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Research article

Modulation of spermidine and spermine levels in maize seedlings subjected to long-term salt stress

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Abstract

Salinity is one of the major abiotic stresses affecting plant agriculture worldwide. Polyamines, a group of aliphatic amines, are known to accumulate under salt stress conditions in different plant systems, resulting in presumed protective effects, acting as free radical scavengers, stabilizing cellular membranes and maintaining cellular ionic balance under these conditions. In the present study, we measured the polyamine content in maize leaves of semi-hydroponically grown seedlings subjected to 1 and 7 days of salt stress. We observed that the maize plants tend to maintain or accumulate the levels of spermidine and spermine, while putrescine levels fluctuate depending on the NaCl concentration. The effect of salt stress on the expression of the main genes involved in polyamine biosynthesis was also assessed. Our data show a time and NaCl dependent regulation of the *Zmspds2* and *Zmspds1* genes, suggesting that the former might be hyperosmotic responsive while the later NaCl responsive. Interestingly, the maize *adc*, *Zmspds1* and *Zmspds2* genes are regulated at the transcriptional level by the plant growth regulator abscisic acid. A connection between polyamine metabolism, abiotic stress and abscisic acid is discussed.

Keywords: Abscisic acid; Polyamines; Salt stress; *Zea mays*

1. Introduction

Salinity is one of the major abiotic stresses affecting plant agriculture worldwide. Most of the commercially important crops are salt sensitive and as a consequence salinity is becoming a threat to food supply [11]. High salt-stress disrupts homeostasis of water potential and ion distribution which results in plant molecular damage, growth arrest and even death [47]. In order to achieve salt tolerance, plants rely on three main strategies: detoxification, homeostasis re-establishment and growth regulation. These processes involve the production of stress proteins, the accumulation of compatible solutes (sugars, amino and organic acids, betaines and polyamines) and the expression of different set of genes [39,47].

Polyamines (PAs) are small organic cations, involved in both developmental [23] and stress responses in plants [7]. Putrescine (Put) biosynthesis in plants can be addressed by two decarboxylases: ornithine decarboxylase (ODC; EC 4.1.1.17) and arginine decarboxylase (ADC; EC 4.1.1.19). The higher PAs, spermidine (Spd) and spermine (Spm) are synthesized by spermidine and spermine synthase (SPDS; EC 2.5.1.16 and SPMS; EC 2.5.1.22) by the successive addition of aminopropyl groups to Put. The aminopropyl moiety is derived from methionine, which is first converted into *S*-adenosylmethionine (SAM) and then decarboxylated via *S*-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50).

Abbreviations: ABA, abscisic acid; ADC, arginine decarboxylase; ODC, ornithine decarboxylase; PAs, polyamines; PCR, polymerase chain reaction; Pro, proline; Put, putrescine; RT–PCR, reverse-transcriptase polymerase chain reaction; SAMDC, S-adenosylmethionine decarboxylase; Spd, spermidine; SPDS, spermidine synthase; Spm, spermine; SPMS, spermine synthase.

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PA accumulation under salt stress has been reported for both mono and dicotyledonous plants [7]. In this sense, seedlings of *Lupinus luteus* accumulate Put and Spd in leaves in response to NaCl [26]. Accumulation of Spd and Spm rather than Put has been reported in rice and tomato salt tolerant cultivars in comparison to the salt sensitive cultivars [25,40]. The function of PAs is presumed to be protective, acting as free radical scavengers, stabilizing cellular membranes and maintaining cellular ionic balance [4,10,12].

Transcriptional regulation of some polyamine biosynthetic genes, mainly *adc*, has been reported under salt stress. Accumulation of the *adc* transcript was observed in apple shoots subjected to 300 mM NaCl [15]. Brassica species such as *Arabidopsis thaliana* and *Pringlea antiscorbutica* accumulate the *adc2* transcript in response to salt stress [19,44], while in mustard (*Brassica juncea*) the *adc3* transcript is accumulated in this response [31]. In the case of *samdc* genes, an up-regulation was observed while treating mustard leaves with 400 mM of NaCl [18]. In a previous work, we reported the up-regulation of two maize *spds* transcripts (*Zmspds2A* and *Zmspds2B*), both generated by alternative splicing, in a detached leaf system with short-term salt treatments [37]. Despite the importance of these findings, we were interested in knowing whether longer salt stress treatments lead to higher PAs (Spd and Spm) accumulation and if this is a result of the transcriptional regulation of the *adc*, *samdc*, *Zmspds1* and *Zmspds2* polyamine biosynthetic genes. In addition, evidence for the induction of PA biosynthetic genes at the transcriptional level by abscisic acid is presented.

