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ORIGINAL ARTICLE

Differential effects of cold, osmotic stress and abscisic acid on polyamine accumulation in wheat

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Abstract The effects of cold, osmotic stress and abscisic acid (ABA) on polyamine accumulation were compared in the moderately freezing-sensitive wheat (*Triticum aestivum* L.) variety Chinese Spring (CS) and in two derived chromosome 5A substitution lines, CS(*T. spelta* 5A) and CS(Cheyenne 5A), exhibiting lower and higher levels of freezing tolerance, respectively. When compared with the other treatments, putrescine (Put) and spermidine (Spd) levels were much greater after cold treatment, spermine (Spm) following polyethylene glycol-induced (PEG) osmotic stress and Spm and cadaverine (Cad) after ABA treatment. During 3-week cold stress, the Put concentration, first exhibited a transient increase and decrease, and then gradually increased. These alterations may be due to changes in the expression of genes encoding the enzymes of Put synthesis. The Put content was higher in the freezing-tolerant chromosome 5A substitution line than in the sensitive one after 3 weeks of cold. In contrast to cold, ABA and PEG induced a continuous decrease in the Spd level when applied for a period of 3 weeks. The Spm content, which increased after PEG and ABA addition, was twice as high as that of Put during ABA treatment at most sampling points, but this difference was lower in the case of PEG. The Cad level, induced to a great extent by ABA, was much lower when compared with that of the other polyamines. The present experiments indicate that cold, osmotic stress and ABA have different effects on polyamines, and that some of these changes are affected by chromosome 5A and correlate with the level of stress tolerance.

Keywords Abscisic acid · Cold stress · Osmotic stress · Polyamine · Wheat

Abbreviations

ABA	Abscisic acid
ADC	Arginine decarboxylase
Agm	Agmatine
Cad	Cadaverine
cDNA	Complementary deoxynucleic acid
CS	*Triticum aestivum* cv. Chinese Spring
CS(Ch5A)	Chinese Spring (Cheyenne 5A) chromosome 5A substitution line
CS(Tsp5A)	Chinese Spring (*T. spelta* 5A) chromosome 5A substitution line
DAO	Diamine oxidase
dNTP	Deoxyribonucleotide triphosphate
DREB/CBF	Drought responsive element-binding factor/C-repeat-binding factor
DTT	Dithiothreitol
Fr-A1 and Fr-A2	Freezing tolerance A1 and A2 locus
FW	Fresh weight
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
LDC	Lysine decarboxylase
M-MLV	Moloney-murine leukemia virus
ODC	Ornithine decarboxylase
oligodT_{23}	23mer Oligonucleotide dT
PAO	Polyamine oxidase
PCR	Polymerase chain reaction

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PEG	Polyethylene glycol
Put	Putrescine
RNA	Ribonucleic acid
RT	Reverse transcriptase
SAM	*S*-Adenosylmethionine
SAMDC	*S*-Adenosylmethionine decarboxylase
Spd	Spermidine
Spm	Spermine
SSAT	Spermidine/spermine N^1-acetyltransferase

Introduction

Adverse environmental conditions result in significant yield losses in crops. Positively charged polyamines are involved in the stress response through their interaction with negatively charged macromolecules, such as DNA, RNA, proteins and phospholipids, resulting in changes in the physical and chemical properties of the membranes, in the structure of nucleic acids and in the enzyme activities (Galston and Kaur-Sawhney 1990; Bouchereau et al. 1999; Alcázar et al. 2006b). In addition, polyamines are able to detoxify the reactive oxygen species accumulating during abiotic stress (Groppa and Benavides 2008; Rider et al. 2007).

Specific enzymes with amino acid decarboxylase activity are involved in the biosynthesis of polyamines: ornithine decarboxylase (ODC, EC 4.1.1.17) and arginine decarboxylase (ADC, EC 4.1.1.19) for putrescine (Put, 1,4-diaminobutane) and agmatine (Agm, 4-guanidino-butylamine), and lysine decarboxylase (LDC, 4.1.1.18) for cadaverine (Cad, 1,5-diaminopentane). Put is a precursor for spermidine [Spd, *N*-(3-aminopropyl)-1,4-diaminobutane] and spermine [Spm, *N,N′*-bis(3-aminopropyl)-1,4-diaminobutane] through the sequential transfer of aminopropyl groups donated by decarboxylated *S*-adenosylmethionine (SAM), in reactions catalyzed by Spd synthase (EC 2.5.1.16) and Spm synthase (EC 2.5.1.22), respectively. Decarboxylated SAM is produced from SAM by the enzyme SAM decarboxylase (SAMDC, EC 4.1.1.50). In the return direction of the interconversion pathway, Spd and Spm can be acetylated by spermidine/spermine N^1-acetyltransferase (SSAT, EC 2.3.1.57) to produce compounds suitable for oxidation by polyamine oxidase (PAO, EC 1.5.3.3). Oxidoreduction reactions for the degradation of diamines are catalyzed by diamine oxidases (DAO, EC 1.4.3.6) (Bagni and Tassoni 2001; Martin-Tanguy 2001; Medina et al. 2003; Seiler 2004). In nature, polyamines occur as free molecular bases, but are often conjugated to small molecules, such as phenolic acids, or to various macromolecules, such as proteins (Bagni and Tassoni 2001; Martin-Tanguy 2001).

