ENGEENERING OF NEW PLANTS CULTIVARS WITH IMPROVED ABIOTIC STRESS TOLERANCE

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Abstract

Abiotic stress is the main cause of yield loss in agriculture. Plant cell response to stress includes expression of numerous stress genes and changes of concentration of protective metabolites. Over-expression of the stress genes or transcriptional regulators controlling the stress response allows engineering of new varieties of plants demonstrating enhanced stress tolerance.

Keywords: *reactive oxygen species, heat shock proteins, osmolytes.*

Introduction

Agronomically important plants suffer from environmental stresses like salinity. drought and flooding, high-light stress, freezing and heat and cold stresses. Abiotic stress is the major limitation of plant productivity causing average yield losses of more than 50% for major crops (Boyer, 1982; Hawkes, 1990; Bray et al., 2000). Drought and heat stress (HS) appears to be especially detrimental: even moderate HS results in loss of 10-15% wheat yield (Wardlaw and Wrigly, 1994). Accordingly, radical temperature changes due to global climatic alterations could have negative consequences in the near future. Therefore, engineering of new plant cultivars with enhanced abiotic stress tolerance becomes especially important (Iba, 2002; Maestri et al., 2002; Valliyodan and Nguyen, 2006; Southworth, et al., 2000). To achieve this goal it is necessary to improve existing knowledge about molecular and genetic basis of stress tolerance in order to identify novel beneficial genes and to use them in breeding program.

Mechanisms of cell damage upon abiotic stress

Environmental stresses results in damages of plant cell, which are either specific or common for different abiotic stresses. HS results in denaturation of proteins leading to the decrease or complete loss of activities of some enzymes (Inaba and Sato, 1976; Downs and Heckathorn, 1998;

Feller et al., 1998; Panchuk et al., 2002; Basha et al., 2004). Drought, high salinity and freezing impose osmotic stress, which affects conformation and charge interaction of macromolecules and disorder normal cellular activities (Xiong and Zhu, 2002). Also, alterations in fluidity and composition of cellular membranes, protein-protein and protein-lipid interactions were reported (Blum, 1988; Maestri et al., 2002; Iba, 2002; Chaves et al., 2003).

In plant tissues under optimal grow conditions, about 1% of the total oxygen consumption goes to production of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals in chloroplasts, mitochondria and peroxisomes. However, an excessive generation of ROS causes oxidative damage of proteins, nucleic acids and membrane lipids (Suzuki and Mittler, 2006; Asada and Takahashi, 1987; Noctor and Foyer, 1998; Asada, 1999; Moller, 2001; Apel and Hirt, 2004). An important consequence of HS is an increased production of ROS due to changes in stability of membranes and/or inactivation of proteins involved in the electron transport and/or scavenging of ROS in different intracellular compartments (Downs and Heckathorn, 1998; Davidson and Schiestl, 2001; Panchuk et al., 2002; R. Pyrizhok and I. Panchuk, unpublished results). High-light stress enhances production of ROS in chloroplasts due to over-reduction of electron transport chain (Niyogi, 1999; Karpinski et al., 1999). Also, increased generation of ROS was observed upon osmotic stress (O'Kane et al., 1996; Sairam and Tyagi, 2004). Remarkably, ROS have not distractive role in the cell, but also act as signal molecules activating cellular stress response (Neill et al., 1999; Dat et al., 2000; Noctor et al., 2000; Volkov et al., 2006).

Molecular mechanisms of abiotic stress response and engineering of plants with enhanced stress tolerance

The abiotic stress response in plants includes changes in flow of metabolites through different pathways, suppression of pathways that may be involved in the production of ROS during stress, and transcriptional induction of various defense genes/proteins such as heat shock proteins (HSP), stress-related proteases, ROS-scavenging enzymes, etc. (Vierling, 1991; Dat et al., 2000; Iba, 2002; Mittler, 2002; Panchuk et al., 2002; Xiong and Zhu, 2002; Sairam and Tyagi, 2004; Valliyodan and Nguyen, 2006). Accordingly, over-expression of the respective stress-genes appears to be an attractive biotechnological tool to engineer plants with the enhanced stress tolerance.

