ISSN 1021-4437, Russian Journal of Plant Physiology, 2023, Vol. 70:48. © Pleiades Publishing, Ltd., 2023.

RESEARCH PAPERS

Spermidine and Spermine Converted from Putrescine Improve the Resistance of Wheat Seedlings to Osmotic Stress

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Received December 2, 2022; revised December 9, 2022; accepted December 9, 2022

Abstract—Polyamines play an important role in the plant resistance to drought and osmotic stress (OS). However, the precise function of putrescine conversion to spermidine and spermine is not clear in wheat seedling leaves under OS. Changes in the levels of three major free polyamines, putrescine, spermidine and spermine, were investigated in the seedlings of two wheat cultivars *Triticum aestivum* L. Jinmai 98 (drought-resistant) and Wenmai 6 (drought-sensitive) under PEG 6000 OS. Furthermore, the activity of arginine decarboxylase and *S*-adenosylmethionine decarboxylase was determined. In addition, experiments with exogenous polyamines and the polyamine biosynthesis inhibitors were also implemented to supply more evidence. Under OS, spermidine and spermine levels increased more markedly ($P < 0.05$) in drought-resistant Jinmai 98 than in drought-sensitive Wenmai 6, suggesting that free spermidine and spermine, which were converted from free putrescine, were possibly involved in the resistance of seedlings to OS. Treatment with exogenous spermidine treatment enhanced the OS-induced increase in endogenous spermidine and spermine content in drought-sensitive Wenmai 6, accompanied with an increase in resistance, as judged by a decrease in the relative permeability of plasma membrane of seedling leaf and an increase in the relative water content of seedling leaves. The suggestion was further testified by treatment with methylglyoxal-bis guanylhydrazone and *o*-phenanthrolin. In sum, it could be inferred that spermidine and spermine converted from free putrescine in leaves functioned in an increase in resistance of wheat seedlings to OS.

Keywords: *Triticum aestivum*, polyamine, osmotic stress, conversion, wheat seedling

DOI: 10.1134/S1021443722602993

INTRODUCTION

Area under soil drought is spreading rapidly worldwide. Drought greatly affects plant growth and development, and thereby poses severe threats to agricultural productivity and sustainability [1, 2]. Therefore, it is increasingly interesting to analyze the mechanism underlying the resistance of crop to drought. Wheat seedlings are frequently subjected to soil drought stress. Water deficit causes the disorder of physiological metabolism in wheat seedlings, reduces the productivity of photosynthesis and prevents the normal development. Therefore, it is highly important to explore the wheat drought resistance mechanism at the seedling stage.

Abbreviations: ADC—arginine decarboxylase; D-Arg—D-arginine; DW—dry weight; FW—fresh weight; OS—osmotic stress; MGBG—methylglyoxyl-bis (guanylhydrazone); PEG—polyethylene glycol; SAMDC—*S*-adenosylmethionine decarboxylase; SLRPMP—seedling leaf relative plasma membrane permeability; SLRWC—seedling leaf relative water content; SW—saturation weight.

It is well known that endogenous plant growth regulators can regulate many physiological processes, such as morphogenesis, embryogenesis, cell division, growth and development, seed formation and senescence, and response to adverse environments [3, 4]. Polyamines are plant growth regulation substances with positive charges and strong biological activity. Three polyamines, putrescine, spermidine and spermine, are the most common members of the polyamine family. The diamine putrescine is mainly produced from arginine by the action of arginine decarboxylase (ADC; EC 4.1.1.19), which is one of the key enzymes in putrescine biosynthesis [5], characterized by absolute specificity for L-arginine and is thereby inhibited potently and exclusively by D-arginine (D-Arg) [6]. Putrescine can be converted to spermidine and spermine by being linked with one and two aminopropyls, respectively. Many processes and factors are involved in putrescine derivation to spermidine and spermine. Among these, *S*-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50) is the most important enzyme

and is potently and exclusively inhibited by methylglyoxal-bis guanylhydrazone (MGBG) [6, 7].

Polyamines widely exist in plants and plays important regulatory roles in plant growth, development, morphogenesis and response to environmental stress [3, 4, 8, 9]. Therefore, the balance of three main free polyamines is vital in different growth and development processes [6]. However, the significance of putrescine conversion to spermidine and spermine is not clear. This research aimed to illuminate the significance of putrescine conversion by estimating the dynamic changes in levels of endogenous free polyamine accumulation in leaves of wheat seedlings under PEG OS, which was used to simulate natural drought, with two wheat cultivars, Jinmai 98 (drought-resistant) and Wenmai 6 (drought-sensitive) as experimental materials. To get more insight into polyamine metabolism, the activities of key enzymes, ADC and SAMDC, which are involved in free putrescine biosynthesis and putrescine conversion, were also determined. To further testify our finding, exogenous putrescine, spermidine and spermine, which elevated endogenous polyamine contents, were applied to the experiment. Furthermore, treatments with inhibitors, D-Arg and MGBG, which decreased endogenous free putrescine and putrescine conversion, respectively, were also additionally implemented in this research, to show whether the decreases in three forms of polyamines could affect wheat drought resistance, which was judged by seedling leaf relative plasma membrane permeability (SLRPMP) and seedling leaf relative water content (SLRWC). With the aforementioned results, it would be suggested that the conversion of free putrescine to free spermidine and spermine, rather than free putrescine itself, functioned in enhancing wheat resistance to OS. Furthermore, the finding could address the issue of whether free putrescine accumulation at an earlier stage of OS could bring about or alleviate injury.

MATERIALS AND METHODS

Plant Material and Stress Treatment

The typical wheat (*Triticum aestivum* L.) cultivars, Jinmai 98 (drought-resistant) and Wenmai 6 (drought-sensitive), were applied in the research. Wheat seeds were permitted to be used in this research in accordance with the legislation and guidelines of China. The study evaluated the difference in drought tolerance between the two cultivars.