Stress-induced changes in polyamine content, which may derive from altered synthesis, degradation, transfer or conjugation with other molecules, are affected by abscisic acid (ABA), as shown in maize, where the reduced endogenous ABA level in mutants or the reduced synthesis due to chemical inhibition resulted in lower polyamine concentration (Liu et al. 2005). Following ABA treatment, increases were observed in polyamine levels and in the desiccation tolerance of sugarcane embryos, again confirming the role of ABA in the control of polyamine concentrations (Nieves et al. 2001).

The importance of Put in the response to low temperature stress was demonstrated in tomato leaves, in which exogenous Put decreased cold-induced electrolyte leakage, while the inhibition of Put synthesis increased membrane damage (Kim et al. 2002). The accumulation of Put was also observed in alfalfa and wheat during cold hardening, with a decrease in the activity of ADC, a key enzyme of Put synthesis in the control plants, and no change in treated ones (Nadeau et al. 1987). In addition, the Put, Spd and Cad contents increased after cold hardening in a winter wheat genotype, while only the concentrations of the polyamines Spd and Spm increased in a spring wheat variety, indicating the involvement of polyamines in the response to low temperature stress (Szalai et al. 2009).

Osmotic stress resulted in higher Put and Spd levels in cereal leaf protoplasts, which must have been the result of increased synthesis, because their accumulation was prevented by the inhibition of the ADC enzyme (Flores and Galston 1984). The inhibitors of the alternative Put biosynthetic enzyme, ODC, did not affect the Put levels. Osmotic stress induced an increase in Cad, Put and diaminopropane (an oxidation product of Spd or Spm), but reduced the Spd concentration in rape leaf disks and whole seedlings, while only small changes were observed in the Spm and Agm levels (Aziz et al. 1997). The inhibition of various enzymes involved in the polyamine metabolism in rape indicated that osmotic stress did not block Spd synthesis, but stimulated Spd oxidation. In sorbitol-treated lupin, the amount of Put increased both in the roots and leaves, but the activity of the ADC enzyme was only higher in the roots, suggesting the translocation of Put from the roots to the shoots (Legocka and Kluk 2005). Studies on the effect of osmotic stress on polyamines show that coordinated changes occur in their synthesis, degradation and transfer in stressed plants.

Chromosome 5A is a major regulator of freezing tolerance and vernalization requirement in wheat and carries several genes involved in the response to abiotic stress (Galiba et al. 2009). Changes induced by osmotic agents, and by low and high temperature in glutathione,

carbohydrate and free amino acid levels were influenced by this chromosome in stressed wheat (Galiba et al. 1992; Simon-Sarkadi and Galiba 1995; Vágújfalvi et al. 1999; Kocsy et al. 2000; Simon-Sarkadi et al. 2007). The effect of chromosome 5A on Spd content was demonstrated in callus cultures subjected to osmotic stress by comparing substitution lines developed from parents with different levels of tolerance (Galiba et al. 1993). In the present study, the effect of ABA, cold and osmotic stress on polyamine accumulation was compared in chromosome 5A substitution lines differing in their stress tolerance at the whole plant level to obtain new information about the involvement of polyamines in the adaptation of plants to stress conditions and about the possible regulatory role of chromosome 5A.

Materials and methods

Plant material

The experiments were carried out on a specific genetic system consisting of the moderately freezing-sensitive (spring habit) recipient wheat variety *Triticum aestivum* cv. Chinese Spring (CS), and the freezing-tolerant (winter habit) Chinese Spring (Cheyenne 5A) [CS(Ch5A)] and the freezing-sensitive (spring habit) Chinese Spring (*T. spelta* 5A) [CS(Tsp5A)] chromosome 5A substitution lines. The following survival data in a freezing test were obtained for these genotypes after 3 weeks at 2°C in a previous experiment: CS: 16.0%, CS(Tsp5A): 7.1% and CS(Ch5A): 67% (Kocsy et al. 2000). These genotypes also differed in their tolerance to osmotic stress, because the following survival data were detected after their treatment with 19% polyethylene glycol (PEG 4000) for 3 weeks: CS: 39% CS(Tsp5A): 28% and CS(Ch5A): 72%. In these tests, the survival of the plants was evaluated on a scale from 0 (dead) to 5 (no damage) after 2 weeks of recovery following the stress treatments. The seeds were obtained from the Martonvásár Cereal Gene Bank (Agricultural Research Institute of the Hungarian Academy of Sciences, Martonvásár, Hungary).

