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Research article

Exogenous applications of Polyamines modulate drought responses in wheat through osmolytes accumulation, increasing free polyamine levels and regulation of polyamine biosynthetic genes

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ABSTRACT

Polyamines (PAs) can improve drought stress tolerance in plants; however, very limited information is available on the mechanism of action of exogenous application by different methods under drought stress in wheat. The present study investigates the mechanism through which seed priming and foliar spraying with PAs protect wheat plants from drought stress. 10 days old wheat seedlings were exposed to drought stress by withholding water alone or with 100 μM PAs solutions (putrescine, Put; spermine, Spm; and mixture of Put and Spm for 10 h seed-priming or three foliar sprays during withholding water. Drought stress impaired the wheat growth and altered the osmoprotectants, endogenous PAs levels, PAs biosynthetic genes expression and weight of 1000 grains compared to the corresponding control values. Exogenously applied PAs improved cell water status, accumulated osmoprotectants and PAs and up-regulated PAs biosynthetic genes, *ADC*, arginine decarboxylase; *DHS*, deoxyhypusine synthase; *ODC*, ornithine decarboxylase and *SAMDC*, S-adenosyl methionine decarboxylase. Put significantly regulate the endogenous PAs by both methods of application, however, Spm and mixture of Put and Spm could positively regulate the endogenous PAs and the biosynthetic gene expression by foliar spraying rather than seed priming. The data provide evidence that maintenance of water economy through stabilized cellular structure is an important strategy of drought tolerance by PAs in wheat.

1. Introduction

Plants are exposed to multiple stresses under field condition which adversely affect their physiological processes. Drought stress is in the centre of concern among many environmental challenges to agricultural production worldwide as it can reduce grain yield. Edmeades et al. (1994) estimated an average yield loss of 17–70% in grain yield due to drought stress. The potential yield and water-limited yield of wheat need to increase in order to cope with future demand for food, which is a result of the growing population (Hawkesford et al., 2013), and also to reduce the negative impacts on crop productivity of global climate change (Lobell and Gourdji, 2012). Due to the complex nature of stresses, multiple sensors, rather than a single sensor, are responsible for a plant's response to stress. In general, various nitrogen-containing compounds accumulate in plants in response to environmental stress such as amino acids (arginine, proline), quaternary ammonium (glycine betaine), and polyamines (PAs) (Ahmad et al., 2012). PAs including spermidine (Spd), spermine (Spm), and putrescine (Put) are small ubiquitous nitrogenous compounds. They are a recent addition to the class of plant growth regulators and considered as a secondary messenger in signalling pathways (Liu et al., 2007; Kusano et al., 2008). PAs are widely implicated in cell division and differentiation, root elongation, floral development, fruit ripening, leaf senescence, programmed cell death, DNA synthesis, gene transcription, protein translation, and chromatin organization (Zhang et al., 2011; Tavladoraki et al., 2012; Silveira et al., 2013). In addition, PAs was thought to be involved in the regulation of grain development that is supported by the finding that the PAs content of normal maize kernels was significantly higher than that of aborted kernels, and the PAs content was positively correlated with the endosperm nuclei number (Liang and Lur, 2002).

Abbreviations: ADC, arginine decarboxylase; *DHS*, deoxyhypusine synthase; FAs, free amino acids; GB, glycine betaine; *ODC*, ornithine decarboxylase; PAs, polyamines; Put, putrescine; RGR, relative growth rate; *SAMDC*, S-adenosyl methionine decarboxylase; Spd, spermidine; *SPDS*, spermidine synthase; Spm, spermine; *SPMS*, spermine synthase.

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The accumulating and the extensive variation in PAs levels under stress conditions suggested that PAs function in adaptive responses to various environmental stresses. Changes in PA levels have been observed in various plant species subjected to a range of abiotic stresses, including drought, high salinity, low and high temperatures, nutrient deficiency, and others (Liu et al., 2007). In some cases, it has been observed that the three most abundant PAs, Put, Spd and Spm, show substantial increases in abundance following abiotic stress (Yang et al., 2007). However, in most cases, only one of the three PAs shows a significant increase.

Plants can be prepared to better tolerate abiotic stress conditions through the exogenous application of chemical compounds as well as the transgenic approaches. PAs is a promising chemical. In this context, the transgenic plants over-producing PAs displayed better stress tolerance than their wild-type counterparts (Kusano et al., 2008), the exogenous PAs application could be employed to improve the tolerance against several abiotic stresses (Li et al., 2013; Sagor et al., 2013). Exogenous application of Put and Spd substantially improved the drought tolerance in soybean (Nayyar et al., 2005). In addition, seed priming with PAs was effective in improving the emergence and seedling growth in sunflower (Farooq et al., 2007).

Alcazar et al (Alcázar et al., 2006a). have reported PA biosynthesis pathway in detail in *Arabidopsis thaliana*. PAs synthesis starts with the synthesis of the diamine Put. This step is done in Arabidopsis by decarboxylation of arginine (Arg) by arginine decarboxylase (ADC), and two additional successive steps involving agmatine iminohydrolase (AIH) and N-carbamoylputrescine amidohydrolase (CPA) activities. Put is then converted into higher molecular weight PAs (Spd and Spm) by Spd synthase (SPDS) and Spm synthase (SPMS) respectively, which add aminopropyl groups. Both enzymes use decarboxylated S-adenosylmethionine (dcSAM) as donor of aminopropyl moieties, resulting from the decarboxylation of SAM in a reaction catalyzed by SAM decarboxylase (SAMDC). Drought induces expression of *ADC2*, *SPDS1*, and *SPMS* (Alczar et al., 2006); *ADC1*, *ADC2*, and *SAMDC2* expression are also induced by cold (Cuevas et al., 2009; Urano et al., 2003). Thus, the expression of several genes involved in PAs biosynthesis is strongly induced by one or more abiotic stresses.