Wheat seeds were uniformly planted in plastic pots (20 × 10 × 15 cm) with pores in the bottom after sterilizing the seed surface for 5 min with 0.1% $HgCl_2$ (w/v) and rinsing three times with distilled water. The pots with seeds were then placed in plastic turnover boxes with Hoagland solution. Turnover boxes were placed in a photoperiod and temperature-controlled chamber with 14 h light at 20°C/10 h night at 10°C regimes, and an illuminance of 200 μmol/(m^2 s). After the second leaf complete extension, seedlings were thinned to 10 seedlings per pot and various treatments were performed. For OS treatment, the leaves of the seedlings were sprinkled with dH_2O, and the roots were placed in Hoagland's solution supplemented with 20% PEG 6000 (–0.55 MPa). For OS + D-Arg treatment, the leaves were sprayed with 1 mM D-Arg and roots were in Hoagland's solution supplemented with 20% PEG (–0.55 MPa). For OS + D-Arg + putrescine treatment, the leaves were sprinkled with 1 mM D-Arg + 1 mM putrescine and the roots were in Hoagland solution supplemented with 20% PEG (–0.55 MPa). For OS + spermidine treatment, the leaves were sprayed with 1 mM spermidine and roots were in Hoagland solution supplemented with 20% PEG (–0.55 MPa). For OS + MGBG treatment, leaves were sprinkled with 0.5 mM MGBG and roots were in Hoagland solution supplemented with 20% PEG (–0.55 MPa). The samples of leaves sprinkled with deionized water and roots in Hoagland solution without PEG were used as a control.

Concentration of chemicals (including 20% PEG, 1 mM D-Arg, 1 mM putrescine, 1 mM spermidine and 0.5 mM MGBG) was determined in preliminary experiments. All Hoagland solutions mentioned above were renewed every 2 days. Due to the similar effect to exogenous spermidine, treatment with exogenous spermine was not shown here. The polyamines and inhibitors were purchased from Sigma-Aldrich (USA). The determination of a dose of the abovementioned reagents was based on our preliminary experiments. Seedling leaves of treated groups were sprayed with a solution containing the above reagents, with addition of 0.1% ethanol, and 0.01% (v/v) Tween-20; 10 mL per pot at 5:30 am and 06:30 pm each day. The control group was sprayed with water containing 0.1% ethanol and 0.01% (v/v) Tween-20. The seedling leaves were collected at 5:30 a.m. on days 0, 2, 4, 6, 8, 10, and 12 after treatment.

Assessment of the Relative Water Content (SLRWC) in Seedling Leaves

Fresh seedling leaves were weighed to obtain fresh weight (FW). Then, they were immediately immersed in dH_2O to fully absorb water until the weight of seedling leaves was constant, which was regarded as the saturation weight (SW). Then, the sample was put in an oven at 110°C for 5 min and then dried at 75°C until the sample weight was constant. The dried constant seedling leaf weight was named the leaf dry weight (DW). SLRWC was estimated using the formula:

$$\mathrm{SLRWC}(\%) = (\mathrm{FW} - \mathrm{DW})/(\mathrm{SW} - \mathrm{DW}) \times 100.$$

Assessment of the Relative Plasma Membrane Permeability (SLRPMP) in Seedling Leaves

Wheat SLRPMP was assessed according to the method described by Jahan et al. [10] with minor adjustments. 1 g of wheat seedling leaf was immersed into 10 mL deionized water. Then, under dark conditions, the sample was incubated in a water bath at 25°C for 2 h. The original electrical conductivity (OEC) of the water medium containing seedling leaves was determined with a portable conductivity meter DDB-11A (Guangzhou Ruibin Technology Co., China). Afterwards, the seedling leaves were boiled for 20 min at 100°C, cooled to 25°C, and left to stand for 30 min. The terminal electrical conductivity (TEC) was determined. DEC represents EC of deionized water. SLRPMP was assessed by the formula:

$$\mathrm{SLRPMP}(\%) = (\mathrm{OEC} - \mathrm{DEC})/(\mathrm{TEC} - \mathrm{DEC}) \times 100.$$

Assay of Arginine Decarboxylase (ADC) in Seedling Leaves

ADC was extracted and assayed by the method of Nam et al. [5] with modification. 2 g of wheat leaf was homogenized with 5 mL of pre-cooled extract solution, which was composed of 50 mM phosphate buffer (pH 6.3), 5 mM EDTA, 0.1 μM PMSF, 40 μM phosphopyridoxal, 5 mM DTT, and 20 mM vitamin E in an ice bath and filtered with 4 layers of gauze. The solution was precipitated by 20–50% $(NH_4)_2SO_4$. The precipitate was re-dissolved in 10 mM phosphate buffer (pH 6.3), dialyzed overnight at 4°C, and then added to a double volume of ice-cold acetone. After centrifugation at 5000 *g* for 5 min at 4°C, the precipitate was re-dissolved in 10 mM phosphate buffer, dialyzed overnight at 4°C and centrifuged at 15000 *g* for 15 min. The supernatant was used to measure enzyme activity. The ADC reaction system, including 100 mM Tris HCl buffer (pH 7.5), 5 mM EDTA, 40 μM phosphopyridoxal, 5 mM DTT, and ADC extraction buffer, was placed in a water bath at 37°C for 2 min, and added with 0.2 mL 25 mM Tris HCl buffer (pH 7.5). The reaction lasted for 60 min at 37°C and was terminated by adding $HClO_4$ to a final concentration of 5%. The sample was centrifuged at 3000 *g* for 10 min. Then, 0.5 mL of the supernatant was added to 1 mL of 2 N NaOH and 10 μL of benzoyl chloride, vortexed vigorously for 20 s, left for 60 min at 25°C and added to 2 mL of saturated NaCl. After mixing, 2 mL of diethyl ether was added, vortexed vigorously for 20 s, and centrifuged at 1500 *g* for 5 min. Collected 1 mL of diethyl ether was evaporated at 50°C and precipitate was dissolved in 3 mL of methanol. ADC activity in solution was calculated by using absorbance value at 254 nm wavelength obtained with UV/VIS spectrophotometer. 1 nmol agmatine/(g FW)/h was regarded as one enzyme activity unit.

