Phytochemistry xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Deciphering the protective role of spermidine against saline–alkaline stress at physiological and proteomic levels in tomato

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ARTICLE INFO

Article history:
Received 10 January 2014
Received in revised form 23 November 2014
Available online xxxx

Keywords:
Tomato (*Solanum lycopersicum* L.)
Solanaceae
Proteomics
Saline–alkaline stress
Spermidine

ABSTRACT

In this research, the protective effect of spermidine (Spd) in mitigating saline–alkaline stress in tomato (*Solanum lycopersicum* L.) at physiological and proteomic levels were examined. The results showed that saline–alkaline stress induced accumulation of H_2O_2 and $O_2^{\cdot-}$, and increased the activities of antioxidase (SOD, CAT, and POD). Spermidine efficiently alleviated the inhibitory role of saline–alkaline on plant growth and inhibited saline–alkaline stress-induced H_2O_2 and $O_2^{\cdot-}$ accumulation. Proteomics investigations of the leaves of tomato seedlings, responding to a 75 mM saline–alkaline solution and 0.25 mM Spd, were performed. Maps of the proteome of leaf extracts were obtained by two-dimensional gel electrophoresis. An average of 49, 47 and 34 spots, which appeared repeatedly and that significantly altered the relative amounts of polypeptides by more than twofold, were detected for seedlings treated with saline–alkaline solution (S) compared to normal solution (CK), saline–alkaline plus spermidine (MS) compared to CK, or S versus MS, respectively. Thirty-nine of these proteins were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry and were classified into five functional categories, including energy and metabolism, signal transduction, amino acid metabolism, protein metabolism, and stress-defense response. Proteomics analysis coupled with bioinformatics indicated that Spd treatment helps tomato seedlings combat saline–alkaline stress by modulating the defense mechanism of plants and activating cellular detoxification, which protect plants from oxidative damage induced by saline–alkaline stress.

1. Introduction

With increased intensive agricultural practices in many countries, soil salinity becomes a severe problem. Munns and Tester (2008) reported that more than 6% of global land area and 20% of irrigated lands are affected by soil salinity. Two distinct types of plant stresses, alkaline salt stress and neutral salt stress, are referred to as alkalinization and salinization, respectively (Shi and Yin, 1993). Soil salinization and alkalinization often take place simultaneously in nature. Soil that is naturally saline and alkaline is very complex. Saline–alkaline stress reduces plant growth and leads to wilting or death. Ion toxicity, nutrient limitation, high-osmotic stress, and oxidative stress due to increased salinity may be the main reason of seriously disrupted protein synthesis due to inhibition of normal enzymatic activities (Rajaei et al., 2009). These can reduce enzymatic activities or even degrade some proteins (Guo et al., 2012). As a result of genotypic differences and various environmental conditions, plants have developed different adjustment mechanisms to reduce injuries from salt. The levels of polyamines and the activities of S-adenosylmethionine decarboxylase and diamine oxidase increase significantly in tomatoes exposed to saline–alkaline stress (Hu et al., 2012). This also increases contents of ammonium salts, and the activities of NADH-dependent glutamate dehydrogenase. In addition, the

Abbreviations: ROS, reactive oxygen species; Spd, spermidine; Spm, spermine; 2-DE, two-dimensional gel electrophoresis; MALDI-TOF/TOF-MS, matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; Rubisco, ribulose 1,5-bisphosphate carboxylase; SBPase, sedoheptulose-1,7-bisphosphatase; OEE2, oxygen-evolving enhancer protein 2; RuBP, ribulose-1,5-bisphosphate; CER, ceramidase; ABP, auxin-binding protein; AMT, aminomethyl transferase; GS, glutamine synthase; GST, glutathione S-transferase; MDAR, monodehydroascorbate reductase.

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http://dx.doi.org/10.1016/j.phytochem.2014.12.021
0031-9422/

activities of glutamate pyruvate transaminase, nitrate reductase, NADH-dependent glutamate synthase, glutamine synthetase, and glutamate oxaloacetate transaminase are reduced (Zhang et al., 2013). Ions and reactive oxygen species (ROS) may also accumulate and cause harm to plant cells.

Homeostasis of intracellular Na^+/K^+, which can be maintained by Na^+ sequestration and extrusion, is related to expression of *AtNHX1*, *AVP1*, *AtSOS1*, *AtSOS2* and other stress-related genes (Liu et al., 2000; Shi et al., 2000). Additional stress-related genes include *SKC1* in rice (Ren et al., 2005) and *GhNHX1* in tobacco (Wu et al., 2004). Adaptation of plants to stress is associated with profound proteomic changes (Kosová et al., 2011). Salt stress alters gene expression, which affects protein expression (Hasegawa et al., 2000; Seki et al., 2003). During adverse conditions, many regulatory proteins rapidly adopt active conformations (Guo et al., 2010; Ngara et al., 2012). Proteomics is an integrated analysis of proteins based on a particular organism, tissue, or cell at a given time (Ngara et al., 2012). Different proteomics approaches can be applied to identify the molecular networks involved in the stress reaction of plants (Ngara et al., 2012). These methods have been used to study the expression of proteins related to salt stress in potato (*Solanum tuberosum* L.) (Aghaei et al., 2008), watermelon (*Citrullus lanatus* L.) (Yang et al., 2012), foxtail millet (*Setaria italica* L. cv Prasad) (Veeranagamallaiah et al., 2008), and cucumber (*Cucumis sativus* L.) (Li et al., 2013).