Growth conditions

The seeds were germinated between two layers of wet filter papers at 25°C for 1 day, then at 4°C for 2 days to synchronize germination and for a further 2 days at 25°C. After germination, the seedlings were grown in hydroponic culture using half-strength Hoagland nutrient solution (Hoagland and Arnon 1950) in an autumn–winter type growth chamber (Conviron PGV-36, Controlled Environments Ltd., Canada) at 18/15°C day/night temperature and 70/75% relative humidity with 16 h illumination at 270 µmol m^{-2} s^{-1} for 12 days prior to the stress period. Cold stress at 2°C day/night, and treatment with 0.1 mmol l^{-1} ABA or 15% PEG (w/v) lasted for 21 days, under the cultivation conditions described above. ABA and PEG were added to the nutrient solutions, which was changed each week during the whole experiment. Aeration was carried out using aquarium pumps. Shoots were collected from the seedlings for biochemical analysis at the beginning of the treatments and after 1, 3, 7 and 21 days of treatment. Ten plants from each line were investigated in each experiment, and the experiments were repeated three times.

Analysis of polyamines

Samples (300–600 mg fresh weight, FW) crushed in liquid nitrogen were extracted with 2 ml cold 10% trichloroacetic acid for 1 h with gentle agitation on a shaker at room temperature (Laboshake LS 500i, C. Gerhardt GmbH & Co. KG, Germany). The extracts were then centrifuged (Heraeus Labofuge 400R, Thermo Fischer Scientific Inc., Germany) at 5,000 rpm for 10 min. The supernatants were filtered through a 0.2 µm pore membrane filter (Sartorius AG, Germany) and stored at −20°C. The analysis of polyamines was carried out on an automatic amino acid analyzer (Amino Acid Analyzer AAA400, Ingos Ltd., Czech Republic) equipped with an Ostion LG ANB ion-exchange column (6 × 3.7 cm). The polyamines were separated by stepwise gradient elution using a Na^+/K^+–citric buffer system (Ingos Ltd., Czech Republic). Colorimetric detection was accomplished at 570 nm after post-column derivatization with ninhydrin reagent (Csomós and Simon-Sarkadi 2002).

Analysis of gene expression by semiquantitative reverse-transcription PCR

Total RNA was extracted using the RNeasy Plant Mini Kit (Qiagen, Germany) according to the instructions of the supplier. During the first-strand cDNA synthesis, a mixture of 1 µl RNA (1 µg), 0.8 µl 70 µM oligodT_{23} and 5.7 µl H_2O was first incubated at 70°C for 10 min, then 1 µl (200 U) M-MLV reverse transcriptase (Promega, USA), 0.125 µl (5 U) RNasin (Promega), 2 µl 100 mM DTT (Reanal, Hungary), 2 µl 10 mM dNTP, 4 µl 5 × RT buffer (Promega) and 4.375 µl H_2O was added and the solution was kept at 25°C for 10 min, then at 42°C for 60 min. Gene sequences coding for ADC, ODC, SpdS, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were obtained from the TIGR Plant Transcript Assemblies wheat section database (http://plantta.jcvi.org/index.shtml). The accession numbers and primers designed for the

sequences are shown in Table 1. The PCR amplification mixture (20 µl) contained 2 µl (0.1 µg) cDNA, 0.1 µl (0.5 U) Go*Taq* DNA polymerase, 0.5 µl 10 mM dNTP, 2 µl 10 mM $MgCl_2$, 0.6 µl each of 10 mM forward and reverse primers, 2 µl 5 × Go*Taq* buffer (Promega) and 12.2 µl H_2O. Amplification by PCR involved 30 (ADC, SpdS), 35 (GAPDH) or 36 cycles (ODC) of denaturation at 95°C for 30 s, primer annealing at 59°C (55°C for *GAPDH*) for 30 s, and extension at 72°C for 90 s. The exponential phase of amplification was determined for each gene by analyzing the gel electrophoresis pattern of the PCR products generated by various numbers of cycles. The constitutively expressed *GAPDH* gene was used for the normalization of cDNA concentrations (Kellős et al. 2008).

Statistical analysis

Samples from three independent experiments were analyzed using the statistical computer package of Microsoft Excel 2007. The relative standard deviations of the data were below 10% in each case.

Results

The applied treatments reduced the growth of the plants and resulted in less if any visible damage to the shoots (data not shown). Four polyamines were detected in the moderately freezing-sensitive CS wheat variety and in the freezing-sensitive CS(Tsp5A) and freezing-tolerant CS(Ch5A) chromosome 5A substitution lines during ABA treatment, cold and osmotic stress. The main polyamine was Spd, followed by Put, Spm and Cad. The concentration of the polyamines was usually higher after 3-week treatment when compared with the corresponding controls.

