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Short communication

Heat stress alters the expression of salt stress induced genes in smooth cordgrass (*Spartina alterniflora* L.)

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ABSTRACT

To understand the tolerance mechanism associated with high temperature stress in a halophyte smooth cordgrass (*Spartina alterniflora*), a few selected salt-induced genes including transcription factors were studied for their transcript abundance. The mRNA analysis of eight genes and nine transcription factors known to be involved in various salt tolerance mechanisms revealed temporal and tissue-dependent variation in their expression under high temperature stress. Differential response of genes under heat and salt stress (reported earlier) indicated different mode of action in the metabolic pathway in response to different environmental cues, and a few common genes responsive to multiple stresses showed temporal and tissue-dependent variation in their expression. This study demonstrates that *S. alterniflora* could be a potential source of candidate genes conferring tolerance against high temperature in addition to salt tolerance for crop improvement.

1. Introduction

A variety of abiotic stresses, such as temperature extremes, drought, salinity etc. affect plant growth and development leading to loss of crop productivity worldwide. In nature, temperature changes are more frequent than any other abiotic factors. The complexity of heat stress is characterized by variation in intensity, duration and rate of increase in temperature. It is usually accompanied by drought stress due to the physical loss of water at high temperature. Plants, being sessile, have, therefore evolved complex tolerance mechanisms for continued survival and reproduction under heat stress. Understanding these processes requires the identification and analysis of major genes that underlie stress-regulatory networks. Furthermore, identification of these tolerance genes would help in designing crop plants with enhanced thermotolerance. Moreover, there exists a crosstalk among the different mechanisms to adapt to changing external environments [31]. The abundance of information on expressed sequence tags (ESTs) available in the public domain helps to accelerate the progress toward understanding the genetic mechanisms that control plant responses to a given edapho-climatic condition.

Spartina alterniflora (Loisel.), commonly known as smooth cordgrass, is a halophyte capable of withstanding salinity as high as double the strength of sea water [6]. It has also the ability to withstand high temperatures of up to 35 °C. Its growth appeared to be more robust in heated water [2]. It is necessary to have first hand information on the transcriptional response of genes induced by one stress (for example salt stress in this case) to another stress in such a halophytic grass. Transcript profiling through cDNA-AFLP and EST analysis unraveled genes exhibiting transcriptional induction/repression under stress and therefore could give clues into processes underlying stress tolerance, especially in the species like *S. alterniflora* with no prior genome information [4,5]. In an effort to identify genes that could be potential candidates in multiple stress tolerance for their further use in multigene-engineering, cDNA libraries were made from both leaf and root tissues of smooth cordgrass under salinity stress [5]. Here, we report the change in the expression profiles of a few selected salt-induced genes including transcription factor genes in response to heat stress.

2. Materials and methods

2.1. Plant material and heat stress treatments

Young (3- to 4-leaf stage), uniform, clonally propagated [under non-saline conditions inside a greenhouse with 14 h light (250–270 µmol m^{-2} s^{-1}) and 10 h dark at 26 °C/18 °C day/night temperature] plants of smooth cordgrass (*S. alterniflora* cv. Vermilion) were grown in 4 inch sand-filled plastic pots with bottom and side drainage holes as described earlier [5]. Ten plants

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were subjected to heat stress inside a temperature and illumination control incubator (Scheldon Manufacturing Inc, Cornelicus, Oregon, USA) maintained at a constant day/night temperature of 38 °C with a photoperiod regime of 12h:12h light:dark. Leaf and root tissues were collected at different time points i.e., 0 h, 1 h, 24 h, 48 h, and 1 week after exposure to heat stress and stored frozen at −80 °C for further use.

2.2. Reverse transcription polymerase chain reaction (RTPCR)

Total RNA was extracted from the frozen root and leaf tissues of the control (before heat stress) and heat-stressed plants using an RNeasy plant minikit (Qiagen, Valencia, CA, USA). Qualitative and quantitative assessments of total RNA employed formamide-denaturing gels by running 2 µl of total RNA along side an RNA ladder (Invitrogen, Carlsbad, CA, USA).

The RNA, extracted from the root tissues harvested after 1 week of heat stress, was degraded and therefore, was not included in the downstream applications. First strand cDNA was synthesized from 2 µg of total RNA using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA, USA). One hundred nanogram of the first strand cDNA [quantitated using an ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA)] was used for further PCR analysis, using the gene-specific primers. The primers and PCR conditions were essentially same as described earlier [5].

2.3. Real-time reverse transcription PCR (RTi-RTPCR)

The fold-change in the mRNA abundance of *PMP3* (plasma membrane protein) and nine transcription factor genes (see legends in Fig. 3) was determined in the tissues under stress conditions against the unstressed control. The *α-tubulin* gene of *S. alterniflora* (GenBank Acc. No. EH277253) was used as the reference gene. The primers were designed with Primer Express software (Applied Biosystems, Foster City, CA, USA). The primer sequences and their details have been reported earlier [5].