Assay of S-Adenosylmethionine Decarboxylase (SAMDC) Activity in Seedling Leaves

SAMDC activity was evaluated by examining the release of $^{14}CO_2$ using ^{14}C-labeled substrates according to the method of Kaur-Sawhney and Shin [11] with a few modifications. 2 g of wheat leaf was homogenized with 5 mL of 100 mM phosphate buffer (pH 7.6) and then centrifuged at 25000 *g* for 20 min at 4°C. The supernatant was used for SAMDC activity assessing. The assay solution consisted of the aforementioned supernatant supplemented with 0.2 mM EDTA, 0.1 M Tris-HCl buffer (pH 8.3) and 2 mM 2-mercaptoethanol. 0.5 nM *S*-adenosyl-L-[carboxyl-^{14}C] methionine was added to the assay solution, and the reaction was continued for 30 min at 30°C. Then, 0.4 mL of 1 M KH_2PO_4 was used to quench the reaction, and $^{14}CO_2$ was collected in a glass test tube. 1 μL of $^{14}CO_2$ min^{-1} was defined as one SAMDC activity unit.

Extraction and Quantification of Free Polyamines

The extraction and quantification of free polyamines were conducted by the method of Quinet et al. [12] with minor adjustments. 2 g FW of wheat leaf was homogenized in 5 mL $HClO_4$ (5%, v/v), and homogenate was left for 1 h at 4°C. Then, after centrifugation at 21500 *g* for 30 min the supernatant was collected for the free polyamine assay. After benzoylation with benzyol chloride, free putrescine, spermidine and spermine were quantified by HPLC Waters 2695 (Waters, USA). A C-18 reverse-phase separation column was used, with 1,6-hexanediamine as the internal standard and 254 nm as the detecting wavelength. The polyamine sample was eluted at 25°C from the separation column using a Perkin-Elmer Series 410 pump (USA) at 0.6 mL/min.

Statistical Analysis

The experiment was performed three times, i.e. three biological replicates, and three technical replicates were carried out in every biological replicate. Therefore, the data shown in the paper were averages of 9 values ± SE. The data were analyzed by SPSS 16.0 and Microsoft Excel (SPSS Inc., USA). The deviation of the averages was statistically evaluated by two-way analysis of variance (ANOVA) and Duncan's method was used to compare means at the $P < 0.05$ level. The significant differences among multiple groups were indicated by different letters.

