Journal of Plant Physiology 193 (2016) 12–21

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

The wheat MAP kinase phosphatase 1 alleviates salt stress and increases antioxidant activities in *Arabidopsis*

Ikram Zaidi[a], Chantal Ebel[a,b], Nibras Belgaroui[a], Mouna Ghorbel[a], Imène Amara[a], Moez Hanin[a,b,*]

[a] *Laboratory of Biotechnology and Plant Improvement, Center of Biotechnology of Sfax, BP1177, 3018 Sfax, Tunisia*
[b] *University of Sfax, Institute of Biotechnology, BP "1175", 3038 Sfax, Tunisia*

ARTICLE INFO

Article history:
Received 8 December 2015
Received in revised form 19 January 2016
Accepted 19 January 2016
Available online 16 February 2016

Keywords:
MAPK signaling
TMKP1
Durum wheat
Oxidative stress
Salt stress tolerance

ABSTRACT

Mitogen-activated protein kinase phosphatases (MKPs) are important negative regulators in the MAPK signaling pathways, which play crucial roles in plant growth, development and stress responses. We have previously shown that the heterologous expression of a durum wheat MKP, TMKP1, results in increased tolerance to salt stress in yeast but its particular contribution in salt stress tolerance in plants was not investigated. Here, TMKP1 was overexpressed in *Arabidopsis thaliana* and physiological changes were assessed in transgenic plants exposed to stress conditions. Under salt stress and especially LiCl, the TMKP1 overexpressors displayed higher germination rates in comparison to wild type plants. The enhancement of salt stress tolerance was accompanied by increased antioxidant enzyme activities, namely superoxide dismutase, catalase and peroxydases. Such increases in antioxidant activities were concomitant with lower malondialdehyde, superoxide anion O_2^- and hydrogen peroxide levels in the TMKP1 transgenic seedlings. Moreover, we provide evidence that, in contrast to the *Arabidopsis* ortholog AtMKP1, TMKP1 acts as a positive regulator of salt stress tolerance via its ectopic expression in the *Arabidopsis mkp*1 mutant.

1. Introduction

Because plants cannot escape unpredictable environmental changes, they have developed different strategies to regulate their stress responses. Previous studies have shown the existence of several cellular signal transduction pathways connecting stress perception to gene expression (Shinozaki et al., 2003; Yamaguchi-Shinozaki and Shinozaki, 2006; Tran et al., 2007). Reversible protein phosphorylation, catalyzed by protein kinases and protein phosphatases, constitutes the common basis for complex signal transduction pathways that allows the cell to respond properly to a broad set of environmental signals. Mitogen-activated protein kinases (MAPK) are considered the major players in these signaling pathways and are involved not only in various stress responses, but also in hormone signaling and in the regulation of cell division and developmental processes (Rodriguez et al., 2010; Meng and Zhang, 2013). The activation of MAPKs is strictly regulated via phosphorylation of the conserved TXY motif by an upstream MAPK kinase. However, the duration and the intensity of this activation must be tightly regulated and MAPKs undergo dephosphorylation through protein phosphatases, including serine-threonine protein phosphatases (PSTPs), protein tyrosine phosphatases (PTPs) and dual-specificity phosphatases (DSPs) (Kyriakis and Avruch, 2001; Owens and Keyse, 2007). MAPK phosphatases (MKPs) constitute a group of specialized DSPs able to fully inactivate MAPKs by dephosphorylating them at both Ser/Thr and Tyr residues (Camps et al., 2000; Theodosiou and Ashworth, 2002; Jeffrey et al., 2007; Bartels et al., 2010). In plants, MKPs constitute an attractive group of proteins as their structure show typical domains that were not found in other eukaryotic MKPs. Beside the catalytic domain they harbor a gelsolin homology and calmodulin (CaM) binding domains (Bartels et al., 2009).