The changes caused in Put by the treatments are illustrated for all the genotypes in Fig. 1. After an initial transient increase and decrease, the Put concentration gradually increased after 7 days in CS (Fig. 1a) and CS(Ch5A) (Fig. 1c), while in the case of CS(Tsp5A) (Fig. 1b) a continuous increase was observed during the 3-week cold stress. The Put content was higher after 3-week cold stress in the freezing-tolerant CS(Ch5A) chromosome substitution line than in the sensitive genotypes, CS(Tsp5A) and CS. Following osmotic stress induced by PEG, the Put content showed similar changes in CS and CS(Tsp5A). In the two chromosome substitution lines, the amount of Put exhibited transient increases and decreases during the first week of ABA treatment, with two maximums after 1 and 7 days, followed by a gradual decrease. The reverse tendency was observed in CS during the first 7 days of treatment.

After an initial decrease, the Spd concentration gradually increased during the 3-week cold stress in all genotypes (Fig. 2). In contrast to cold, a continuous decrease in the Spd level was induced by ABA and PEG during the 3-week treatment. While cold stress induced a large increase in the Spd (Fig. 2) and Put (Fig. 1) concentrations, their levels were only slightly affected, if at all, by the other two treatments.

In the ABA treatment, the Spm level (Fig. 3) exhibited a first peak after 1 day, and a second after 7 days. The amount of Spm was also increased by PEG, but this change was smaller as compared to the effect of ABA, except after 21 days. Like ABA, PEG also induced a transient increase in Spm content at the beginning of the treatment with a maximum after 1 day, but from then on the Spm concentration increased continuously except in the case of CS(Tsp5A) (Fig. 3b), where a saturation curve was observed.

The cadaverine (Cad) level was much lower when compared with the other polyamines, with two maximums after 1 and 7 days of ABA treatment in CS (Fig. 4a) and CS(Ch5A) (Fig. 4c), while in the case of CS(Tsp5A) (Fig. 4b), a continuous increase was observed during the first week, followed by a decrease in all genotypes. The Cad levels were similar to those observed in control plants after 21 days of PEG treatment. The same Cad concentrations were detected following 3 weeks cold and ABA treatment.

Table 1 Primers for arginine decarboxylase *(ADC)*, ornithine decarboxylase (*ODC*), spermidine synthase (*SpdS*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) genes

Name	Accession	Primer sequence (forward/reverse)	Product (bp)
ADC	TA61493_4565	5′-CAATGCCGTACCTGTCGTTC-3′	130
		5′-AACTCCCACTCCTCGTCATC-3′	
ODC	TA89456_4565	5′-GTCGACGTGTACGTCTTCTCG-3′	113
		5′-TGGATCGACGACGGCCTCTA-3′	
SpdS	TA63060–4565	5′-GGATGGTTCTCCGAGATCAG-3′	104
		5′-ACCAGCACGTCTTGGTAGT-3′	
GAPDH	CAA33620	5′-AGGGTGGTGCCAAGAAGGTTG-3′	623
		5′-GTAGCCCCACTCGTTGTCGTA-3′	

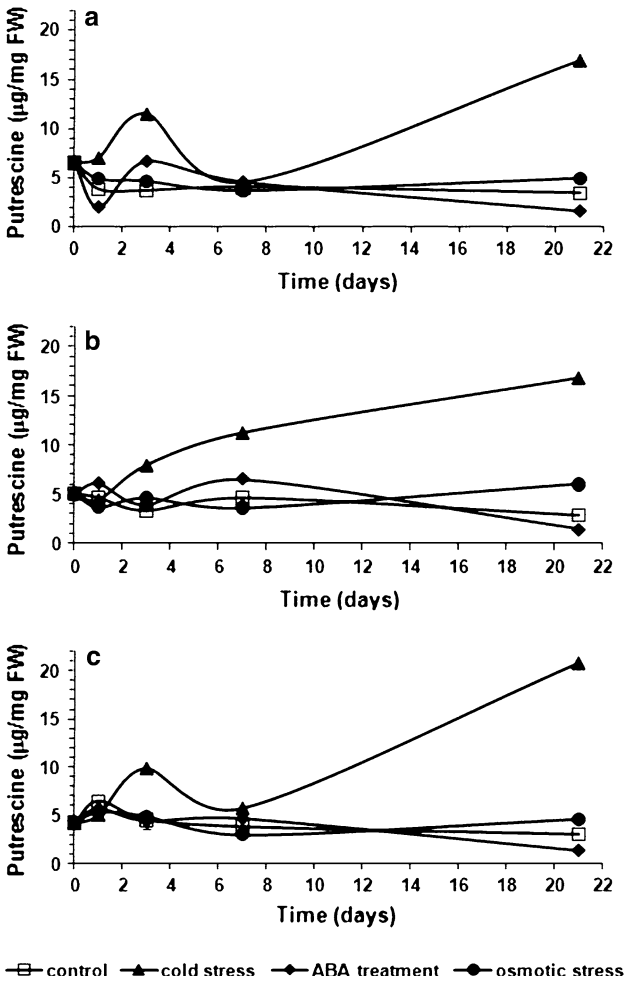

Fig. 1 Effect of different treatments on putrescine content in the freezing-sensitive CS (**a**) and CS(Tsp5A) (**b**) and in the freezing-tolerant CS(Ch5A) (**c**) wheat genotypes. All treatments were repeated three times with similar results. The relative standard deviations of the data were below 10% in each case