One hundred nanogram of the first strand cDNA from leaf and root tissues of unstressed control and heat-stressed plants were used for the *RTi-RTPCR* analysis in triplicate, using the SYBRGreen™ master mix (Bio-Rad, Hercules, CA, USA). A thermal cycler profile of 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 60 °C for 1 min, followed by a melt curve analysis, was used in the MyiQ™ single color real-time PCR detection system (Bio-Rad). A serial dilution of 100, 20, 4, 0.2 and 0.04 ng of first strand cDNA was used for all transcripts to generate a standard curve by plotting the threshold cycle (Ct) values against log(ng cDNA), and to ensure that the efficiencies of the individual transcripts were equal. The fold-change accumulation of the mRNA (ratio) was calculated according to the method described by Pfaffl [20].

3. Results and discussion

3.1. Temporal and tissue-dependent variation in expression of salt-induced genes under heat

The transcript abundance of the eight selected genes (see legends in Fig. 1) varied in response to heat stress in both leaf (Fig. 1A) and root (Fig. 1B) tissues. The genes were, in general, upregulated under heat stress to varying degrees at different time points. The accumulation of transcripts was highest at 1 h under heat stress with the exceptions of *vATPase*, *INO1*, *GAPDH*, and *ZFP*. The *vATPase* showed a sudden decline in transcription in response to heat stress at 1 h but there was significant increase in its transcript accumulation in subsequent time points. *CTP* showed abundance of its transcript at all time points under heat stress. The leaves accumulated higher levels of *HSP90* and *PMP3* at 1 h and 1 week of heat stress than at 0 h and other time points. The root also behaved in a similar fashion as the leaf especially with respect to increased accumulation of transcripts at 1 h of heat stress. But the transcript level was either reduced afterwards or remained the same as in unstressed control (Fig. 1B). The only exception was *HSP90*, which showed an increasing trend of its transcript production with time in root. It was interesting to note that in the root tissue *ZFP* was accumulated only after 1 h and there was no induction of its transcript subsequently. However, a high molecular weight transcript of *ZFP* accumulated under stress (Fig. 1B). Although the exact mechanism is far from clear, it is possible that the transcription factor might have triggered another member of its family that has the same priming site, or the factor itself underwent a concatemerization under stress.

HSP90 chaperone acts as a coregulator of signal transduction mechanisms, and its role in adaptive response at cellular level to sudden temperature changes in the natural environment is well documented [3]. The upregulation of *HSP90* under heat stress in rice leaf has been shown earlier [18]. There are few reports on the influence of heat on the expression of *H^+-ATPase*. It was shown that *H^+-ATPase* activity in leaf cells subject to heat shock was enhanced by moderate heat pretreatment in maize seedlings [14]. It is possible that increased hydrolysis of ATP was required to maintain the given H^+ pumping of the vacuole as heat stress leads to changes in the permeability of the vacuolar membrane. Aside from its role in the Na^+/K^+ uptake mechanism, *PMP3* is speculated to be involved in substrate transport through plasma membrane since temperature stresses like heat and cold could act as inducers of *AcPMP3* within 1 day of treatment [11]. This was further evident from the quantitative RTPCR result, which shows a 7-fold increase in its transcript accumulation in the leaf under heat stress than the unstressed control (Fig. 2). There was a very little increase (0.2-fold) with no significant change in the cumulative amount of its transcript in root under heat stress. *OsCTP* from rice was shown to be upregulated under submergence and drought stress but there was no change in its transcript level under cold stress [21]. Although role of cation exchangers have been documented in heat stress, the exact mechanism of its regulation under heat tolerance is yet to be established. The strong induction of *INO1*, leading to accumulation of di-*myo*-inositol-phosphate in response to heat stress, is documented in *Pyrococcus* [26], but it showed no upregulation when the *Passiflora edulis* plants were subjected to heat stress at 37 °C [1]. Accumulation of *ADH2* protein has been reported under cold stress in rice seedling but not in maize [7], whereas the activity of *Adh* was high at increased environmental temperature in *Drosophila* flies [23]. The anaerobic response of maize is analogous to heat shock response in many plants [22], which suggests that *ADH2* might have a common role in its response to these two stresses. Similarly, during dehydration, *GAPDH* mRNA has been shown to increase in the resurrection plant *Craterostigma plantagineum* [30]. The expression of *GAPDH* was increased in response to various abiotic stresses including heat in oyster mushroom *Pleurotus* [12]. These results indicated that there is a crosstalk among the tolerance mechanisms in response to different abiotic stresses.

3.2. Transcript analysis of the transcription factor genes

Nine salt-induced ESTs of *S. alterniflora* homologous to transcription factor (TF) genes of either rice or *Arabidopsis* were analyzed by *RTi-RTPCR* for their transcript abundance in leaf and root tissues under heat stress. Histone deacetylase (HD), C3H4-type Zinc finger protein (*ZFP1216*), and global transcription factor (*GTF1557*) showed a strikingly high accumulation of mRNA in root tissues, while C2H2-type zinc finger protein (*ZFP353*) and general regulatory factor (*GRF1632*) showed high abundance of mRNA in leaf tissues (Fig. 3). The varying expression pattern and level of