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Transgenic Approaches to Develop Abiotic Stress Tolerant Crop Plants: Rice as the Model Example

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Abstract

Abiotic stress is a major limiting factor for crop productivity. The nature and extent of abiotic stress on crop plants are rapidly increasing with destabilization of environmental balance and deterioration of soil productivity. Transgenic approach has the potential of introducing specific genes from stress tolerant plant species in major crops. Rice is the principal food grain crop for more than half of the world population and faces different kinds of stresses in varying environments. No other crop species is grown in diverse ecology as in case of rice. Being one of the model plant for transgenic research and a premier crop species, it has been targeted several times for development of transgenic genotypes having resistance to salinity, drought, cold, or nutritional stresses. Several path breaking achievements have been made in this exciting research area in recent years, which is discussed here.

1. Introduction

Abiotic stresses including salt, drought, cold, dehydration, heat, heavy metal/pollutant and nutritional stresses are the major bottlenecks of crop productivity in large part of the world. Rice, a principal crop, is grown in around 130 mha allover the world. It is the principal food crop of Asia and feeds half of the human population. An estimated 30% of the rice land experiences salt stress, 20% faces drought conditions, and 10% is prone to low temperature stress at both seedling and flowering stages (Parker, 2004). This clearly shows the productivity limitation caused by abiotic stresses on rice.

Unlike animals, which primarily employ avoidance mechanism when exposed to stress, plants cannot escape stress. Therefore they employ their own mechanisms to cope up and tolerate the stress. This has led to development of unique genetic, physiological and morphological mechanisms in plants that involve different stress response pathways. Although the nature of these abiotic stresses are different, all or most of them affect some common functionality and cellular behavior of plant, such as destruction of membrane stability, generation of active oxygen species, reduction in photosynthetic activity, destabilization in signal transduction and so on. Therefore, it is often observed that the abiotic stresses in plant often share common multiple signaling pathways (Seki et al., 2003). For example, the metabolic pathways those lead to production of proline and glycinebetaine production are activated against a variety of stresses. Recent developments in structural and functional genomics reveal that several genes are involved in stress response pathway in crop plants, many of which are common to multiple abiotic stress response.

Transgenic plant development is a novel approach for crop improvement that allows incorporation of genes from unrelated species to the crop species. This approach is unique for developing abiotic stress tolerance in crops, since most of the crops have been domesticated through thousands of years to exclude certain characters that help wild plants to survive better in the natural environment. For example, xerophytes have better tolerance to drought and dehydration stresses, while halophytes can grow in saline environment where the crop species would fail to either germinate or grow. Advances in genetic engineering has enabled researchers to access the corresponding genes governing tolerance to abiotic stresses and transfer these genes to crop genetic background for higher crop productivity in limiting environments (Wang et al., 2003; Satya, 2007).

The transgenic approaches for developing abiotic stress tolerance has earlier been reviewed by Grover and Minhas (2000), who observed little achievement on transgenic rice for abiotic stress tolerance. In their review they also detailed the genetic transformation techniques of rice. Therefore, in the present review, we will rather concentrate on the new developments in transgenic rice development pertaining to abiotic stress tolerance.

2. Salinity Stress

Soil salinity is one of the major abiotic stresses which is decreasing agricultural production through hampering the use of large terrestrial areas of the world for agricultural production. So, there is a need to produce salt-tolerant crops. Physiologically, salinity enforces an beginning water stress due to the presence of relatively high solute concentrations in the soil; induces ionic distresses arises from disturbed K^+/Na^+ ratios and responsible to increased Na^+ and K^+ concentrations which are lethal to plants. Plants express two types of responses to salinity, viz. (i) salt-sensitive plants limit the uptake of salts and maintain their osmotic pressure by compatible solutes (sugars, proline and glycinebetaine) production and accumulation and (ii) salt tolerant plants maintain a high cytosolic K^+/Na^+ ratio into their cells through sequestration and accumulation of salt into the cell vacuoles and hence controlling the salt concentrations in the cytosol. Plants use two mechanisms, viz. (i) Na^+ ions extrusion out of the cell and (ii) vacuolar compartmentation of Na^+ ions to maintain a high cytosolic K^+/Na^+ ratio which is mandatory for plant growth in salt affected soils (Yamaguchi and Blumwald, 2005). One of the major approaches for developing salinity tolerance in crop plants is development of transgenic plants through metabolic engineering which includes incorporation and integration of novel genes or alteration of expression levels of in-house genes (Bohnert and Jensen, 1996; Yamaguchi and Blumwald, 2005). The majority of transformation experiments have used model systems, viz. rice, tobacco and *Arabidopsis* as experimental material (Table 1).