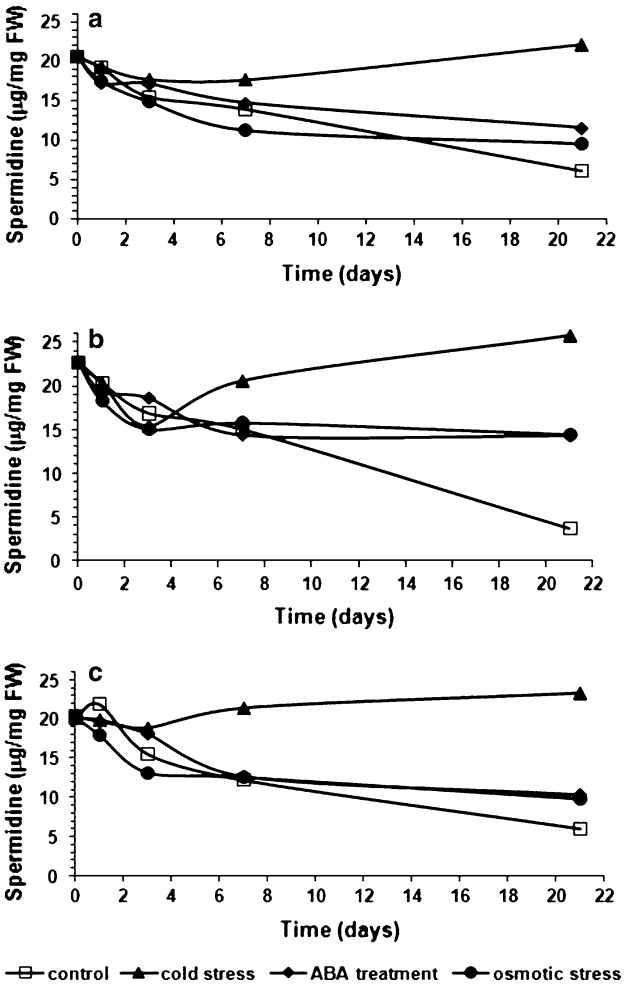

Fig. 2 Effect of different treatments on spermidine content in the freezing-sensitive CS (**a**) and CS(Tsp5A) (**b**) and in the freezing-tolerant CS(Ch5A) (**c**) wheat genotypes. All treatments were repeated three times with similar results. The relative standard deviations of the data were below 10% in each case

When compared with the control, greater changes were observed in the relative amounts of the four polyamines in all the genotypes after 3 and 21 days of cold, after 1 and 7 days of ABA treatment and after 7 and 21 days of PEG treatment (Fig. 5). These tended to be a decrease in Spd and an increase in the Put ratio during cold stress, and a decrease in Spd and a significant increase in Spm ratio during ABA and PEG treatments in all genotypes.

Chromosome 5A could be seen to influence the polyamines after the cold and PEG treatments. After 21 days of cold stress, the Put content was 23% higher in the freezing-tolerant line CS(Ch5A) than in the sensitive genotypes CS and CS(Tsp5A), while the Spm and Cad contents were lower. There was a correlation between freezing tolerance and polyamine levels (with r values of 0.99 for Put, −0.92 for Spm and −0.78 for Cad). After 7 days of PEG treatment, the Spm content was also lower in CS(Ch5A) than in the other two genotypes, and there was a negative correlation ($r = -0.97$) between Spm level and tolerance to osmotic stress.

Cold-induced changes in the expression of genes encoding enzymes involved in polyamine synthesis were also investigated to see whether the correlation observed between polyamine concentrations and freezing tolerance also existed at the transcript level. The expression of the *ADC* gene increased after 1 day at 2°C, while a higher transcript level was only observed for the *ODC* gene after 7 days (Fig. 6). The expression of the Spd synthase gene was only slightly affected by cold. The transcript levels detected for each gene were similar in all genotypes at the individual sampling points.