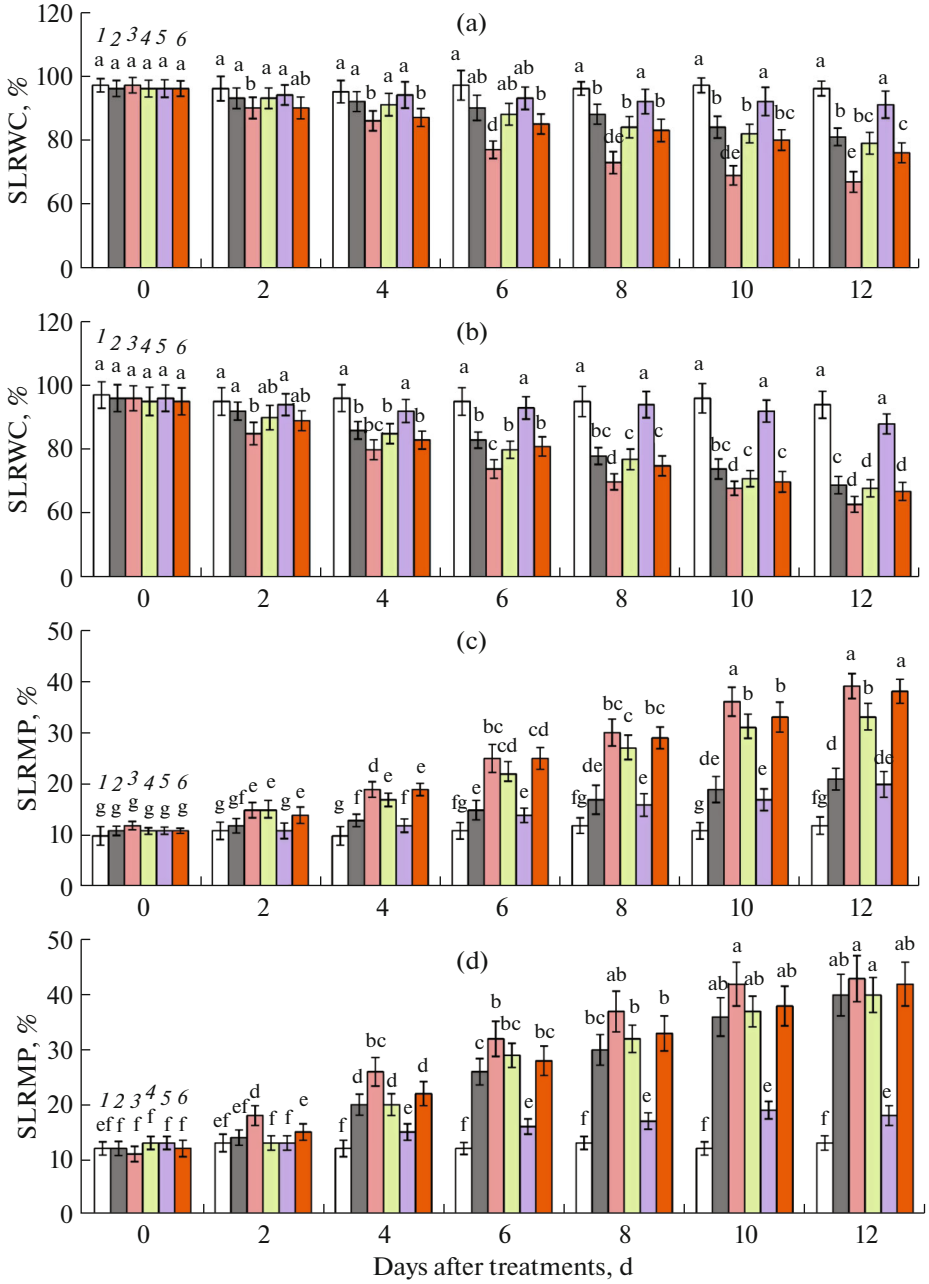

Fig. 1. Dynamic changes in content of SLRWC in (a) Jinmai 98 and (b) Wenmai 6 cultivars, and SLRPMP in (c) Jinmai 98 and (d) Wenmai 6 under the effect of PEG, exogenous polyamines and inhibitors, where (*1*) control; (*2*) PEG; (*3*) PEG + D-Arg; (*4*) PEG + D-Arg + putrescine; (*5*) PEG + spermidine; (*6*) PEG + MGBG. The data are means ±SE ($n = 9$), and the means among multiple groups labeled with different letters indicate a significant differences at $P < 0.05$.

RESULTS

Dynamic Changes in SLRWC and SLRPMP under PEG, Exogenous Polyamines and Inhibitors

As displayed in Fig. 1, SLRWC of the two wheat cultivars decreased over the whole OS period, and it decreased more significantly ($P < 0.05$) in drought-sensitive Wenmai 6 (Fig. 1b) than in drought-resistant Jinmai 98 (Fig. 1a). Especially at the end stage of OS, it decreased to 68% in Wenmai 6, whereas in Jinmai 98, it decreased only to 82%. Treatment with D-Arg, an ADC inhibitor of putrescine biosynthesis, aggravated substantially the OS-induced decreases in SLRWC of both cultivars, and in Wenmai 6 and Jinmai 98, it decreased to 62% and 66%, respectively. Application of exogenous putrescine reversed the D-Arg treatment effect on SLRWC. Exogenous spermidine treatment markedly ($P < 0.05$) alleviated the OS-induced decreases in SLRWC of both cultivars, especially of drought-sensitive Wenmai no. 6, while treat-

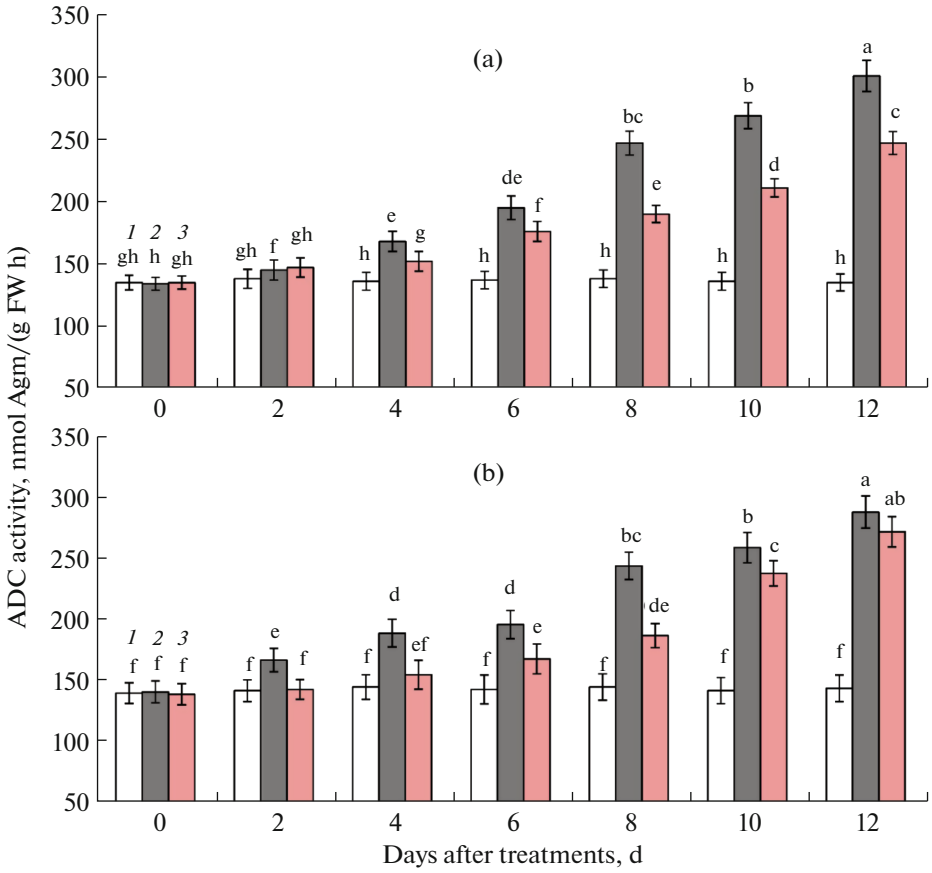

Fig. 2. Dynamic change in ADC activity in seedling leaves of (a) Jinmai 98 and (b) Wenmai 6 cultivars under the effect of PEG and D-Arg, where (*1*) control; (*2*) PEG; (*3*) PEG + D-Arg. The data are means ±SE ($n = 9$), and the means among multiple groups labeled with different letters indicate a significant differences at $P < 0.05$.

ment with the inhibitor MGBG markedly ($P < 0.05$) aggravated the OS-induced decreases in SLRWC of both cultivars, especially of drought-resistant Jinmai 98 (Figs. 1a, 1b).