2. Methods

2.1. Plant material

Seeds of *Zea mays* cv. cafime were surface disinfected with 50% commercial sodium hypochlorite solution (6% free chlorine) for 30 min, and rinsed several times with sterile distilled water. Aseptic seeds were germinated in an agrolite/water support, grown semi-hydroponically in plastic pots filled with a mixture of sand and agrolite (3:1) and sub-irrigated with half strength Hoagland's nutrient solution [17]. Pots, each containing one plant, were maintained in growth chambers under a 12 h light (13,000 lux) and 12 h dark cycle, at 25 ± 2 °C. Ten 10-day-old seedlings were subjected to 1 and 7 days of salt stress by adding 0, 25, 150 and 400 mM of NaCl to the Hoagland's nutrient solution. After each salt treatment, leaves were transferred to liquid nitrogen for subsequent total RNA isolation, and estimation of polyamines, proline and total chlorophyll content. Plant growth under salt stress was estimated as the total shoot height at time n compared with its height at the beginning of the experiment. Height at time n (H_n) from each plant was normalized to its own initial height (H_i) at the beginning of the experiment, according to the formula:

$$\text{Shoot growth} = \frac{H_n - H_i}{H_i}$$

2.2. Proline and total chlorophyll estimation

Proline (Pro) content was determined spectrophotometrically at 520 nm following the ninhydrin method described by Magne and Larher [28], using pure Pro (Sigma) as standard. Free Pro was extracted from leaves of salt stressed maize seedlings (1, 7, 10 and 14 days) by boiling 0.5 g of the vegetal material in 2 ml of distilled water. Then 500 μl of sodium citrate (0.2 mol l^{-1}, pH 4.6) and 2 ml of 1% ninhydrin (acetic acid/water, 60:40, v/v) were added to 500 μl of the vegetal extract. The mix was boiled 1 h, 2 ml toluene was added for the extraction and then centrifuged. Organic phase was read spectrophotometrically.

Total chlorophyll was estimated in 80% acetone extracts obtained from the same samples mentioned above for Pro determination according to Arnon [3]. One-way-ANOVA and Tukey post-test were performed to assess statistical significance between treatments, using GraphPad Prism 3.0 software.

2.3. Free polyamines determination

Free polyamines (Put, Spd and Spm) were estimated as dansyl-derivatives by reversed phase HPLC in leaves of salt-stressed maize seedlings (1 and 7 days) as described by Marcé et al. [29]. In general, 300 mg of the plant material were extracted with 1 ml of 5% (v/v) perchloric acid (PCA) and then incubated overnight at 4 °C. The extracts were recovered by centrifugation and 200 μl of the supernatants were dansylated in a mixture containing 100 μl of saturated Na_2CO_3, 200 μl dansylchloride (5 mg ml^{-1} acetone) and 5 μl of 100 μM 1,7-diamino heptane (HTD) as internal standard. The mixture was incubated overnight in darkness at room temperature. The reaction was stopped by adding 100 μl proline (100 mg ml^{-1}) and dansylated polyamines were extracted with 400 μl toluene. Organic phase was vacuum-evaporated and dansylated polyamines were dissolved in 100 μl acetonitrile and analyzed by HPLC.

2.4. Effect of abscisic acid on the expression of polyamine biosynthetic genes

Leaves of 10-day-old seedlings grown in semi-hydroponia, were detached, and the cut end immersed in a sterile distilled water solution containing 100 and 200 μM ABA at 25 ± 2 °C for 1, 6 and 11 h. Control leaves were immersed in sterile distilled water. After each time point, leaves were transferred to liquid nitrogen and their total RNA was isolated.

2.5. RNA isolation and RT–PCR assays of gene transcripts

Total RNA was isolated from maize leaves using the RNAqueous® Kit (Ambion, USA). After DNase I treatment, first strand cDNA synthesis was performed in a total volume of 30 μl using the SuperScript™ First-Strand Synthesis System for RT–PCR (Invitrogen, USA), as described previously [37]. One microliter of the RT reaction was used as template