Gene xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Gene

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

Research paper

Distinct gene networks drive differential response to abrupt or gradual water deficit in potato

Alfredo Ambrosone [a,1], Giorgia Batelli [a], Hamed Bostan [b], Nunzio D'Agostino [c], Maria Luisa Chiusano [b], Gaetano Perrotta [d], Antonietta Leone [e], Stefania Grillo [a], Antonello Costa [a,*]

[a] National Research Council of Italy, Institute of Biosciences and BioResources, Research Division Portici (CNR-IBBR), Portici (NA), Italy
[b] Department of Agricultural Sciences, University of Naples "Federico II", Portici (NA), Italy
[c] Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di ricerca per l'orticoltura (CREA-ORT), Pontecagnano Faiano (SA), Italy
[d] Italian National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA), TRISAIA Research Center, Rotondella, MT, Italy
[e] Department of Pharmacy, University of Salerno, Fisciano SA, Italy

ARTICLE INFO

Article history:
Received 24 June 2016
Received in revised form 8 October 2016
Accepted 18 October 2016
Available online xxxx

Keywords:
Plant stress and adaptation
Potato
Solanum tuberosum
Water stress

ABSTRACT

Water-limiting conditions affect dramatically plant growth and development and, ultimately, yield of potato plants (*Solanum tuberosum L.*). Therefore, understanding the mechanisms underlying the response to water deficit is of paramount interest to obtain drought tolerant potato varieties. Herein, potato 10 K cDNA array slides were used to profile transcriptomic changes of two potato cell populations under abrupt (shocked cells) or gradual exposure (adapted cells) to polyethylene glycol (PEG)-mediated water stress. Data analysis identified > 1000 differentially expressed genes (DEGs) in our experimental conditions. Noteworthy, our microarray study also suggests that distinct gene networks underlie the cellular response to shock or gradual water stress. On the basis of our experimental findings, it is possible to speculate that DEGs identified in shocked cells participate in early protective and sensing mechanisms to environmental insults, while the genes whose expression was modulated in adapted cells are directly involved in the acquisition of a new cellular homeostasis to cope with water stress conditions.

To validate microarray data obtained for potato cells, the expression analysis of 21 selected genes of interest was performed by Real-Time Quantitative Reverse Transcription PCR (qRT-PCR). Intriguingly, the expression levels of these transcripts in 4-week old potato plants exposed to long-term water-deficit. qRT-PCR analysis showed that several genes were regulated similarly in potato cells cultures and tissues exposed to drought, thus confirming the efficacy of our simple experimental system to capture important genes involved in osmotic stress response. Highlighting the differences in gene expression between shock-like and adaptive response, our findings could contribute to the discussion on the biological function of distinct gene networks involved in the response to abrupt and gradual adaptation to water deficit.

1. Introduction

Water limiting conditions drastically affect plant growth and production. In particular, water deficit, which causes also osmotic stress, severely impairs plant development through the imposition of molecular and physiological changes that, in case of severe and durable water deficit, lead to disorganization of cell membranes, metabolic toxicity and inhibition of photosynthesis (Zhu, 2001). However, even non tolerant plants respond and might adapt to drought by modulating the expression of several genes (Seki et al., 2003; Shinozaki et al., 2003; Urano et al., 2010; Yamaguchi-Shinozaki and Shinozaki, 2005). Usually, stress-related genes govern important cell mechanisms such as protein metabolism, transcriptional and post-transcriptional regulation, signalling and stress perception, ion homeostasis, detoxification and damage repair (Ambrosone et al., 2012; Bray, 2002; Rensink et al., 2005; Shinozaki et al., 2003). However, the survival or death of plants experiencing water deficit is known to depend not only on the plant genetic background, developmental stage and morphology, but also on the severity and duration of the stress event (Leone et al., 1994a). Therefore, while gradual and continuous imposition of water deficit can produce metabolic and molecular modifications that allow a new

Abbreviations: PEG, polyethylene glycol; DEG, differentially expressed gene; qRT-PCR, Real-Time Quantitative Reverse Transcription PCR; TIGR, The Institute for Genomics Research; LOWESS, locally weighted linear regression; PGSC, potato genome sequencing consortium; EST, expressed sequence tag; ABA, abscisic acid.