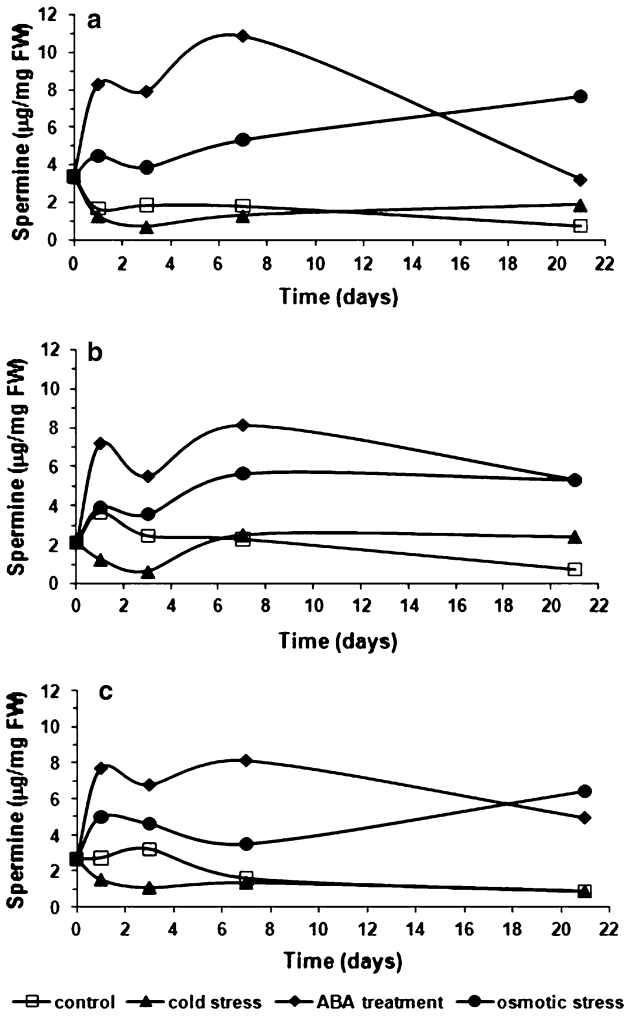

Fig. 3 Effect of different treatments on spermine content in the freezing-sensitive CS (**a**) and CS(Tsp5A) (**b**) and in the freezing-tolerant CS(Ch5A) (**c**) wheat genotypes. All treatments were repeated three times with similar results. The relative standard deviations of the data were below 10% in each case

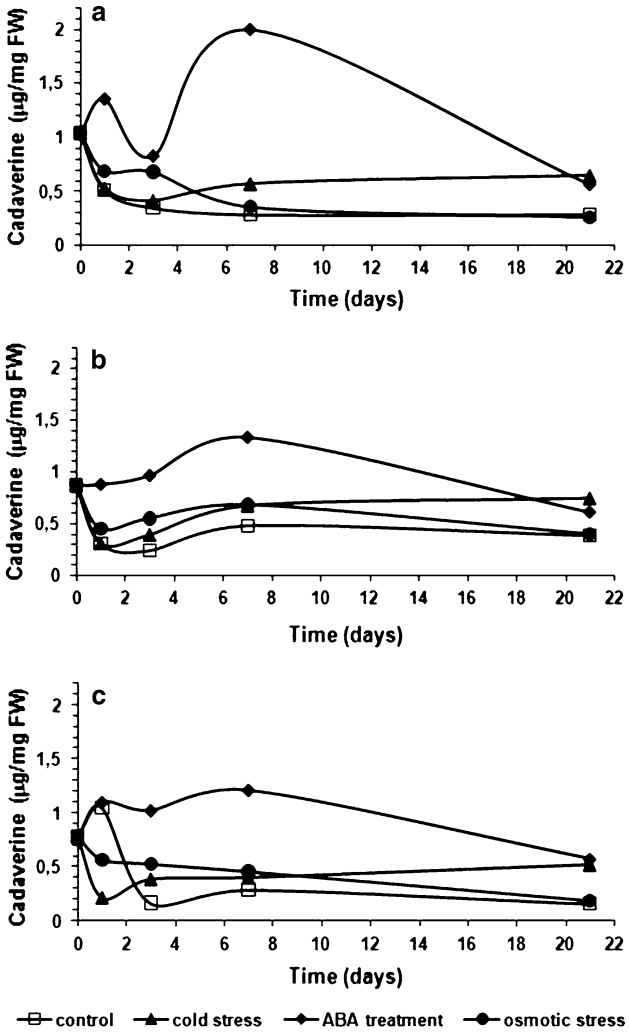

Fig. 4 Effect of different treatments on cadaverine content in the freezing-sensitive CS (**a**) and CS(Tsp5A) (**b**) and in the freezing-tolerant CS(Ch5A) (**c**) wheat genotypes. All treatments were repeated three times with similar results. The relative standard deviations of the data were below 10% in each case

Discussion

The present study demonstrated that the various abiotic stresses and plant hormones have specific effects on the time course of changes in the concentrations of individual polyamines during a 3-week treatment period. During the first week of the treatments, great alterations rapidly occurred in their levels, which could be a sign of disturbed metabolism rather than their involvement in the initial adaptation process, since these alterations were observed in all three wheat genotypes irrespective of their stress tolerance. However, during the second and third week, much slower changes were detected, if at all, in the polyamine levels, which could indicate the adjustment of the metabolism to the altered environmental conditions.

