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Unravelling the molecular cues of plant adaptation or survival to water deficit

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Abstract. When experiencing water deficit, glycophyte plants undergo physiological and biochemical changes aimed at limiting cellular damages and rescuing a new cellular homeostasis. Discriminate irreversible cell injury from adaptive rearrangements to water stress, is quite critical since only the latter plant response is compatible with active growth and development sustaining, ultimately, plant yield. An up-dated view of the molecular basis of cellular response to drought stress and of key functions activated able to protect cellular processes is summarized in the paper. It also reported a functional approach developed to identify genes with a crucial role in the adaptation to water deficit based on the systematic comparison of potato cell populations exposed abruptly or gradually to PEG-induced low water potential. Gradually adapted cells were characterized by distinctive metabolic adaptations (proline accumulation, changes in membrane lipid composition, de novo synthesis, etc) which enable them to grow actively at non-permissive water stress conditions. Differential gene expression in response to shock or gradually increasing water deficit was monitored by microarray technology, using the 1K TIGR potato cDNA array. More than 100 genes belonging to different functional categories were up-regulated in response to stress conditions. However, only a few induced genes were common to both cell populations, confirming that different gene networks mediate shock or long-term response to water deficit.

Keywords. Potato – Water stress/adaptation – Gene expression – Microarray.

Etude des signaux moléculaires d'adaptation ou de survie de la plante à la pénurie d'eau

***Résumé**. La pénurie d'eau provoque chez les plantes glycophytes des modifications physiologiques et biochimiques qui visent à limiter les dommages cellulaires et à rétablir une nouvelle homéostasie.*

Distinguer entre les dommages cellulaires irréversibles et le nouvel arrangement adaptatif provoqué par le stress hydrique est très critique, puisque seule l'adaptation est compatible avec la croissance active et le développement qui soutiennent la production de la plante.

Une vision actualisée de la base moléculaire de la réponse des cellules à la pénurie d'eau et des fonctions-clés activées, est résumée dans cet article. En outre, on y décrit l'approche fonctionnelle développée pour identifier les gènes qui ont un rôle crucial dans l'adaptation à la pénurie d'eau. Cette approche est fondée sur une comparaison systématique des populations cellulaires de pommes de terre qui sont exposées, brusquement ou graduellement, à un potentiel hydrique bas, induit par le PEG. Les cellules graduellement adaptées ont été caractérisées par une adaptation métabolique typique qui leur donne la possibilité de croître activement en conditions non permissives.

Les technologies microarray (1K potato TIGR cDNA) ont détecté une expression génique différentiée, en réponse à l'adaptation brusque ou graduelle à la pénurie d'eau. Une centaine de gènes appartenant à différentes catégories fonctionnelles se sont surexprimés en cas de stress hydrique. Toutefois un petit nombre de gènes induits se sont révélés communs aux deux populations cellulaires, confirmant que les réponses choc et de long terme à la pénurie d'eau, sont arbitrées par différents réseaux de gènes.

***Mots-clés.** Pomme de terre – Pénurie d'eau/adaptation – Expression génique – Microarray.*

I – Introduction

Fluctuating or permanent stress conditions affect the cultivation of most of the crops, causing yield instability and loss. This scenario is even more aggravated by the predicted forthcoming global changes in climate, the foreseen extremization of environmental conditions, the continuous increase of world population, the ever increasing deterioration of arable land and the scarcity of fresh water, all underscoring the importance of developing stress-resistant crops able to sustain growth and productivity in stressful environments. Drought is one of the major abiotic stresses affecting plant growth and reducing crop productivity being the 28% earth land represented by soils too dry for crop production (Bray, 2006). Agronomic amendments (irrigation, soil correction) might be still adopted to reduce the impact of drought stress on yield stability, but the additional costs for such modifications could be in many cases not economically advantageous. The increased occurrence of drought and his severity, imposes to select new tolerant varieties. Conventional breeding for drought resistance has been a basic approach for a long time and some successes have been achieved in crops such as maize (Hoisington *et al.*, 1996), wheat (Zhao *et al.*, 2000) and rice (Zhang *et al.*, 2006). However, a big gap remains between the current resistance levels and what is needed to guarantee yield stability for most of the major crops. Drought stress response and/or tolerance is a complex trait and is the result of the coordination of biochemical and physiological changes at the cellular and molecular level. Many of these changes involve a large number of genes, most of which are directly involved in the activation of adaptive mechanisms. The enormous body of information recently gathered on the molecular bases of plant response to stress signals and the identification of key genetic determinants in this process opened new opportunities for the development of tolerant plants based on marker assisted selection approaches as well as on innovative biotech approaches through targeted genetic manipulation of crop plants.