As displayed in Figs. 1c and 1d, SLRPMP of the two wheat cultivars increased throughout the whole period of PEG treatment, and the parameter increased more significantly ($P < 0.05$) in drought-sensitive Wenmai 6 (Fig. 1d) than in drought-resistant Jinmai 98 (Fig. 1c). It increased to 41 and 20% at the end treatment stage in Wenmai 6 and Jinmai 98, respectively. D-Arg treatment aggravated dramatically the OS-induced increases in SLRPMP of both cultivars and SLRPMP of Wenmai 6 and Jinmai 98 increased to 42 and 40%, respectively. Application of exogenous putrescine reversed the D-Arg treatment effect on SLRPMP of Jinmai 98. Exogenous spermidine treatment markedly ($P < 0.05$) alleviated the OS-induced increase in SLRPMP of both cultivars, especially of drought-sensitive Wenmai 6, while the treatment with inhibitor MGBG markedly ($P < 0.05$) aggravated the OS-induced increases in SLRPMP of both cultivars, especially of drought-resistant Jinmai 98 (Figs. 1c, 1d).

Dynamic Changes in ADC Activity in Leaves under PEG and Inhibitor D-Arg

The ADC activities in seedling leaves of both cultivars rose continuously with the prolonged stress time (Fig. 2). At the end stage of OS, the ADC activities were 299 and 290 nmol Agm/(g FW)/h in Jinmai 98 (Fig. 2a) and Wenmai 6 (Fig. 2b), respectively. The results indicated that there was no marked difference between the two cultivars. D-Arg inhibited ADC activity over the whole OS period.

Dynamic Changes in Putrescine Level in Leaves under PEG, Exogenous Polyamines and Inhibitors

As displayed in Fig. 3, free putrescine contents in seedling leaves of both cultivars increased drastically at the earlier stage of OS, while on the 4th day, the contents began to decrease. However, the decrease in free putrescine at the later stage of OS was more significant in drought-resistant Jinmai 98 (Fig. 3a) than in Wenmai 6 (Fig. 3b). For example, at the end stage of OS treatment, the free putrescine reached 139 and 370 nmol/(g FW) in Jinmai 98 (Fig. 3a) and Wenmai 6 (Fig. 3b), respectively. D-Arg inhibited the OS-induced

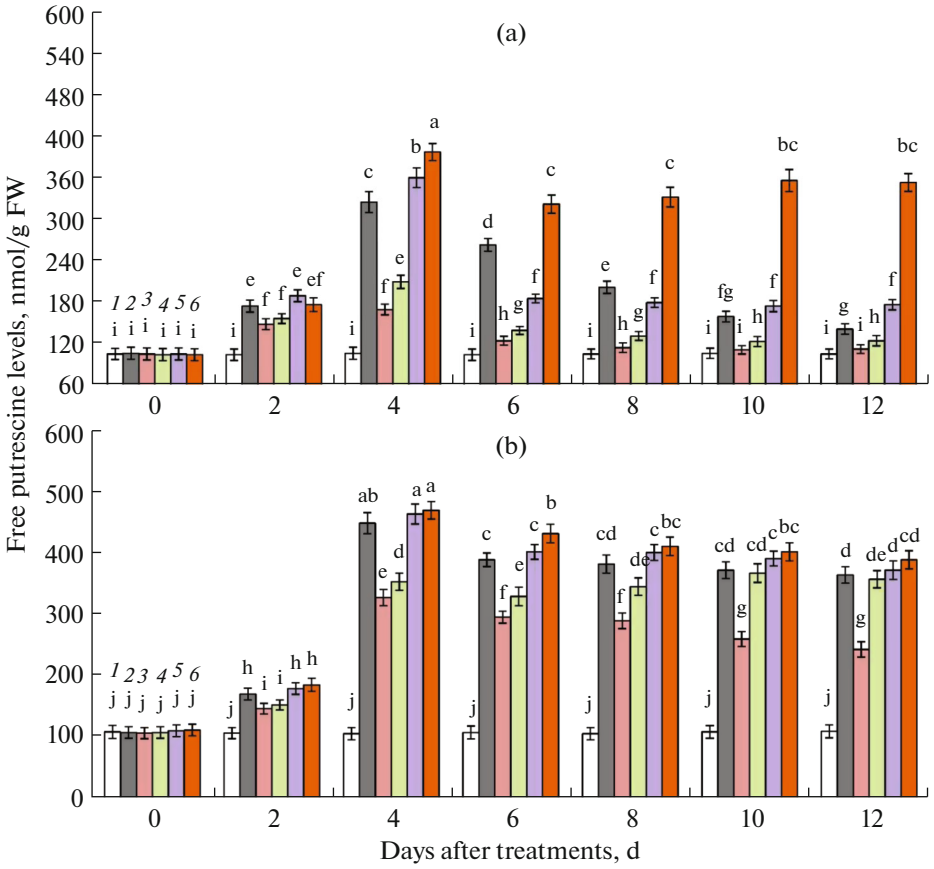

Fig. 3. Dynamic change in free putrescine level in seedling leaves of (a) Jinmai 98 and (b) Wenmai 6 cultivars under the effect of PEG, exogenous polyamines and inhibitors, where (*1*) control; (*2*) PEG; (*3*) PEG + D-Arg; (*4*) PEG + D-Arg + putrescine; (*5*) PEG + spermidine; (*6*) PEG + MGBG. The data are means ±SE ($n = 9$), and the means among multiple groups labeled with different letters indicate a significant differences at $P < 0.05$.

increase in free putrescine of the two cultivars. Application with exogenous putrescine, spermidine or inhibitor MGBG enhanced the OS-induced increase in endogenous free putrescine and the effect of MGBG was more significant than the others.

Dynamic Changes in Spermidine Level in Leaves under PEG, Exogenous Polyamines and Inhibitors

As shown in Fig. 4, the free spermidine contents in leaves of both cultivars increased continuously throughout the whole period of PEG treatment. However, the increase was more significant ($P < 0.05$) in drought-resistant Jinmai 98 (Fig. 4a) than in Wenmai 6 (Fig. 4b) during the later period (from the 4th to the 12th day). Application of exogenous putrescine and spermidine enhanced the OS-induced increase in endogenous free spermidine, while inhibitor D-Arg or MGBG treatment inhibited the increase dramatically. The trends of change in free spermine were similar to those of free spermidine in seedling leaves of both wheat cultivars under PEG, exogenous polyamines, and inhibitors (Fig. 5).