The correlation between cold-induced changes in polyamine levels (Put, Spm, Cad) and freezing tolerance after 3 weeks of cold treatment in wheat indicates the involvement of polyamines in adaptation to low temperature. In a similar study, cold-induced alterations in Put content were found to depend on the level of freezing tolerance when other wheat genotypes with different levels of freezing tolerance were compared (Szalai et al. 2009). The increase in polyamine content observed in the present study originated from the increased transcription of the *ADC* and *ODC* genes. The early increase in polyamine content was based on the arginine pathway, but the ornithine pathway also contributed to the later elevation in their amounts, as shown by the increased transcript levels of the corresponding genes. In contrast, only the inhibition of the arginine pathway affected Put levels in rice (Lee et al. 1997). Because similar expression levels were found for *ADC* and *ODC* genes at the individual sampling dates for

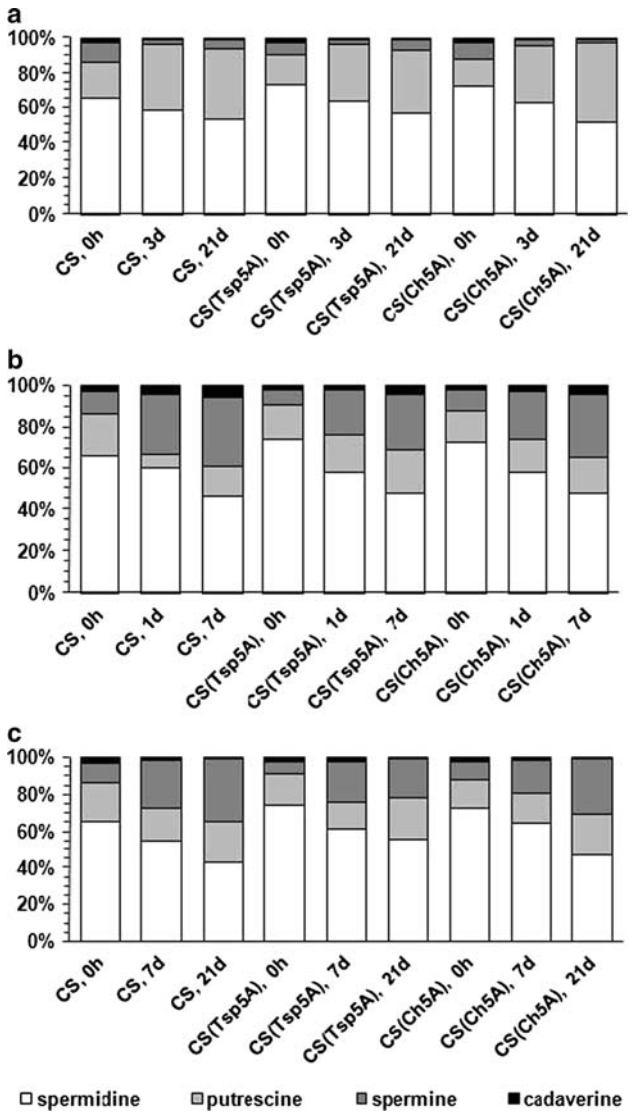

Fig. 5 Changes in the relative ratio of polyamines during cold stress (**a**), ABA treatment (**b**) and osmotic stress (**c**) in the freezing-sensitive CS and CS(Tsp5A) and in the freezing-tolerant CS(Ch5A) wheat genotypes. All treatments were repeated three times with similar results

the three wheat genotypes in the present study, the differences detected in the polyamine levels may result from the post-transcriptional regulation of their synthesis. Indeed, the higher Put content in a cold-tolerant rice cultivar compared with a sensitive cultivar was based on the higher ADC activity (Lee et al. 1997). *Arabidopsis* mutants with defective Put biosynthesis (*adc1, adc2*) displayed reduced freezing tolerance when compared with wild-type plants, demonstrating the protective role of this polyamine (Cuevas et al. 2008). The role of Put in the stress response was also demonstrated in transgenic *Arabidopsis* overexpressing the gene encoding the ADC enzyme, which resulted in increased Put content and the downregulation of several genes encoding transcription factors involved in the response to abiotic stress (Alcázar et al. 2005).

Although a relationship was found between osmotic stress-induced changes in Put and Cad contents and drought tolerance in wheat callus cultures (Galiba et al. 1989), no such correlation was found after osmotic stress at the whole plant level in the present study. The induction of polyamine synthesis in *Arabidopsis* subjected to osmotic stress took place at the transcriptional level, since a higher expression of the *ADC* gene was observed (Urano et al. 2003). In addition, transgenic rice overexpressing the *ADC* gene exhibited increases in both Put, Spd and Spm contents and in drought tolerance (Capell et al. 2004). The modification of ADC enzyme activity by stress treatment was demonstrated in cereals (Flores and Galston 1984). These observations show that osmotic stress influences polyamine contents at several levels.

The effect of ABA on polyamine levels, observed for Spm and Cad in wheat, was also demonstrated in ABA-treated sugarcane (Nieves et al. 2001). Exogenous ABA increased the Put and Cad contents during the first 24 h of application in chickpea (Bueno and Matilla 2008). The reduction in endogenous ABA levels, either as a result of mutation or due to the use of a chemical inhibitor, led to a decrease in polyamine levels in maize (Liu et al. 2005).