II – Molecular basis of plant response to osmotic stress

Plants have developed a wide variety of adaptive strategies to cope with environmental stresses; accordingly, plant cells have evolved signalling pathways to perceive and integrate different signals from their surroundings and to respond by modulating the expression of the appropriate genes (Knight and Knight, 2001). As complex trait, water stress tolerance is the result of the coordination of biochemical and physiological alterations at the cellular and molecular level, such as the increased level of ABA, the accumulation of various osmolytes and proteins coupled with an efficient antioxidant system. Many of these mechanisms have been characterized and have been found to exist in both tolerant and non-tolerant plants (Taji *et al.*, 2004). It is now clear that the difference between tolerant and non-tolerant crops at the molecular level involves a large number of genes. It has been estimated that the response to a stressful environment involves ca. 2000 genes, most of which are up-regulated upon stress (Bonhert *et al.*, 2001; Huang *et al.*, 2008). However, it is still unclear how many and which genes are directly involved in the activation of adaptive mechanisms. In fact, up-regulated genes do not necessarily have a role in adaptation, some might be induced because of stress-caused cell injury (Bray, 1997).

In the last decade different approaches have been adopted to dissect the complex molecular and biochemical mechanisms underlying plant stress response, to identify the genes involved and to establish their essential contribution to stress tolerance and protection. Several of the isolated genes, associated to the stress response and/or tolerance, have shown to be common to different environmental stresses, sharing a physiological osmotic component as determinant of the stress signal (water deficit, salt and freezing stress).

The characterization of a large number of stress-induced genes has significantly contributed to the understanding of the complex response to stress signals. Many of the genes, induced upon stress conditions, encode polypeptides, with putative protective roles in the stressed cells, such

as in ion transport (uptake, extrusion and sequestration of ions), membrane stabilization and chaperone functions, and small organic molecules, called compatible solutes, osmolytes and osmoprotectants (proline, glicyne betaine, sugars) (Xiong and Zhu, 2002). Manipulation of the expression of this class of genes has long been the most common approach, first, to demonstrate their role and function in stress tolerance and, secondly, to produce stress-tolerant transgenic plants. Genes belonging to the different categories above described, cloned from plants or other organisms, have been over-expressed, under the control of strong constitutive promoters, in model and crop plants, with the final result to increase tolerance to a specific or more than one environmental constraints (Table. 1). Though an increase in the level of tolerance to the stress under study has been claimed in many cases, tolerance of the over-expressing transgenic plants has been evaluated only rarely in field trials under realistic stress conditions. Additionally, only in few cases the possible negative pleiotropic effects of the genetic manipulation on the plant phenotype have been thoroughly discussed (Umezawa *et al.*, 2006). Recently, research activities in the field evolved from the study of single genes directly involved in cellular stress tolerance (functional genes) to the identification and characterization of key regulatory genes involved in stress perception and transduction and able to rapidly and efficiently activate the complex gene network acting downstream the signalling cascade. In this context protein kinases and phosphatases emerged as key players in stress signalling processes in yeast and animals, and also in plants (Jonak *et al.*, 2002) triggering stress-induced transcription factors able to directly activate the downstream machinery of genes involved in stress-protection and relief. The identification of key transcription factors (TFs), as CBF/DREB, has opened the possibility to obtain transgenic plants with a coordinated induction of the entire network of genes involved in stress tolerance. Infact TF over-expressing transgenic plants revealed cold and dehydration tolerance by activation of the target genes with reduced negative pleiotropic effects when they were expressed under the control of inducible promoters (Kasuga *et al.*, 1999; Hsieh *et al.*, 2002). The complexity of the events occurring in response to stress have been recently approached by genomics tools; progress in the mass-scale profiling of the transcriptome, proteome and metabolome has allowed a more holistic approach in investigations of drought tolerance based on the measurement of the concerted expression of thousands of genes and their products. High-throughput mRNA profiling has been applied to investigate the changes in gene expression in response to dehydration (Ozturk *et al.*, 2002). Collectively, the transcriptome profiling experiments conducted on drought-stressed plants have confirmed the central role of transcription factors while unveiling the complex hierarchy of the regulatory network that differentially modulates the expression of dehydration signature genes in a tissue-specific manner (Shinozaki and Yamaguchi-Shinozaki, 2007).

