the non-Spd application under drought stress. This result indicates that exogenous Spd can effectively alleviate a series of physiological changes caused by drought stress in tall fescue.

Although drought affects the photosynthesis of tall fescue, exogenous Spd can alleviate the effects of drought stress in this study. In summary, our study suggests that the exogenous application of Spd resulted in higher F_J and F_P and the lower values of S_m, N, δR_0, ABS/RC and TR_0/RC than those observed with the non-Spd application under drought stress. However, the underlying mechanism of Spd action under drought stress in photosynthetic apparatus still needs further elucidation.

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