* Corresponding author.
E-mail address: antcosta@unina.it (A. Costa).
[1] Present Address: Instituto de Ciencias de Materiales de Aragón (ICMA), Universidad de Zaragoza-CSIC, 50009 Zaragoza, Spain.

http://dx.doi.org/10.1016/j.gene.2016.10.024
0378-1119/

cellular homeostasis and active growth even in not optimal environmental conditions, a short exposure to severe water stress may activate genes involved in the prevention/repair of cellular damage, as well as genes involved in the stress perception/signalling function (Ambrosone et al., 2011; Leone et al., 1994b).

Many varieties of *S. tuberosum*, the most commonly consumed potato, are very sensitive to water-limiting conditions, which reduce marketable yield (Deblonde and Ledent, 2001; Weisz et al., 1994). Potato is the most important non-grain food crop, and given the increased occurrence of drought and its severity, great efforts need be addressed to understand genetic mechanisms controlling potato plant adaptation to water deficit. In that respect, recently published outcomes from the potato genome sequencing project have provided important genomic information allowing dissecting complex genetic traits of agronomical interest (The Potato Genome Sequencing Consortium, 2011).

Over the past two decades, we deeply investigated the physiological and molecular responses of potato cell cultures exposed to osmotic stresses, demonstrating that they respond in different ways if exposed abruptly or gradually to water deficit elicited by PEG addition into the culture medium (Leone et al., 1994a). Our previous studies indicated that water stress, when imposed gradually to potato cells, is compatible with active cellular growth, primarily due to restored normal protein synthesis (Leone et al., 1994b), proline and polyamine accumulation (Scaramagli et al., 2000) and changes in membrane composition and fluidity (Leone et al., 1996).

Although one can argue that a cellular system may not fully represent the complex *in vivo* plant behaviour to a changeable environment, investigating plant stress response at cellular level allows to identify genes mostly involved in cell stress perception and transduction, as well as in controlling and maintaining cellular homeostasis. Worth of note, several lines of evidence indicate that the experimental conditions used to impose plant water stress frequently do not reflect what really happens in soil. For instance, sudden exposure to severe water stress occur rarely in the field, while more often plants need to adapt to natural fluctuation of environmental conditions which make the physiological response much more complex than a simple experimental binary condition (stress *vs* control). Overall, this suggests that transcriptional changes conferring tolerance to severe water stress may not sustain plant growth under gradual adaptation to water limiting conditions (Claeys and Inzé, 2013; Claeys et al., 2014; Clauw et al., 2015). As consequence, the comprehension of these distinct dynamics is really appealing for genetic improvement programs conceived to select drought-resistant varieties (Blum, 2005).

With this respect, comparative transcription profiles of the response to abrupt or gradual water stress conditions may provide additional information about genes regulated in severe water stress that might belong to early responsive genes, providing initial protection and amplification of primary osmotic/water stress signals, and about genes whose expression is changed during gradual adaptation that might be involved in tolerance to stress conditions.

Because of the complexity of the overall plant response to drought stress, in recent years global transcriptome analyses have replaced the gene-by-gene approaches in different plant species to identify genes whose expression is regulated in response to water stress (Gullì et al., 2015; Iovieno et al., 2016; Kreps et al., 2002; Li et al., 2016; Roche et al., 2008; Seki et al., 2002; Xue et al., 2008) or to other abiotic stresses since it allows the simultaneous analysis of a great number of genes (Legay et al., 2009).