Despite the approach used, many of the stress-induced genes, identified in glycophyte or xero- and halophytes, still have no assigned functions (Bouchez and Hofte, 1998; Grillo *et al.*, 2006). Different functional genomic strategies based on reverse and forward genetics approaches in model as well as in crop species, have rapidly developed in recent years and are now routinely used to define key functions (Bohnert *et al.*, 2006). Though the ”gain and loss” approach has highlighted the contribution of specific stress-induced genes in the physiological processes of stress tolerance, there is, however, no clear evidence that the agronomic performance of crop plants under stressful conditions can be improved by simply overexpressing one/few genes.

The advance in plant genomic research with new information on genome sequence and structure for an increasing number of crops and the establishment of powerful bioinformatic platforms for data management and analysis (Chaves *et al.*, 2003; Cattivelli *et al.*, 2007) were also crucial in providing new tools for plant breeders to approach the complexity of plant response to drought. The massive development of molecular marker technologies (RFLPs, RAPDs, SSPs, AFLPs) and the consequent generation of high density genetic maps for economical important crops, is leading nowdays to the identification of major Quantitative Trait Loci (QTLs) contributing to stress tolerance (Tuberosa and Salvi, 2006). The identification of genes underlying QTL by mapping the stress-induced genes (candidate gene approach), although is still a long way off, will ultimately

provide the indication of the effective contribution of single/multiple candidate genes to stress tolerance and additionally will provide simple and efficient tools for effective molecular marker assisted (MAS) breeding for stress tolerance. It is becoming clear that the success of breeding for stable high yield will be only possible when a true integration of traditional breeding with plant physiology will be achieved using a multidisciplinary approaches based on plant genomics and advanced modelling.

Table 1. Transgenic plants tolerant to water stress obtained by over-expressing plant and microbial genes belonging to different functional categories (adapted from Grillo *et al.*, 2006).

Product	Gene	Origin	Host	Reference*
Osmolytes Metabolism				
Fructan	SacB	B. subtilis	Tobacco	Pilon-Smits et al., 1995
Trialose	Tps1	Saccaromyces	Tobacco	Romero et al., 1997
Poliamine	ADC	D. stramonium	Rice	Capell et al., 2004
Prolin	P5CS	Arabidopsis	Petunia	Yamada et al., 2005
Protective Proteins				
LEA	HVA1	Barley	Rice	Xu et al., 1996
Chaperone	Bip	Soya	Tobacco	Alvim et al., 2001
LEA	LLA23	Lilium	Arabidopsis	Yang et al., 2005
Detoxificant Enzymes				
Peroxidase	APX3	Arabidopsis	Tobacco	Yan et al., 2003
Superoxide dismutase	Mn-SOD	Tobacco	Alfalfa	McKersie et al., 1996
Transcriptional Factors				
DREB1/CBF	ZmDREB1A	Mais	Arabidopsis	Qin et al., 2004
DREB1/CBF	DREB1A/CBF3	Arabidopsis	Arabidopsis	Kasuga et al., 1999
AP2/ERF	SHN1/WIN1	Arabidopsis	Arabidopsis	Aharoni et al., 2004
bZip	ABF3	Arabidopsis	Rice	Oh et al., 2005
MYB	CpMYB10	C. plantagineum	Arabidopsis	Villalobos et al., 2004
Signal Trasduction				
MAPKKK chinase	NKP1	Tobacco	Mais	Shou et al., 2004
Famesyl transferase	ERA1	Arabidopsis	Oilseed	Wang et al., 2005
Others				
Ionic pump H^+	AVP1	Arabidopsis	Arabidopsis	Gaxiola et al., 2001
Malic enzyme	Chi-NADP-Me	Tobacco	Tobacco	Laporte et al., 2002
Expoxi-dioxigenase (ABA biosynthesis)	AtNCED3	Arabidopsis	Arabidopsis	Iuchi et al., 2001