It has been firmly established that salt tolerance is a complex trait which is determined by several sub-traits, which may in turn be determined by several genes. By comparing different genes combinations in different genetic background, researchers will be able to develop salt tolerant germplasms (Yamaguchi and Blumwald, 2005).

Table 1: Transgenic plants expressing salt tolerant genes*

Gene	Gene product	Source	Cellular role	Target plant and reference
P5CS	D1-pyrroline-5-carboxylate synthetase	Mothbean	Proline accumulation	Rice (Zhu, 1998)
AtNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Arabidopsis*	Na$^+$ Vacuolar sequestration	*Arabidopsis* (Apse et al., 1999)
AtNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Arabidopsis*	Na$^+$ vacuolar sequestration	Tomato (Zhang and Blumwald, 2001), *Brassica* (Zhang et al., 2001)
Apo-Inv	Apoplastic invertase	Yeast	Sucrose accumulation	Tobacco (Fukushima et al., 2001)
AVP1	Vacuolar H$^+$ Pyrrophosphate	*Arabidopsis*	Vacuolar acidification	*Arabidopsis* (Gaxiola et al., 2001)
SAMDC	S-adenosylmethionine decarboxylase	*Tritordeum*	Biosynthesis of the polyamines	Rice (Roy and Wub, 2002)
AgNhX1	Vacuolar Na$^+$/H$^+$ antiporter	*Atriplex gmelini*	Na$^+$ vacuolar sequestration	Rice (Ohtaa et al., 2002)
HVA1	LEA3 accumulation	Barley	Membrane stabilization	Rice (Rohila et al., 2002)
AtSOS1	Plasma membrane Na$^+$/H$^+$ antiporter	*Arabidopsis*	Na$^+$ extrusion	*Arabidopsis* (Shi et al., 2003)
SOD2	Plasma membrane Na$^+$/H$^+$antiporter	*Schizosacchaomyce pombe*	Na$^+$ extrusion	Rice (Zhao et al., 2006)
GhNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Gossypium hirsutum*	Na$^+$ vacuolar sequestration	Tobacco (Wu et al., 2004)
BnNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Brassica napus*	Na$^+$ vacuolar sequestration	Tobacco (Wang et al., 2004)
AtNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Arabidopsis*	Na$^+$ vacuolar sequestration	Maize (Yin et al., 2004), Wheat (Xue et al., 2004)
HbNHX1	Vacuolar Na$^+$/H$^+$ antiporter	*Hordeum brevisubculatum*	Na$^+$ vacuolar sequestration	Tobacco (Lu et al., 2005)
nhaA	Plasma membrane Na$^+$/H$^+$ antiporter	*E. coli*	Na$^+$ extrusion	Rice (Wu et al., 2005)
TaSTRG	Na$^+$/H$^+$ antiporter	*Triticum aestivum*	Lower intracellular Na$^+$/K$^+$ ratios	Rice (Zhou et al., 2009)

*Modified from Yamaguchi and Blumwald (2005)

The areas under rice cultivation, particularly in south-east Asia, are continuously being exposed to saline environment due to inundation, indiscriminate use of fertilizers and depletion of ground water level. Consequently, attempts have been made to develop salt tolerant transgenic rice that can grow under these environments.