Dynamic Changes in SAMDC Activity in Seedling Leaves under PEG and Inhibitor MGBG

SAMDC activity in seedling leaves of the control group was approximately invariable and began to increase slightly at the end stage (Fig. 6). Nevertheless, OS obviously elevated SAMDC activity over the whole treatment period. More interestingly, the OS-induced increase in SAMDC activity was much more significant ($P < 0.05$) in drought-resistant Jinmai 98 (Fig. 6a), than in drought-sensitive Wenmai 6 (Fig. 6b), especially from the 4th to the 12th day of OS treatment period. On the 12th day, it increased by 92.9 and 55.8% in Jinmai 98 and Wenmai 6, respectively. MGBG treatment could inhibit significantly ($P < 0.05$) the OS-induced increase in SAMDC activity.

DISCUSSION

Selection of Wheat Cultivars with Different Drought Resistance

It was vital to select wheat cultivars with different drought resistance because by analyzing the differences between the different wheat cultivars in dynamic

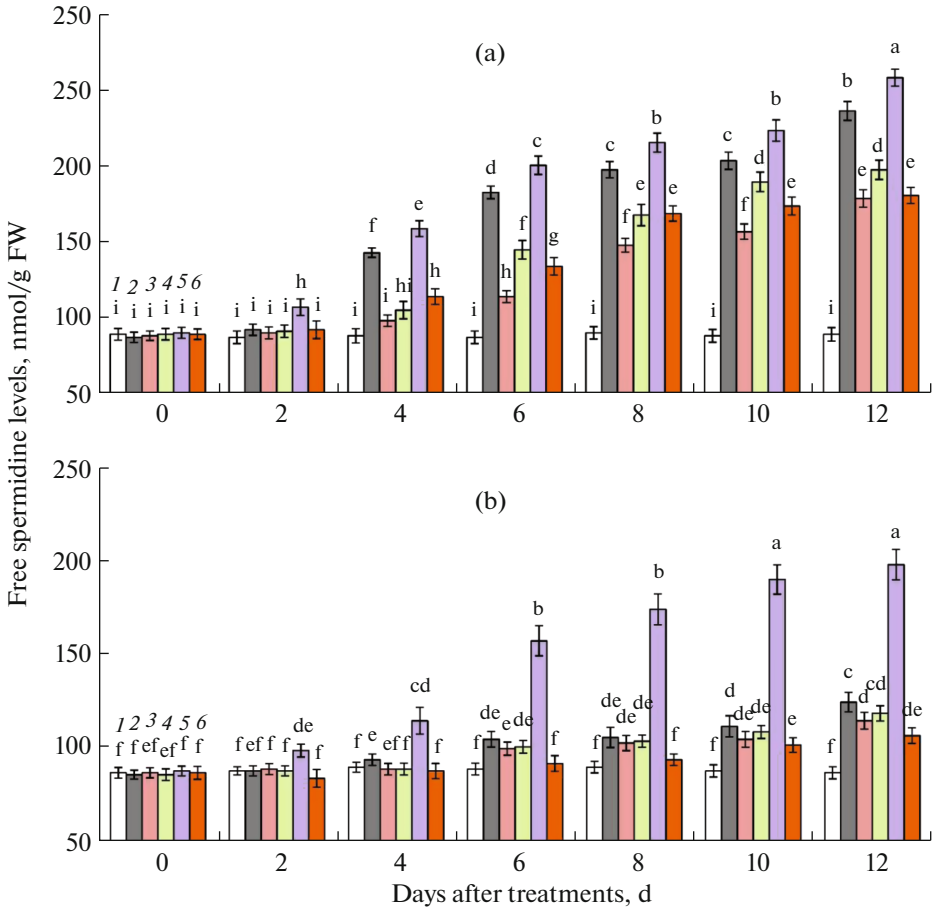

Fig. 4. Dynamic change in free spermidine level in seedling leaves of (a) Jinmai 98 and (b) Wenmai 6 cultivars under the effect of PEG, exogenous polyamines and inhibitors, where (*1*) control; (*2*) PEG; (*3*) PEG + D-Arg; (*4*) PEG + D-Arg + putrescine; (*5*) PEG + spermidine; (*6*) PEG + MGBG. The data are means ±SE ($n = 9$), and the means among multiple groups labeled with different letters indicate a significant differences at $P < 0.05$.

changes in different polyamine types and forms in seedling leaves during the treatment period of OS, the proper conclusions could be drawn from the experiment. Based on the following two reasons, two wheat cultivars, Jinmai 98 and Wenmai 6, were selected as experimental materials in the study. First, Jinmai 98 was planted in drought ecotope of Northwest China, while Wenmai 6 was distributed in rain-rich ecotope of Central China. Second, obvious reaction of crops to abiotic stresses is displayed as growth inhibition, which is usually quantified by biomass and production accumulation [13, 14]. Under OS, the decrease in SLRWC (Figs. 1a, 1b) and the increase in SLRPMP (Figs. 1c, 1d) were much more marked in Wenmai 6 than in Jinmai 98. Therefore, by analyzing the differences in plant physiological parameters, SLRWC and SLRPMP, which are closely associated with OS and membrane injury, respectively, it could be inferred that Jinmai 98 and Wenmai 6 were drought resistant and sensitive, respectively.