HS results in production of HSP, which have been classified into a number of families based on their molecular mass (HSP100, HSP90, HSP70, HSP60 and small (s) HSP), most of which function as molecular chaperones (Jakob and Buchner, 1994; Boston et al., 1996; Lee et al., 1997; Löw et al., 2000; Sun et al., 2001). The expression of sHSP is a signature of HS response in plants. Plants are unique in the number sHSP produced upon HS; no isoforms are expressed in vegetative tissue under non-stress conditions (Jakob and Buchner, 1994; Schöffl et al., 1998; Volkov et al., 2005). HSP are necessary for thermotolerance: in maize, expression of 45 kDa HSP correlated with thermotolerance (Ristic et al., 1998). In carrot, over-expression of HSP17.7 provides an increased thermotolerance (Malik et al., 1999). In *Arabidopsis*, HSP101 was identified as a major component of thermotolerance (Hong and Vierling, 2000), because plants expressing less than usual amount of HSP101 had a reduced capacity to acquire heat tolerance, whereas transgenic plants constitutively expressing HSP101 possess enhanced thermotolerance (Queitsch et al., 2000). It could be expected that the antioxidative enzymes are required for thermotolerance because HS induces secondary oxidative stress in plants. In accordance, in wheat acquired thermotolerance correlated with activities of ROS-scavenging enzymes (Sairam et al., 2000).

The HS response is primarily regulated at the transcriptional level by HS transcription factors (HSF), which are activated by stress for a binding to HS promoter elements (HSE). Plants appear to contain a larger number of different HSF than animals. Although human and animal cells express up to four different HSF, 21 different HSF genes have been identified in *Arabidopsis* (Nover et al., 2001). AtHSF-A1a (HSF1) and AtHSF-A1b (HSF3) are the regulators, which become activated very early in the HS response and are necessary for efficient expression of HS genes (Lohmann et al., 2004). In tomato, HSF-A1a is a master regulator of HS induced gene expression, including synthesis of HSF-A2 and HSF-B1. Tomato HSF-A2 was expressed only after HS treatment and represents a dominant HSF in plants subjected to repeated cycles of HS and recovery. HSF-B1 acts as coregulator enhancing activity of HSF-A1a and/or HSF-A2 (Banival et al., 2004). Also, HSF-A2 of *Arabidopsis* serves as a regulatory amplifier of a subset of genes during the HS response (Schramm et al., 2006).

Recent investigations have shown that not only conventional HSP genes are controlled by HSF upon HS. Other genes encoding key enzymes in biochemical pathways related to environmental responses like ascorbate peroxidase 2 (APX2) and galactinol-synthase 1 have been identified as targets of HSF regulation in HSF3 (HSF-A1b) transgenic *Arabidopsis* plants (Panchuk et al., 2002; Panikulangara et al., 2004; Busch et al., 2005) indicating that not only chaperons but also other proteins are required for HS response and adaptation.

Transgenic over-expression of HSF in *Arabidopsis* resulted in synthesis of heat-inducible HSP and antioxidative enzymes (APX) at non-stress temperatures. The plants demonstrate a moderate increase in basal thermotolerance that was also associated with protection from oxidative bleaching of seedlings induced by HS (Lee et al., 1995; Prändl et al., 1998). This suggests that one aspect of thermotolerance in *Arabidopsis* is an increased ability to either prevent or repair heat-induced oxidative damage (Lee et al., 1995; Prändl et al., 1998; Döhr et al., 2001; Panchuk et al., 2002).

HSP/HSF appears also to play a role in the responses to other abiotic stresses: e.g., expression of HSP/HSF can be induced by drought (Bray, 2002; Rizhsky et al., 2002). However, not all HSP mRNA elevated during HS were also elevated during drought or combination of drought and HS, and transcripts encoding for four HSP were specifically induced during drought. Expression pattern of HSF was also different during drought and heat treatment (Rizhsky et al., 2002). Induction of HSP/HSF upon different kinds of stresses seems to be due to the activation of hydrogen peroxide signalling. Oxidative stress induces expression of HSP and other chaperones in plants, which can provide protection against oxidative stress. In tomato and rice, mitochondrial HSP22 and chloroplastic HSP26, respectively, are induced by hydrogen peroxide (Banzet et al., 1998; Lee et al., 2000). In cyanobacteria and *Arabidopsis*, high light and hydrogen peroxide, respectively, induced the mRNAs of some HSP and HSF (Desikan et al., 2001; Hihara et al., 2001; Pnueli et al., 2003). Recently, it was demonstrated that hydrogen peroxide is an important component in HS-activated gene expression in *Arabidopsis* (Volkov et al., 2006).