2.1. Increased ROS scavenging

In one of the earlier works, Tanaka et al. (1999) transferred yeast mitochondrial *Mn-SOD* gene into rice protoplasts through electroporation by using the chloroplast targetting signal of glutamine synthetase gene. They confirmed the accumulation of yeast *Mn-SOD* into the chloroplasts of transgenic lines by immunogold labeling experiments. Total SOD activity in the transformants was about 1.7 times higher than control under non-stressed conditions. Upon salt stress (100 mM NaCl), the SOD activity decreased at faster rate in control plants compared to transgenic lines. The ascorbate peroxidase activity of the transformants was about 1.5 times higher than that in the control plant at high levels of salinity. This indicated that salinity stress can be mitigated through ROS scavenging pathway. Supporting evidences can be obtained from Zhao et al. (2006) who found that transgenic rice plants expressing the plasma membrane Na$^+$/H$^+$ antiporter *SOD2* gene from yeast (*S. pombe*) maintained high cytosolic K$^+$/Na$^+$ ratio and accumulated more Ca^{2+} and Mg^{2+} in their shoots compared to controls.

The transgenic lines maintained higher photosynthetic activity, root proton exportation capacity, reduced ROS generation and increased root vesicles *P-ATPase* hydrolytic activity.

2.2. Modification of polyamines and osmolyte production

Zhu et al. (1998) incorporated D1-pyrroline-5-carboxylate synthetase (*P5CS*) gene of proline biosynthesis pathway from *Vigna aconitifolia* into the genome of rice plant via particle bombardment. The *P5CS* transgene expressed under control of stress-inducible promoter led to stress-induced overproduction and accumulation of proline in transgenic rice plants. They noticed an increase in biomass under salt-stress and water-stress conditions of second-generation (R_1) transgenic rice plants as compared to control.

S-adenosylmethionine decarboxylase (*SAMDC*), a key enzyme of polyamines biosynthesis, was transferred into the rice (*Oryza sativa* L.) through *Agrobacterium*-mediated transformation. The source of *SAMDC* gene was *Tritordeum* plant. The ABA-induced *SAMDC* gene expression conferred 3-4 times increase in polyamine, viz. spermidine and spermine levels and hence increased seedling growth of transgenic plants as compared to non-transgenic controls under sodium chloride stress (Roy and Wub, 2002). Wu et al. (2005) engineered a bacterial *nhaA* gene into *japonica* rice and found high level of expression in the transgenic rice. The rate of germination, plant growth, and average seed yield plant^{-1} of the transgenic lines were better than those of control lines under salt stress. The over-expression of *nhaA* gene enhanced osmoregulation by activating the biosynthesis of proline in transgenic lines.

Zhou et al. (2009) identified and cloned salt tolerance-related gene (*TaSTRG*) of salt-tolerant wheat (*Triticuma estivum* L.) mutant RH8706-49. The rice plants engineered through *Agrobacterium*-mediated transformation over-expressed *TaSTRG* gene and the transgenic lines exhibited higher rate of survival, plant fresh weight, chlorophyll, proline and soluble sugar contents, lower intracellular Na^+/K^+ ratio as well as significantly higher levels of expression of proline synthetase and transporter genes as compared to control under salt stress.

2.3. Other approaches

Halophytes have higher capability to thrive in saline soils. In a pioneer study, *AgNHX1* a gene from a halophyte, *Atriplex gmelini* was engineered into a salt-sensitive rice cultivar Kinuhikari. The transgenic lines exhibited eight-fold higher activity of the vacuolar-type Na^+/H^+ antiporter compared to that in wild-type rice plants and survived for 3 days at the concentration of 300 mM NaCl. This indicated that over-expression of the Na^+/H^+ antiporter gene in rice plants significantly enhances salt tolerance (Ohtaa et al., 2002).