This study was aimed to capture and compare transcriptional profile changes in potato cells exposed to abrupt (shock) or gradual imposition (adaptation) to water stress by using a potato 10 K cDNA array slides containing approximately 10,000 potato cDNA clones. By exploiting an alternative route towards the identification of water stress responsive genes, we provided evidences that distinct gene networks are regulated in potato cell exposed to abrupt or gradual adaptation to water stress. Furthermore, to verify whether genes involved in the response of the whole plant to stress can be identified by an *in vitro* simplified cellular system, a selected number of stress responsive genes in potato cells was monitored in potato plants subjected to a mild drought-stress treatment. In conclusion, we demonstrated that potato cells respond to water deficit according to the intensity and the duration of water limiting conditions providing unique molecular signatures for potato stress adaptation *in vitro* and *in vivo*.

2. Materials and methods

2.1. Cell cultures, plant growth and stress treatments

Potato cell cultures were obtained from leaf callus of potato plants and subcultured every 7 days. Cells were maintained in a modified Murashige and Skoog medium in a rotary shaker at 28 °C in the dark as described in Leone et al., 1994a. Gradual acclimation to low water potential was achieved by transferring cells to nutrient medium containing increasing concentrations of PEG 8000 (5, 10, 15 and 20% w/v). Cells were maintained at each PEG concentration for at least 2 subculture cycles and then for 45 subculture cycles at 20% PEG. For shock conditions, control cells were transferred and maintained for 24 h into a medium containing 20% PEG.

Four-week-old potato plants were grown in greenhouse in pots containing peat:perlite (1:1) soil and subject to water stress for seven days by withholding water. Control potato plants of the same developmental stage were maintained in condition of regular water regime (daily watered). Stomatal resistance was measured daily during a seven-day treatment with a leaf porometer (AP4, Delta-T Devices). In each plant, measurements were carried out on basal, middle stem and apical leaves.

2.2. RNA isolation

Total RNAs were extracted from at least three biological replicates of control cells, cells subjected to PEG treatments and plant tissues (500 mg fresh weight) by the guanidinium isothiocyanate method according to Chomeczynski and Sacchi, 1987. The concentration of RNA samples was determined through spectrophotometric analysis. The extraction purity (absence of any contaminants, such as proteins, polysaccharides and phenol) was estimated by measurement the absorbance ratio OD260/280. The samples were considered for further analysis when the ratio A OD260/A OD280 had a value between 1.7 and 2.0. The RNA samples were examined by electrophoresis on agarose gel at 1,2% to check the absence of any degradation and contamination of residual genomic DNA. Total cell RNA (50 mg) from control, treated cells and plant tissues were incubated for 30 min at 37 °C with 20 units of RNAse-free DNase I (Gibco BRL, Life Tecnologies, USA). After extraction with phenol/chloroform (3:1) and ethanol precipitation in the presence of 0.3 M sodium acetate, the RNA was redissolved in 20 μL of diethyl pyrocarbonate-treated water:

2.3. Preparation of labelled probes and array hybridisation

The potato 10 k cDNA array version 3 provided by TIGR (The Institute for Genomics Research) was employed in the present study. Preparation of labelled probes, microarray hybridisation and data analysis was carried out in the TRISAIA Research Center (ENEA). To minimize the cyanine incorporation bias, we adopted the "dye-swap" hybridization (Dobbin et al., 2003) for each combination of probes (stressed *vs* control cells, adapted *vs* control cells). At least three technical and two biological replicates were carried out per hybridization.

RNA labelling was achieved using direct-labelling methods. For each direct-labelling reaction, 40 μg of DNA-free total RNA were reverse transcribed using SuperScript II reverse transcriptase (Invitrogen) and oligo-dT primer in the presence of Cy3- or Cy5-labelled dUTP. Unincorporated

Please cite this article as: Ambrosone, A., et al., Distinct gene networks drive differential response to abrupt or gradual water deficit in potato, Gene (2016), http://dx.doi.org/10.1016/j.gene.2016.10.024