Significance of Free Putrescine Accumulation in Seedling Leaves at the Earlier Stage of PEG OS Treatment

It is well known that no unanimous conclusion can be drawn regarding the significance of free putrescine accumulation under abiotic stresses. Some previous researches have showed that free putrescine build-up can cause injury and decrease the resistance of plants to environmental stresses, because oxidation and degradation products of putrescine, such as H_2O_2, amido aldehyde and propylene aldehyde, can be cross-linked with proteins and nucleic acid to lead to cell senescence and apoptosis [15, 16]. However, some recent studies have suggested that putrescine can alleviate the stress-induced injury symptoms and improve the resistance of plants subjected to abiotic stresses [17, 18]. Disagreement on this issue might be attributed to different treatment periods and experimental materials, especially to the different resistant cultivars. Therefore, to address the issue clearly, dynamic changes in free putrescine throughout the whole stress period were examined, with two wheat cultivars with different

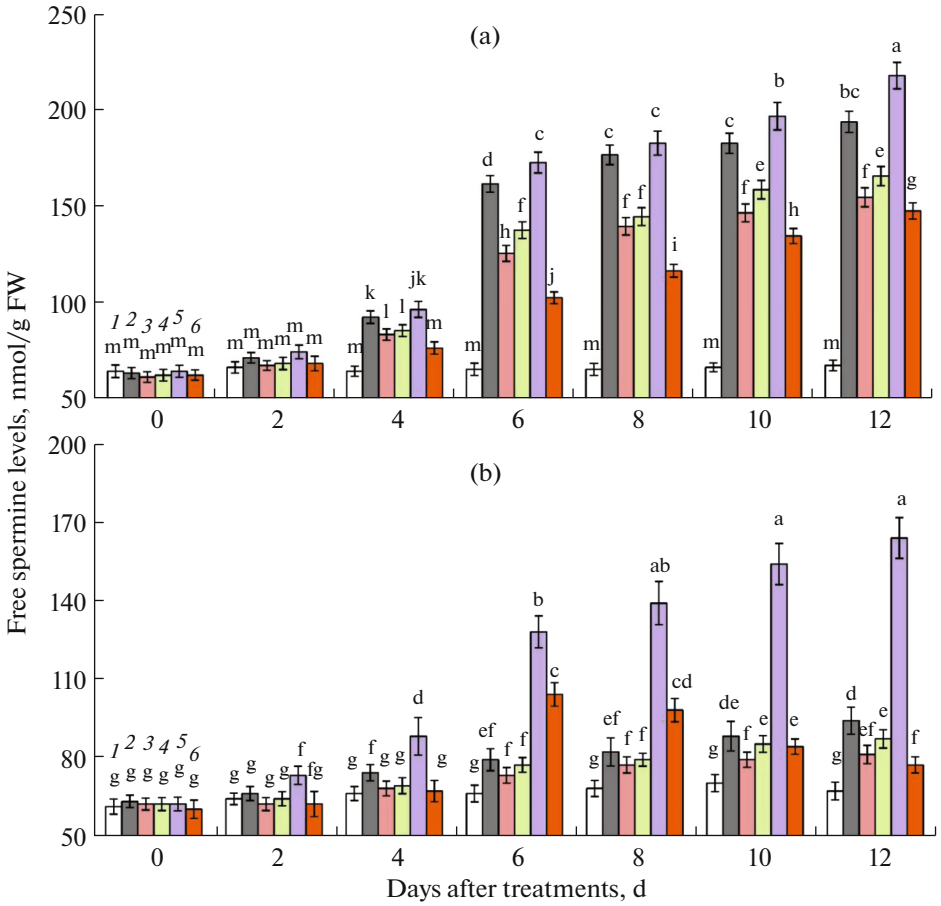

Fig. 5. Dynamic change in free spermine level in seedling leaves of (a) Jinmai 98 and (b) Wenmai 6 cultivars under the effect of PEG, exogenous polyamines and inhibitors, where (*1*) control; (*2*) PEG; (*3*) PEG + D-Arg; (*4*) PEG + D-Arg + putrescine; (*5*) PEG + spermidine; (*6*) PEG + MGBG. The data are means ±SE ($n = 9$), and the means among multiple groups labeled with different letters indicate a significant differences at $P < 0.05$.

drought resistances as experimental materials in the research. The results showed that OS could lead to a substantial increase in free putrescine not only in the drought-sensitive wheat cultivar, but also in the drought-resistant cultivar at the earlier stress stage (Fig. 3). Furthermore, coupled with the significant decreases in the free putrescine level and ADC activity, which was inhibited by D-Arg (Fig. 2), the resistances of the two cultivars decreased markedly, as judged by the two representative parameters, SLRWC (Figs. 1a, 1b) and SLRPMP (Figs. 1c, 1d). The results suggested that free putrescine accumulation at the earlier stage of PEG treatment might be essential for plant resistance to stress. It has been well documented that free putrescine could play a crucial role in signal transduction under abiotic stresses [3, 19]. However, our present study revealed that free putrescine build-up could be regarded as only one possibility for plant resistance. Whether the increased free putrescine level induced by OS could function in enhancing resistance would depend on whether free putrescine could be converted to spermidine and spermine at the later stage of osmotic stress.

Significance of Free Putrescine Conversion to Free Spermidine and Spermine in Seedling Leaves at the Later Stage of PEG Treatment

From the result that the OS-induced increases in free spermidine (Fig. 4) and spermine (Fig. 5) were more significant ($P < 0.05$) in the drought-resistant cultivar than in the drought-sensitive cultivar during the later period, we could conclude that free spermidine and spermine might enhance osmotic stress resistance. Furthermore, by analyzing the dynamic changes in the three free polyamines, we could find that in the drought-resistant cultivar, the increases in free spermidine (Fig. 4) and spermine (Fig. 5) were in good agreement with the decrease in free putrescine (Fig. 3) and all changes in free polyamines began simultaneously on the 4th day of OS. Therefore, it could be inferred that the OS-induced increases in free spermidine and spermine were mainly from free putrescine conversion. The inference was further confirmed with the application of the inhibitors, D-Arg and MGBG. D-Arg inhibited free putrescine biosynthesis, coupled with the decrease in free spermidine and spermine, and more importantly, MGBG inhib-