Rohila et al. (2002) attempted to develop transgenic rice plants for enhancing abiotic stress tolerance through *Agrobacterium*-mediated transformation of *HVA1* gene from barley (encoding LEA 3 protein) under the control of a constitutive or a stress-inducible promoter in a Pusa Basmati 1 rice variety. Homozygous transformants of third generation (R_2) transgenic plants were exposed to high salinity and water stress and which exhibited good cell integrity and growth after exposure to salt and water stresses, as compared to non-transformant control plants. The improved salt and drought tolerance of transgenic Pusa Basmati 1 rice plants might be due to high levels of *LEA3* accumulation in the leaves.

3. Drought and Dehydration Stress

Water is the single most limiting factor for plant growth, as plants require substantial amount of water, much of which is lost through transpiration. Rice being a water loving plant requires the highest volume of water for cultivation in a crop season. Therefore, effect of drought stress is more prominent on rice severely limiting growth, panicle initiation and flowering of plants in the field. This results in drastic reduction in rice yield, which often goes down to less than 0.5 t ha^{-1} instead of potential yield of 5-6 t ha^{-1} in Asian countries.

Dehydration may result from either drought or heat stress. However, the responses to dehydration in both the stress environments are similar. Recent reports show that ABA responsive transcription factors (ABF) play an important role in dehydration tolerance. Similar to drought stress, it involves both ABA sensitive and ABA independent response pathways.

3.1. Over-expression of drought signaling pathway genes

A variety of genes are activated in response to drought stress in crop plants. These can be sub-divided further in two groups depending on their response to abscisic acid (ABA)—ABA responsive genes and genes that do not response to exogenous ABA application. The ABA responsive genes share common sequence known as ABA responsive elements (ABARE) in the promoter region, indicating common mechanism for regulation of these genes. A transcription factor that binds to the ABARE sequence has also been identified in *Arabidopsis*, rice and maize. However, studies have revealed that ABA is not only involved in drought tolerance but also play crucial role in salinity, dehydration and cold tolerance mechanisms (Seki et al., 2002). A second group of ABA independent genes are DREB (Drought Responsive Element Binding) genes, with a common motif DRE/CRT. This was first isolated in *Arabidopsis* and later identified in rice as OsDREB genes (Doubuzet et al., 2003). However, there are certain genes that share both ABF and DREB target sequences, such as RD29 in *Arabidopsis*.

Oh et al. (2005) developed transgenic rice plants that constitutively expressed *CBF3/DREB1A* (*CBF3*) and *ABF3*, *Arabidopsis* genes that function in ABA-independent and ABA-dependent stress-response pathways, respectively. They showed that *CBF3* in transgenic rice elevated tolerance to drought and high salinity, and produced relatively low levels of tolerance to low temperature exposure. On the other hand, *ABF3* in transgenic rice increased tolerance to drought stress alone.

Mitogen Activated Protein Kinases and their Kinases (MAPK, MAPKK) are also involved in signal transduction in drought response pathway. Ning et al. (2010) have identified a MAPK Kinase Kinase (MAPKKK) gene in rice from a drought hypersensitive mutant, producing a protein DSM1. They found that the protein is expressed under salt, drought and ABA stress, but not by cold stress. Upon over-expression, the transgenic plant exhibits tolerance to dehydration stress at seedling stage.

3.2. Other approaches

In order to investigate the possible role of *LOX3* gene under stresses, Liu et al. (2008) constructed a plant expression vector containing antisense cDNA of *LOX3* and transformed two rice varieties namely Wuyunjing 7 and Kasalath by the *Agrobacterium*-mediated method. Antisense *LOX3* gene integration was confirmed by PCR and Southern blot analyses. The sensitivity of T_2 antisense *LOX3* plants to drought stress as compared to non-transgenic plants revealed the possible function of *LOX3* gene in response to drought stress.