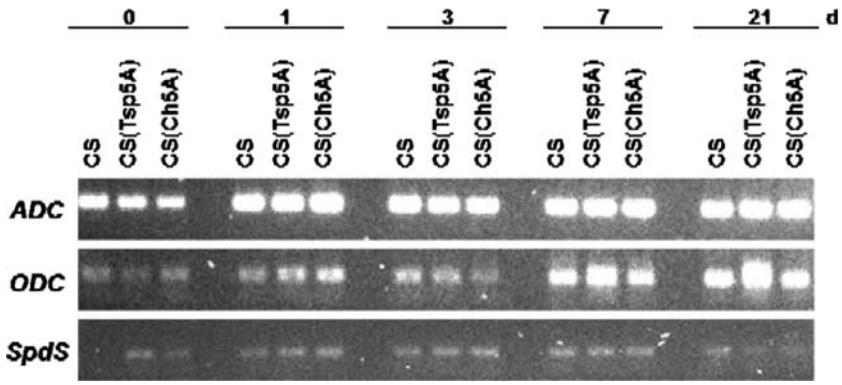

Fig. 6 Effect of cold treatment on the expression of arginine decarboxylase (*ADC*), ornithine decarboxylase (*ODC*) and spermidine synthase (*SpdS*) genes in the freezing-sensitive CS and CS(Tsp5A) and in the freezing-tolerant CS(Ch5A) wheat genotypes. All treatments were repeated three times with similar results

These studies indicate that ABA controls polyamine levels and this regulatory effect may be involved in the protective effect of ABA against abiotic stresses.

Following cold, osmotic stress and ABA treatment, it was expected that the changes in polyamine concentrations would have similar time courses, since all these abiotic stresses result in oxidative stress and the redox and ABA-related signaling pathways are interconnected (Foyer and Noctor 2009). In contrast to this hypothesis, in the present study in wheat higher levels were observed for Put and Spd after cold treatment, for Spm after osmotic stress and for Spm and Cad after ABA treatment compared with the other treatments. These results indicate that despite the possible convergence in certain parts of the various signaling pathways, they may diverge at the end and may induce specific changes in the levels of individual metabolites. Thus, each polyamine may have a special role in the response to different environmental effects. In accordance with this, a Spm-deficient *Arabidopsis* mutant exhibiting drought hypersensitivity was cured by pretreatment with Spm, but not with the other polyamines, Put or Spd (Yamaguchi et al. 2007). As well as being target molecules in stress signaling pathways, polyamines may also be involved in signal transduction, as observed in *Arabidopsis* mutants with defective Put biosynthesis and in transgenic *Arabidopsis* overexpressing Spd synthase (Cuevas et al. 2008; Kasukabe et al. 2004). In Put-defective mutants cold stress led to the reduced expression of the gene coding for the key enzyme of ABA biosynthesis and to the down-regulation of ABA-related genes, but this was compensated by exogenous ABA, indicating that the influence of Put on freezing tolerance is mediated by ABA (Cuevas et al. 2008). This mutual effect of Put and ABA is not surprising because a previous study in rice showed that the inhibition of ABA synthesis also reduced ADC activity and Put content (Lee et al. 1997). Similarly, the drought-induced increase in the expression of ADC, Spd and Spm synthase genes was impaired in ABA-deficient or ABA-insensitive Arabidopsis mutants, indicating the regulatory effect of ABA on the polyamine metabolism at the transcriptional level (Alcázar et al. 2006a).

The present genetic system was designated to demonstrate the possible effect of chromosome 5A, a major regulator of stress responses in wheat, on stress tolerance-related changes in polyamine levels. While at the callus level chromosome 5A affected the accumulation of Spd during osmotic stress (Galiba et al. 1993), in the present study it was found to influence that of Spm, indicating that under stress conditions the polyamine metabolism also depends on the type of tissues and organs. The influence of chromosome 5A on the Spm content was observed after 1 week of osmotic stress, but its effect on the Put, Spm and Cad contents was only detected after 3 weeks in the case of cold, showing the stress- and polyamine-specific nature of its influence. The interesting relationship between chromosome 5A and polyamine levels is shown by the fact that the genes encoding the cold-responsive *DREB/CBF* (drought responsive element-binding factor/C-repeat-binding factor) transcription factors are localized on chromosome 5A (Vágújfalvi et al. 2003), while their expression depends on the Spd level (Kasukabe et al. 2004). The *DREB/CBF* genes are linked to the *Fr-A2* (freezing tolerance A2) locus, but there is also another locus, *Fr-A1*, controlling freezing tolerance on this chromosome; *Fr-A1* may affect polyamine levels, these in turn may control DREB/CBF expression.

The present experiments demonstrated that some of the stress-specific changes induced in polyamine levels in wheat are affected by chromosome 5A and are correlated with the level of stress tolerance.

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