Several plant MKPs have been reported to interact with and inactivate specific MAPKs (Ulm et al., 2002; Zaidi et al., 2010), thus contributing to the control of hormonal signaling, stress responses, growth and development. Indeed, in *Arabidopsis*, by deactivating MPK12, a specific MKP named AtIBR5 (*Arabidopsis* indole-3-butyric acid-response 5) was shown to regulate auxin signaling as illustrated by the *Atibr5* mutant that exhibits reduced root sensitivity to auxin and abscisic acid (ABA) (Monroe-Augustus et al., 2003; Lee et al., 2009). The rice ortholog, OsIBR5, negatively regulates

* Corresponding author at: Laboratory of Biotechnology and Plant Improvement, Center of Biotechnology of Sfax, BP1177, Sfax, 3018, Tunisia. Fax: +216 74 875 818.
E-mail address: moez.hanin@cbs.rnrt.tn (M. Hanin).

http://dx.doi.org/10.1016/j.jplph.2016.01.011
0176-1617/

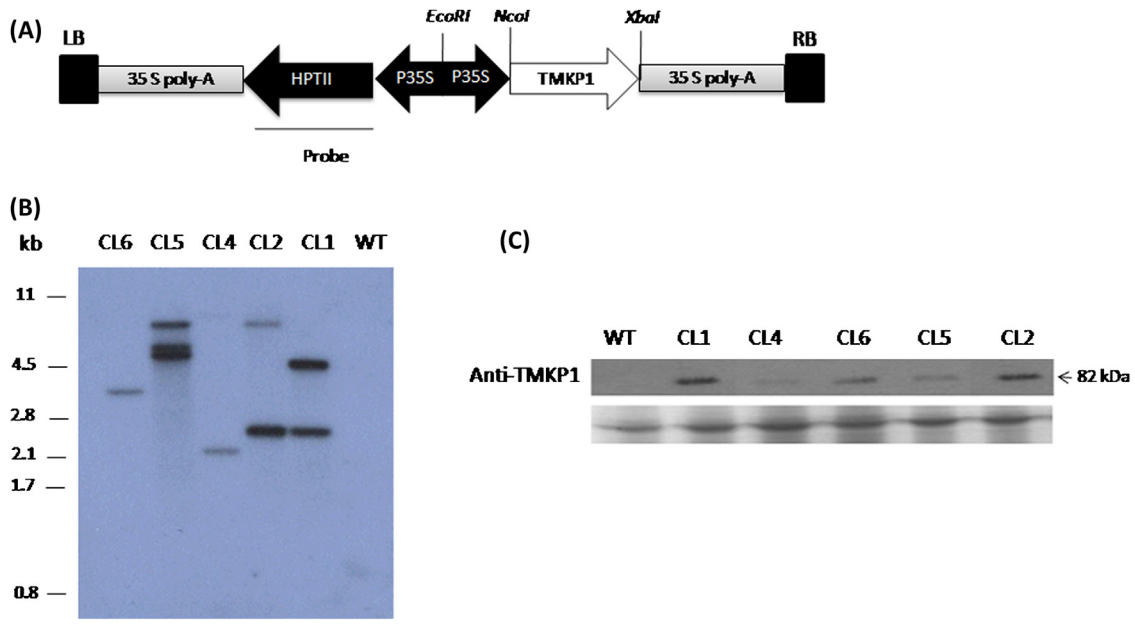

Fig. 1. Molecular analyses of the TMKP1 transgenic lines. (A) Schematic map of the T-DNA insert containing TMKP1, cloned in the binary vector pCAMBIA1302. (B) Southern blot analysis of genomic DNA of *Arabidopsis* T1 transformants digested with *EcoR*I and probed with the *HPT* gene. Untransformed *Arabidopsis thaliana* plants (WT); Lanes 1–5, independent transformants (CL1, CL2, CL4, CL5, CL6). (C) Immunodetection of TMKP1 protein in the T3 homozygous plants from five transgenic lines (CL1, CL2, CL4, CL5, CL6) using the wheat anti-TMKP1 antibody. WT protein extract was used as control. On lower panel, protein loading controls are shown on 10% SDS–PAGE stained with Coomassie Brilliant blue. The size of TMKP1 protein is indicated.