Fang et al. (2005) engineered *MnSOD* (Manganese Superoxide Dismutase) from *Pisum sativum* under the control of an oxidative stress-inducible SWPA2 promoter into rice chloroplasts by *Agrobacterium*-mediated transformation to develop drought-tolerant rice plants. *MnSOD*, an important antioxidant enzyme, may play role in the drought tolerance of rice. Drought stress induced by polyethylene glycol (PEG) 6000 revealed the expression of the pea *MnSOD* in transgenic rice plants (T_1). Transgenic plants showed reduced electrolyte leakage and less injury, measured by net photosynthetic rate, as compared to wild type which revealed that SOD is a critical component of the ROS scavenging system in plant chloroplasts. In another study Li et al. (2009) generated transgenic rice plants, with inhibited expression of *RACK1* gene by RNA interference (RNAi), to decipher the possible functions of *RACK1* in responses to drought stress in rice. Under drought stress the transgenic rice plants exhibited significantly lower production of malondialdehyde, reduced peroxidation of membrane and significantly higher activity of the superoxide dismutase in transgenic rice plants than those in wild type rice plants.

For identification of suitable candidate genes for genetic transformation for drought tolerance, Xiao et al. (2009) evaluated seven candidate genes, viz. *CBF3*, *SOS2*, *NCED2*, *NPK1*, *LOS5*, *ZAT10* and *NHX1* by transforming them into rice cultivar Zhonghua 11. They generated 1598 transgenic plants and observed eight events showed higher yield under drought stress. The genes *LOS5* and *ZAT10* performed better than other genes for inducing drought tolerance.

4. High Temperature Stress

Projections of global warming predicted that there would be a temperature rise of about 2-5^{0}C from 1990 to 2100 (Houghton et al., 2001). Such rapid increase in earth temperature poses a direct threat to agriculture since high temperature severely limits crop growth. Peng et al. (2004) showed that with 1^{o}C increase in maximum temperature at night reduced rice yield by 10%. High temperature stress directly affects leaf photosynthesis at physiological level, since leaves are the most exposed parts of plant to direct sunlight. A group of plant proteins, known as heat shock protein (HSP), are synthesized in response to high temperature in plants. These proteins act as chaperones or protectors of other biologically active proteins by reversible binding. Several groups of HSP have been identified in plants, including *hsp60*, *hsp70*, *hsp90* and *hsp100* group of proteins.

Sohn and Back (2007) produced high temperature tolerant transgenic rice plants by incorporation of chloroplast localized ω-3 fatty acid desaturase (*FAD7*) gene of *Arabidopsis thaliana* under the control of the maize ubiquitin promoter, in which the content of dienoic fatty acids was increased as a result of gene silencing of endogenous fatty acid desaturase via co-suppression mechanism. Transgenic rice lines exhibited approximately 1.6 and 2.1 times more growth, higher chlorophyll content and maximum photochemical efficiency of photosystem-II at 35 °C as compared to non-transformed wild type rice.

A rice cultivar Zhonghua 11 was engineered with *SBPase* (a gene encoding rice Calvin cycle enzyme sedoheptulose-,7-bisphosphatase) containing a maize ubiquitin promotor and nopaline synthase terminator sequences via the standard *Agrobacterium*-mediated transformation. Under high temperature stress the resulting transformants over-expressed and accumulated *SBPase* in chloroplasts vis a vis maintained the activation of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) through prevention of the sequestration of Rubisco activase to the thylakoid membranes from the soluble stroma fraction and hence improved the tolerance of CO_2 assimilation to high temperature stress (Feng et al., 2007).

5. Cold Stress

Cold stress affects rice plant at various growth stages, more severely at seedling and flowering stage in particular. At the nursery stage typical response to cold stress is yellowing of rice leaves and seedling death. At the flowering stage the effect is more severe and grain formation is hampered. It has been estimated that cold stress alone is more severe than biotic stresses in some Asian countries, particularly in China and Japan. A few QTLs controlling cold tolerance have been identified in *japonica* rice.

It was noted by Oh et al. (2005) that over-expression of *Arabidopsis* CBF In 2008, Xiang et al. have engineered ICE1, a transcription factor isolated from *Arabidopsis* for developing cold stress tolerant rice plants. The transgenic seedlings had lower mortality than control. An accumulation of proline was noted in the transgenic plant.