* Reference are listed in Grillo et al., 2006

III – Cellular adaptation to water stress: a case study

As pointed out before, drought tolerance is a complex polygenic trait requiring the coordinated regulation of a large number of genes, as also recently confirmed by research studies based on global gene expression analysis in several plant species (Oono *et al.*, 2003; Rensink *et al.*, 2005; Bray, 2006). To discern stress-responsive genes that contribute to increase drought tolerance from those merely activated by a general stress response, our group has developed an *ad hoc* experimental system based on a potato (cv. Desiree) cell populations. Potato cells were exposed to a gradual increase in PEG-mediated low water potential (adapted cells) and to abrupt intense water stress (shocked cells) to study and compare systematically, at physiological and molecular level, the water stress response in these adapted cells and in non-adapted cells (Fig. 1) (Leone *et al.*, 1994a). Adaptation to water stress was found to be associated to several physiological and biochemical changes that include ability to sustain active cellular growth at conditions of water stress and salt conditions otherwise non permissive, accumulation of compatible osmoprotectants, such as proline (Leone *et al.*, 1994a), recovery of protein synthesis (Leone *et al.*, 1994b), changes in gene expression of stress responsive genes (Costa *et al.*, 2002; Ambrosone *et al.*, 2006), higher membrane stability due to changes in membrane lipid composition (Leone *et al.*, 1996).

A more complete description of the repertoire of genes involved in water stress adaptation, was obtained by comparing the global changes in gene expression in adapted potato cells with those induced by abrupt water stress in control cells by microarray analysis, using 1k and 10k cDNA slides from TIGR (The Institute for Genomics Research). In particular from the analysis of 1k, 64 up-regulated genes and 49 down-regulated genes during PEG adaptation, and 45 up-regulated and 22 down-regulated genes in response to PEG water stress were identified. Among the up-regulated genes, only two genes were found to be in shocked and adapted potato cells. Similarly, a limited number of common down-regulated genes, one was identified in the two cell populations. Altogether these data confirm that different gene networks are mediating the short- and long-term cellular response to water stress. Many of the identified genes highly expressed in the adapted potato cells belong to different functional classes, such as protein known to be involved in stress-response (e.g. heat shock proteins), involved in general cellular processes, e.g. protein synthesis, cellular transport, cell wall synthesis, and others. As already found as a results of transcriptome analyses in other crop species, about 20% of the stress responsive genes, identified in adapted potato cells, are highly expressed in stress conditions and matched with sequences in data banks with no assigned biological function. It is possible to speculate that genes up-regulated in shocked cells belong to early responsive genes providing initial protection and amplification of primary osmotic stress signals, while the genes whose expression is changed in adapted cells may be involved in tolerance to stress conditions. These results constitute the scientific and experimental background to further characterize identified genes with unknown functions and establish their role in drought tolerance. This will be approached through quantitative analysis of expression of selected genes in plant tissues in response to water stress and by a comprehensive functional analysis by both forward and reverse genetics approaches using also tools and materials available for the model plant *Arabidopsis thaliana*.

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