drought stress tolerance in transgenic *Nicotiana tabacum*, where it inhibits the activity of the wounding-induced protein kinase (WIPK), following a drought stress (Li et al., 2012). Functional characterizations of additional *Arabidopsis* MKPs were reported during the last decade, i.e. AtPHS1 interacts with and deactivates MPK18 to regulate cortical microtubule functions (Walia et al., 2009), while AtMKP2 is a positive regulator of oxidative stress response and dephosphorylates AtMPK3 and AtMPK6 (Lee and Ellis, 2007). Nevertheless, AtMKP1 remains the best studied MKP in plants and among the most relevant regulators of MAPKs in *Arabidopsis*. AtMKP1 seems to have a pleiotropic function, as it was shown to be involved in the control of various abiotic stress responses, including salinity and UV, and such effect occurs via negative regulation of MPK3 and MPK6 (Ulm et al., 2001, 2002; Bartels et al., 2010). It has also been reported that AtMKP1 acts as a negative regulator of bacterial pathogen resistance and MPK6-mediated pathogen-associated molecular pattern (PAMP)-responses (Bartels et al., 2009; Anderson et al., 2011). The rice ortholog, OsMKP1 deactivates OsMPK3 and OsMPK6 under wounding stress (Katou et al., 2007). Similarly, the tobacco NtMKP1 dephosphorylates WIPK and SIPK (wound and salicylic acid induced protein kinases), the OsMPK3 and OsMPK6 counterparts, respectively (Katou et al., 2005). This NtMKP1-mediated MAPK inactivation is responsible for the negative regulation of wound response and induced resistance against necrotrophic pathogens and *Lepidoptera* herbivores in tobacco (Oka et al., 2013).

We have previously isolated the durum wheat ortholog TMKP1, which with the rice OsMKP1 are the only studied cereal MKPs. TMKP1 interacts with the wheat TMPK3 and TMPK6 (homologous to the *Arabidopsis* and rice MPK3/6 counterparts) and controls their subcellular localization (Zaidi et al., 2010). In addition, the catalytic activity of TMKP1 can be activated by TMPK3 (Zaidi et al., 2010) and also by CaM but only in the presence of the bivalent metallic ion Mn^{2+} (Ghorbel et al., 2015). On the other hand, TMKP1 was shown to be differentially regulated in two durum varieties with marked differences in salt and drought stress tolerance, suggesting a possible role of this phosphatase in regulating the response to these stresses. For this reason, we have explored the effect of TMKP1 heterologous expression on stress resistance in *Saccharomyces cerevisiae* and showed that TMKP1 overexpression resulted in an increased yeast tolerance to salt stress (Zaidi et al., 2012).

In this study, we report that TMKP1 overexpression in *Arabidopsis thaliana* promotes seedling establishment under salt stress and especially LiCl. This growth promotion under stress conditions is associated with an increase in antioxidant activities. Finally, we provide experimental evidence that TMKP1 is a positive regulator of the salt stress response and, most importantly, it may act in an antagonistic manner compared to its AtMKP1 ortholog. Such findings suggest that, despite their shared homology, both MKP may have evolved divergently in monocotyledonous and dicotyledonous plants to ensure distinct regulatory functions in abiotic stress tolerance mechanisms.

2. Materials and methods

2.1. Plant material and growth conditions

A. thaliana (ecotype Columbia, Col-0 and Wassilewskija, WS) plants were grown under long-day conditions (16 h light/8 h dark) at 20–22 °C. *Arabidopsis mkp1* and Line 6 (Ulm et al., 2001) were grown under the same conditions. For germination assays, 100 seeds from the different lines were surface sterilized and sown on MS medium supplemented with various concentrations of NaCl (0–200 mM) or LiCl (0–14 mM). After 2 days of stratification at 4 °C, the plates were transferred to a growth chamber as described. Survival rates were calculated by counting seedlings with green cotyledons after 12 days of growth. These assays were conducted in triplicate using independent seed lots.

2.2. Generation of *TMKP1* Arabidopsis *transgenic lines*

The TMKP1 coding sequence was inserted into the pCAMBIA 1302 vector (CAMBIA) at the *NcoI* and *SpeI* restriction sites. The vector pCAMBIA 1302 carries a selective marker gene (*HPT*),