By regulating the MYB transcription factor, Ma et al. (2009) developed transgenic rice lines having tolerance to chilling stress. They over-expressed a gene *OsMYB3R-2* in rice and observed better tolerance to cold stress, which was primarily due to higher cell division activity and accumulation of proline in the transgenic plant.

6. Submergence Stress

Submergence stress resulted from partial or complete flooding cause oxygen starvation in plant cells. Rice exhibits tremendous variations in reponse to submergence stress and generally is more tolerant than other crop species. Certain rice genotypes can survive long duration (about two weeks) under submerged condition. In general, the adaptive response in hypoxic roots is production of more ethylene, and therefore physiological responses in submerged tissues are influenced by ethylene production. The flood tolerant rice genotypes harbor a gene *Sub1*, which encodes for ethylene response factor proteins. This QTL was first located on chromosome 9 of rice. On further characterization and dissection of this locus, Xu et al. (2006) identified three genes, *Sub1A*, *Sub1B* and *Sub1C* in *Sub1* locus which confers tolerance to submergence in a rice genotype *FR13A*. Of these, *Sub1A* was of greater interest as an ethylene response factor like gene. They over-expressed a variant of *Sub1A* (*Sub1A-1*) in submergence susceptible rice genotype (Liaogeng) and observed that the transgenic plants can survive better under submergence than control. The *Sub 1* locus has also been transferred through marker assisted selection and back cross-breeding in a number of rice genotypes. A prominent example is the development of Swarna-Sub1, a near isogenic line of popular rice variety Swarna in India. The Swarna-Sub1 has the same yield potential as Swarna, but has higher productivity under flooded condition.

7. Nutritional Stress

Both nutritional deficiency and mineral toxicity create stress environment for plant limiting their growth and development. A few transgenic approaches have been initiated to develop nutritional stress resistance in rice.

To develop tolerance to aluminum (Al) toxicity, a bacterial type transporter gene has been identified in rice that confers tolerance to Al stress. This gene was engineered into Al-sensitive rice protoplast that exhibited phenotypic response for Al-toxicity tolerance (Huang et al., 2009).

8. Multiple Stress Tolerance

Recently, a number of transgenic researchers have targeted to tackle multiple stress tolerance in rice, since the abiotic stresses often come in combination. Utilizing the common pathway for various abiotic stress responses, scientists have been able to identify genes that would provide resistance to more than one abiotic stresses.

Wu et al. (2005) showed that expression of *E. coli nha* gene in rice increased the level of salt and drought tolerance in rice. They suggested that *nha* gene over-expression leads to higher accumulation of proline, which results in salt and drought tolerance.

A *Triticum aestivum* salt tolerance related gene (*TaSTRG*) was cloned from wheat and expressed in rice by Zhou et al. (2009). Transgenic rice plants over-expressing *TaSTRG* gene showed higher salt and drought tolerance than the control. This was associated with lower intracellular Na$^+$/K$^+$ ratio under salt stress. Transgenic rice plants had higher survival rate, fresh weight and chlorophyll content, accumulated higher proline and soluble sugar contents.

Transcription factors regulate gene expression by binding to the promoter regions allowing RNA synthesis. A group of transcription factors have been identified in rice that play crucial role in abiotic stress tolerance. Oh et al. (2009) identified a total of 42 such transcription factors that are induced during different abiotic stresses and share an *APETEL2* like domain. They found that over-expression of *AP37* and *AP59*, two of these genes, confer tolerance to drought and high salinity stress at vegetative stages. *AP37* transgenic plants also exhibited increased cold tolerance. Similarly, another leucine zipper transcription factor, *OsbZIP23* when over-expressed in transgenic rice, confers tolerance to both drought and salinity stress. It is presumed that this gene is a key regulator in the ABA-dependant drought/salt tolerance pathway in rice.

9. Conclusion

The volume of information generated in transgenic rice research would obviously benefit development of transgenic genotypes in other crop plants. Increased reports of obtaining multiple stress tolerance in the recent years indicate that the future research direction for abiotic stress tolerance in rice and in other crops will concentrate more on multiple stress tolerance, including both biotic and abiotic stresses.

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