The objectives of the present study were to: 1. To investigate which PAs solution (Put, Spm or Mix) plays the central role in mitigating adversities of drought stress in wheat, 2. To test the role of exogenously applied PAs on enhancing wheat yield parameters under drought stress at post anthesis stage, 3. To assess up to what extent the exogenous applied PAs by different methods could regulate the endogenous PAs concentrations and the expression profile of PAs biosynthetic genes under drought stress in wheat. Hence, to pinpoint if the different methods of PAs application have different strategies for alleviating the damage resulted by drought stress or not.

2. Materials and methods

2.1. Plant materials and growth conditions

Seeds of *Triticum aestivum* var. Sakha 94 were obtained from Wheat Research Department, Agricultural Research Centre, Giza, Egypt. Grains of uniform size were surface-sterilized by treating with a solution of 1% sodium hypochlorite for 10 min, followed by extensive washing with sterile distilled water. PAs concentration used in this study have been optimized in a separate experiment performed on seeds by soaking in the PAs: Put, Spm and mixture of them at concentration (1, 10, and 100 μM). Wheat seeds were soaked in the mentioned concentrations for 10 h and then they were germinated in Petri dishes. Plates were placed in a growth chamber at 22° C, 16 h light (110 μmol $m^{-2}s^{-1}$) and 60–70% relative humidity. A seed was considered to have germinated when the radicle was 2 mm long. Germination percentages were assessed after 1 week of growth, then emerged seeds transferred to pots contain compost and fresh weights of the shoots were measured after another one week. Each PAs treatment was applied to 20 seeds per plate and repeated in four independent experiments. Statistical analyses were performed by one-way ANOVA and the significance of differences between experimental treatments was calculated using the least significant difference (LSD) test with a $P \geq 0.05$. The selected concentrations did not significantly affect seed germination, which was higher than 90% in all PAs concentrations tested (Table 1). However, 100 μM Put, Spm, Put + Spm induced a significant increase in the FW and DW after two weeks. Accordingly, the optimum PAs concentration was 100 μM PAs solution (Put, Spm and Put + Spm) was selected for seed priming and foliar spraying application.

Two experiments were then performed, 1. Pots experiment was carried out to assess the effect of the exogenous application of PAs (Put, Spm and mixture of (Put + Spm)) by seed priming or foliar sprays on the alleviation of water stress adversities. 2. Field experiment was performed to assess the grain yield of the wheat cultivar treated by exogenous PAs application by the two methods and exposed to drought stress at post anthesis stage.

2.1.1. Pots experiment

The experiment was divided into two main groups (group 1, seeds were primed before sowing; group 2, plants were foliar sprayed after applying water stress). Two controls each for general and drought were used. For group 1, seeds were divided into five subgroups; the first 2 subgroups were soaked in tap water then one left as watered control and the other as drought control. Seeds of the other three subgroups were soaked either in 100 μM solutions of Put, Spm or their mixture (100 μM each). All subgroups were left in soaking solutions for 10 h after that, seeds were spread on a filter paper overnight for air-drying then were sown in plastic pots. Thirty seeds were sown in each pot filled with compost and perlite mixtures at a ratio of 2:1. After germination, seedlings were supplied with half strength of Hoagland nutrient solution twice a week. All pots were irrigated with tap water until developing the second leaf (10 DAS) and then irrigation was stopped and the seedlings were thinned to 10 seedlings per pot and the irrigation was continued only for control twice a week throughout the experiment period of 21days after sowing (DAS). The pots were classified based on treatment as follows: Control, Drought, Drought + Put, Drought + Spm and finally Drought + Mixture of (Spm + Put). The experiment was replicated three times and left in the growth chamber under controlled conditions (light intensity

Table 1
PAs effects on germination, seedling growth under. Germination% were taken at 7 days, seedling growth were taken at 14 days after seed priming. Different letters indicate significant differences at $p < 0.05$ compared to plants not exposed to PAs.

Groups	G%	Fw	Dw
Control (water, no PAs)	97.5 ± 1.44 [a]	63.25 ± 1.38 [b]	6 ± 0.00 [b]
1 μM Put	96.25 ± 1.25 [a]	62.5 ± 1.85 [b]	6.25 ± 0.25 [b]
1 μM Spm	96.25 ± 2.39 [a]	64 ± 1.47 [b]	6.25 ± 0.25 [b]
1 μM Put + Spm	95 ± 5.00 [a]	65 ± 1.08 [b]	6 ± 0.00 [b]
10 μM Put	93.75 ± 3.75 [a]	66.75 ± 0.63 [b]	6.25 ± 0.25 [b]
10 μM Spm	95 ± 0.00 [a]	67.5 ± 1.76 [b]	6.5 ± 0.29 [b]
10 μM Put + Spm	95 ± 2.04 [a]	66.75 ± 1.25 [b]	6.5 ± 0.29 [b]
100 μM Put	98.75 ± 1.25 [a]	72 ± 1.08 [a]	7 ± 0.00 [a]
100 μM Spm	95 ± 0.00 [a]	73.5 ± 0.65 [a]	7 ± 0.00 [a]
100 μM Put + Spm	95 ± 0.00 [a]	73.25 ± 2.50 [a]	7.5 ± 0.29 [a]
LSD at $P \geq 0.05$	**4.75**	**2.99**	**0.43**