Polyamines play an important role in growth, cell division, DNA replication, and protein synthesis (Roychoudhury et al., 2011). The major polyamines in plants are spermine (Spm), spermidine (Spd), and their precursor, putrescine (Put). These polyamines act as second messengers that mediate responses to various environmental stressors, including osmotic stressors, changes in salinity, drought, ozone, heavy metals, and ultraviolet exposure (Alcázar et al., 2006; Groppa and Benavides, 2008). Previous studies suggest that exogenous Spd can relieve the nitrogen metabolic disturbances induced by salinity–alkalinity treatment, eventually promoting plant growth (Zhang et al., 2013). These studies provide useful evidence on the effects of exogenous polyamines on plant growth and the accumulation of osmoprotectants for tomatoes exposed to saline–alkaline stress. However, there is a need to identify specific mechanisms by which tomatoes tolerate saline and alkaline stress. Using proteomics tools, to investigate the expression of proteins in plants exposed to saline–alkaline stress, would probably be a better choice for finding out how the Spd-induced cellular activities occurred.

The aim of this study was to identify protein expression changes in response to Spd treatment during saline–alkaline stress in tomato leaf extracts. Two-dimensional gel electrophoresis (2-DE) coupled with matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF-MS) were used. This study will help in understanding the proteomic mechanisms and potential mechanisms of stress resistance induced by Spd during saline–alkaline condition.

2. Results

2.1. Physiological measurements

As compared with the control, salinity–alkalinity stress significantly decreased the plant height, stem diameter, dry and fresh weights of tomato seedlings after 8 days of treatment ($P < 0.05$) (Table 1). Under stress conditions, Spd treatment enhanced plant height, stem diameter, dry and fresh weight by 10%, 6%, 22% and 23%, respectively. These indices showed that saline–alkaline stress noticeably inhibited the growth of tomato seedlings, in addition, Spd partially mitigated injuries to seedlings.

Table 1
Effects of exogenous Spd on plant height, stem diameter, fresh and dry weights of tomato seedlings grown under saline–alkaline combined stress ($n = 10$).

	CK	M	M/S
Plant height (cm)	30.74 ± 0.58a	26.22 ± 1.06c	28.82 ± 0.56ab
Stem diameter (mm)	5.89 ± 0.10a	5.47 ± 0.12b	5.80 ± 0.17a
Fresh weight (g/plant)	33.88 ± 1.23a	18.21 ± 0.42c	22.34 ± 0.77b
Dry weight (g/plant)	4.42 ± 0.18a	2.31 ± 0.04c	2.81 ± 0.12b

Data within the same row followed by different lower-case letters are statistically different at $P < 0.05$, according to Duncan's multiple range test. CK, 1/2 Hoagland solution; M, 75 mM saline–alkaline solution ($NaCl:Na_2SO_4:NaHCO_3:Na_2CO_3 = 1:9:9:1$); MS, sprayed with 0.25 mM Spd and treated with 75 mM saline–alkaline solution.

2.2. The increased activities of antioxidant enzymes efficiently alleviated ROS toxicity in tomato leaves

Saline–alkaline stress significantly reduced superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities ($P < 0.05$), resulting in higher accumulation of H_2O_2 and $O_2^{\cdot-}$ (Fig. 1) compared with the control. In comparison with saline–alkaline stress, Spd significantly ($P < 0.05$) increased SOD and CAT activities and decreased ROS contents. However, there were no significant differences in the POD activities of tomato seedlings treated with saline–alkaline solution compared to saline–alkaline plus Spd treatment.

2.3. Differentially expressed proteins in leaves treated with or without Spd-during saline–alkaline stress

To determine potential mechanisms responsible for Spd-mediated tolerance to saline–alkaline stress in tomato plants, 2-DE whole-protein profiling in leaves were performed. Control and groups treated with 75 mM saline–alkaline solution were compared, with or without 0.25 mM Spd for 4 days. To visualize the extracted proteins, 2-DE separation and silver staining were used (Fig. 2). Image Master 2D Platinum 6.0 software was used to estimate the relative intensities of protein spots, with 3 replicates. The modified TCA/acetone-extraction and silver staining method established an average of more than 950 detectable spots on each 2-DE gel. Compared with the control, saline–alkaline treatment decreased protein yield and detectable spots numbers, while the saline–alkaline plus Spd treatment can dilute this stress-mediated inhibitory effect (Supplementary Table 1). Some differentially displayed proteins were further explored by MALDI-TOF-MS/MS analysis and searched against the NCBI non-redundant database. Subsequent comparisons of samples from the saline–alkaline group, saline–alkaline plus Spd group, and control group showed that 130 protein species were differentially changed in the leaf extract ($P < 0.05$) by more than twofold (Fig. 2). There were 34, 47, and 49 spots of differentially exhibiting protein species on 2-DE gels comparing control and saline–alkaline treatments, control and saline–alkaline plus Spd treatments, saline–alkaline and saline–alkaline plus Spd treatments, respectively. Among 49 differentially proteins exhibiting changes between the saline–alkaline and saline–alkaline plus Spd treatment groups, 40 spots were increased in size in the saline–alkaline plus Spd treatment group, while 9 spots were increased in size in the saline–alkaline treatment group. There were 15 and 12 spots that were newly disappeared and down-accumulated in the saline–alkaline treatment relative to the control group, respectively, while 7 spots were up-accumulated by saline–alkaline stress relative to the control. In addition, it was found that 23 protein spots were increased in size in the saline–alkaline plus Spd group versus the control group, whereas 20 and 4 spots either disappeared or were reduced in abundance,

Please cite this article in press as: Zhang, Y., et al. Deciphering the protective role of spermidine against saline–alkaline stress at physiological and proteomic levels in tomato. Phytochemistry (2015), http://dx.doi.org/10.1016/j.phytochem.2014.12.021