A considerable overlap was found between transcripts involved in the defense of plants against cold, drought and salinity (Oztur et al., 2002; Seki et al., 2001; 2002; Bray, 2002) because these stresses result in water deficit and osmotic disbalance in the cell. On the other hand, a relatively small overlap between transcripts induced during drought or HS was found (Rizhsky et al., 2002; 2004). Plant response to osmotic stress includes increased proteolytic degradations of irreversibly damaged proteins via ubiquitin-mediated pathway and by various proteases and activation of antioxidative enzymes (for review see Ingram and Bartels, 1996). Respectively, over-expression of antioxidative enzymes such as superoxide dismutases provide enhanced low temperature tolerance in tobacco and alfalfa (Sen Gupta et al., 1993 a, b; McKersi et al., 1999; 2000). Ion transporters also play an important role in salt stress tolerance (Apse et al., 2002; 1999; Shi et al., 2000). Accumulation of osmolytes including various carbohydrates (sucrose, manitol, mio-inositol, trehalose, raffinose, galactinol and fructan), amino acids (proline) or their derivates (glycinebetaine) and polyols upon osmotic stress is a well known phenomenon (Loescher et al., 1992; Pattanagul and Madore, 1999). Particularly, accumulation of proline is important for cell protection against osmotic stress. It was also proposed that manitol and proline could be involved in detoxification of ROS (Shen et al., 1997). Genetically engineered plants, which over-accumulate proline, demonstrate enhanced osmotolerance (Kavi Kishor et al., 1995; Nanjo et al., 1999). Also, tobacco and sugar beet plants over-expressing bacterial fructan gene demonstrated increased drought stress tolerance (for review see Valliyodan and Nguyen, 2006). Over-expression of inositol methyl transferase gene, IMT1 in tobacco led to enhanced drought and salt stress tolerance as a consequence of increased accumulation of methylated form of inositol (Sheveleva et al., 1997).

Similar to HS, osmotic stress response is activated mainly at transcriptional level (Reymond, 2000; Seki, 2001; Bray, 2002; Rizhsky et al., 2002; 2004). In *Arabidopsis*, numerous genes responding to water deficit were identified. At least four signaling pathways are involved in osmotic stress response, two of which are abscisic acid (ABA)-dependent and two are ABA-independent (Shinozaki and Yamaguchi-Shinozaki, 2000; Valliyodan and Nguyen, 2006). MYB and MYC transcription factors function as regulators in one of ABA-dependent pathways (Abe et al., 2003), whereas another one is activated via interaction AREB1 (ABA-responsive elements binding) transcription factor of bZIP family with ABA-responsive elements (ABRE) in promoters of target genes (Choi et al., 2000; Uno et al., 2000). Over-expression of rice Myb4 transcription factor in *Arabidopsis* led to enhanced drought tolerance, as a result of increased accumulation of several osmoprotectants (Mattana et al., 2005).

The ABA-independent regulation uses Dehydration-responsive elements/C-repeats (DRE/CRT) for activation of transcription of drought, cold and salt responsive genes. Over-expression of DRE/CRT- binding protein DREB1/CBF in *Arabidopsis* activates transcription of more than 40 stress-inducible genes and results in increased freezing, salt, and drought tolerance (Seki et al., 2001; Fowler and Thomashow, 2002; Maruyama et al., 2004). Also, over-expression of constitutively active form of DREB2A provided significant drought stress but only slight freezing tolerance (Sakuma et al.. 2006).

Numerous studies of molecular basis of abiotic stress were conducted in resent years. However, they were mainly focused on single stress treatments applied in controlled laboratory conditions whereas in field plants are normally exposed to a combination of different stresses. Drought and HS represent a good example of abiotic stresses occurring in nature simultaneously (Mittler et al., 2001; Rizhsky et al., 2002). Remarkably, transcriptome analysis revealed that the response of tobacco and *Arabidopsis* plants to a combination of drought and HS differs from that of plants subjected to drought or HS. Plants subjected to a combination of drought and HS accumulated sucrose and other sugars. In contrast, proline that accumulated in plants subjected to drought did not accumulate in plants during a combination of drought and HS, because increased temperature ameliorate toxicity of proline. Hence, during a combination of drought and HS sucrose replace proline as a major osmoprotectant, underlining ability of plant genome to respond to complex environmental conditions that occur in the field (Rizhsky et al., 2004).

Transgenic plants over-expressing stress-related genes were mainly studied in laboratory conditions and only limited amount of field tests were performed. According to Monsanto, transgenic maize over-expressing transcription factor NF-YB of *Arabidopsis* showed improved drought thermotolerance in field (Valliyodan and Nguyen, 2006). Better understanding of the specific roles of numerous stress-genes and of interplay of different metabolic pathways involved in stress-response will allow engineering of new cultivars of agronomical plants with enhanced stress tolerance that significantly reduces loos of yield.

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