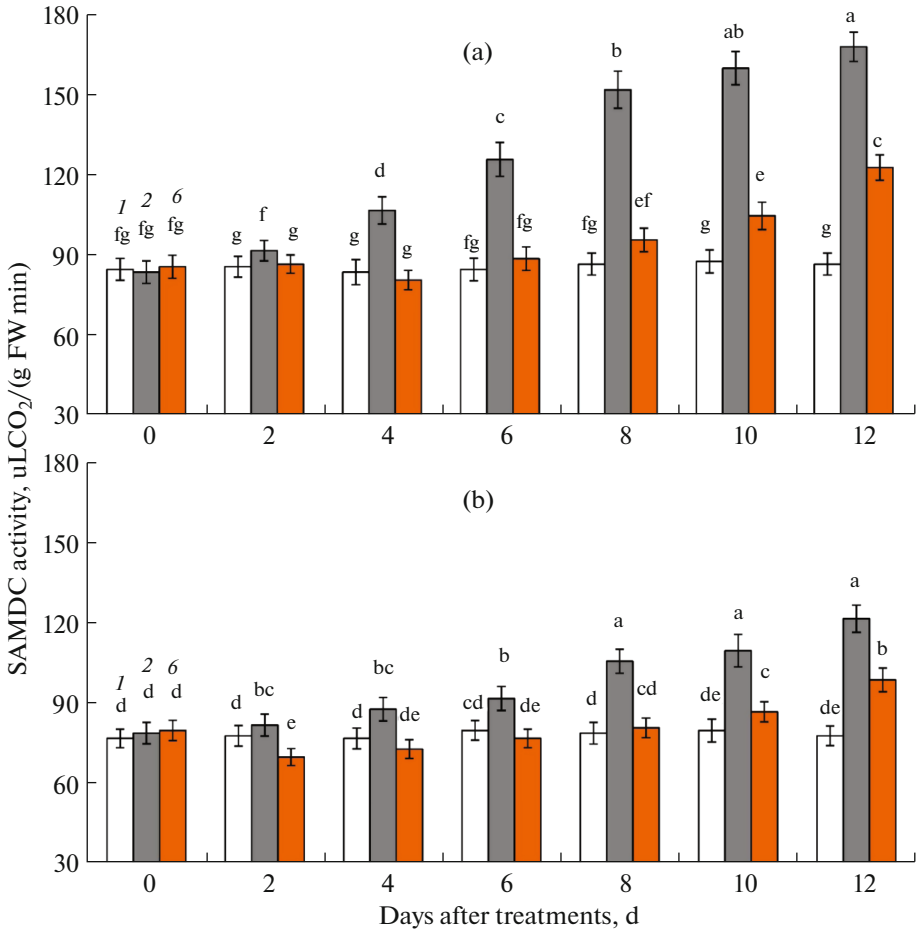

Fig. 6. Dynamic change in SAMDC activity in seedling leaves of (a) Jinmai 98 and (b) Wenmai 6 cultivars under the effect of under PEG and MGBG, where (*1*) control; (*2*) PEG; (*3*) PEG + MGBG. The data are means ±SE ($n = 9$), and the means among multiple groups labeled with different letters indicate a significant differences at $P < 0.05$.

ited the conversion of free putrescine to spermidine and spermine by inhibiting the OS-induced increase in SAMDC activity (Fig. 6), coupled with the decrease in the resistance to OS, as judged by the decreases in SLRWC (Figs. 1a, 1b) and the increase in SLRPMP (Figs. 1c, 1d). Additionally, treatment with exogenous spermidine or spermine (data not shown) led to an increase in endogenous free spermidine and spermine in seedling leaves (Figs. 4, 5), which was coupled with an increase in stress resistance (Fig. 1), and the effect was more especially marked in the drought-sensitive cultivar than in the drought-resistant cultivar, due to the inherently high background level of spermidine and spermine in the latter.

Our finding was in accordance with previous studies [20, 21]. Due to carrying more positive charges, spermidine and spermine could be linked to biomacromolecules, such as acidic proteins and membrane phospholipids, more easily than putrescine, and could play important roles in the response to OS [22, 23]. For example, the research of Farooq et al. [24] suggested that among polyamines, spermine is the most effective in increasing drought tolerance. Additionally, a number of data were recently accumulated to provide testimony for spermidine or spermine significance in regulating cell membrane proteins such as two major vacuolar cation channels and plasma membrane H^+-ATPase [25–29]. Definitely, the precise function of free putrescine in enhancing drought resistance is still not clear, but our research revealed that under OS, conversion of free putrescine to free spermidine and spermine was the main pathway, by which spermidine and spermine were elevated and therefore stress resistance was enhanced.

In overall, in seedling leaves, the conversion of free putrescine to free spermidine and spermine, rather than free putrescine itself, contributed to the OS resistance of wheat. The finding definitely addressed the issue of whether free putrescine accumulation during the earlier stage of OS could bring about or alleviate injury. More specifically, free putrescine build-up could only be regarded as one premise of for plant resistance, and putrescine conversion to spermidine and spermine should be the keys to the issue. This

notion might reasonably explain another phenomenon, in which exogenous putrescine application could lead to different effects on different plant cultivars. Because exogenous putrescine could not be effectively converted to spermidine and spermine in the relatively stress-sensitive cultivars, the excessive accumulated free putrescine would be oxidized by polyamine oxidase, and the degradation products might have adverse effects to plant cells. Conversely, in the stress-resistant cultivars, exogenous putrescine could be converted to spermidine and spermine, which function in enhancing the stress resistance.

FUNDING

This work was funded by National Natural Science Foundation of China (grant no. 31271627), Science and Technology Program of Henan Province (grant no. 212102110016) and Natural Science Foundation of Henan province (grant no. 222300420394).

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any studies involving animals or human participants as objects of research.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Author B.X. Chen designed the experiments. Y.B. Li performed the experiments. Y.B. Li and B.X. Chen drafted the manuscript and all authors revised it.

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