Chapter 9
Abiotic Stress Tolerant Transgenic Plants and Nanotechnology

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Abstract Crop plants are adversely affected by abiotic stresses. Drought is the most widespread and damaging of all environmental stresses. At the global level, significant proportion of cultivable land masses is affected by high salt levels. Heat and cold stresses profoundly affect agricultural yields of major crops. Also, the level of abiotic stresses is on the rise due to both natural and man-made interventions. The ambient temperature is gradually increasing due to the increased levels of CO_2 and other greenhouse gases. The episodes of drought and flooding stress have become more erratic over the years. The production of transgenic crops that can withstand increased level of abiotic stresses is a silver lining to sustain and increase food production in future. Techniques of producing transgenic crops need to be improvised to achieve high frequency transformation. Current experiments deploying nanotechnology tools for gene delivery are extremely relevant in production of new generation of transgenic plants. With such tools, it would be possible to experimentally produce higher number of transgenic lines and screen out the transgenic lines showing desired phenotype in higher numbers. In the ensuing paragraphs, we delve on the current status of abiotic stress tolerant transgenic crops and also project how nanotechnology tools can help in future endeavors.

Keywords Abiotic stress · Genetic transformation · Nanoparticles · Nanobiotechnology · Transgenics

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9.1 Introduction

9.1.1 Abiotic Stresses and Cultivation of Plants

Crop plants are adversely affected by abiotic stress conditions. Major abiotic stresses which affect plants include heat, cold, salinity, drought, flooding/submergence (anoxia), excess light, and chemical toxicities. Abiotic stresses profoundly affect growth and yield of plants. Drought or water stress is among the most important constraints in obtaining higher crop yields worldwide. Water stress affects plants in several ways. During vegetative stages, water stress reduces leaf expansion, photosynthesis, height of plant, and leaf area. Leaf rolling and leaf tip drying are the primary symptoms resulting from drought stress. Cell enlargement is severely affected by water stress. Decrease in water potential closes stomata and decreases transpiration (Kallarackal et al. 1990). The reproductive processes in plants are particularly sensitive to drought stress. Rice is most sensitive to drought for 10 days before flowering to the end of flowering (Yoshida et al. 1981). The water stress at flowering stage inhibits panicle exertion and spikelet filling. This causes high sterility, leading to decreased yield in rice (Ekanayake et al. 1989). The salt stress (mainly sodium chloride) results in Na^+ toxicity as well as physiological water stress to the plants. Estimates show that more than a third of all the irrigated land in the world is presently affected by salinity (Sahi et al. 2006). This is exclusive of the regions classified as arid and desert lands (which comprise 25 % of the total land of our planet). Salt affected lands occur in all climatic regions from deserts to tropical belts of Africa, Latin America, and Polar regions. The loss of farmable land due to salinization is directly in conflict with the needs of the world population. Excess salt levels affect enzyme activities and photosynthesis. The unfavorable Na^+ and Na^+/K^+ ratios adversely affect the grain yield of crops under salt stress conditions (Kaya et al. 2013). Temperature stress is observed when ambient temperature is below (low temperature stress) or above (high temperature stress) the optimal levels. These temperature extremes are injurious to plant growth and development as plants have evolved to grow in a narrow temperature regime (Singh and Grover 2008). In recent years, there has been a general increase in extreme events including floods, droughts, and heat episodes. The man-made changes in the climate of the earth due to the multifarious activities linked to development have become the focus of scientific attention (Grover et al. 2003). The most imminent of climatic changes of the earth is the increase in the atmospheric temperatures due to increased levels of CO_2 and other greenhouse gases. In certain places, climatic extremes such as droughts, floods, timing of rainfall, and melting of snow have also shown erratic trends. This is adversely affecting agriculture through its direct and indirect effects on crops (Grover et al. 2013).

9.2 Genetic Improvement of Plants Against Abiotic Stresses

From the preceding discussion, it is amply clear that crop production can be appreciably enhanced if proper management practices to save plants from such stressful conditions are devised. In simple terms, the crop production is an outcome of G × E equation where G refers to the genotype; E, the environment (El-Soda et al. 2014). It is relatively difficult, expensive, and hence impractical to change the environmental variables for obtaining optimal growth of crops. On the other hand, it is relatively inexpensive if the G factor (i.e., the genotype) can be suitably altered enabling plants to successfully grow, reproduce, and set seeds under stressful conditions. Crops have been genetically altered and improved for a host of different agronomic traits in the long history of the development of agriculture. Plant breeders have been greatly successful in enhancing the yields of major crops employing the conventional Mendelian tools like selection, hybridization, and progeny analysis. However, the response of crops against abiotic stresses is very complex because it involves multiple genes. The application of conventional plant breeding methods has proven difficult for genetic improvement of plants against abiotic stresses. In recent years, newer breeding tools like ‘molecular breeding’ have shown promising results in genetic improvement of plants against salt stress, flooding stress etc. (Jenks et al. 2007). The nonconventional tools of genetic improvement like ‘transgenic tools’ are becoming very significant for genetic improvement of crops against abiotic stresses.

9.3 Births and Growth of Transgenic Technology

Molecular biology science was born essentially in the middle of the twentieth century. The experiments leading to (1) elucidation of DNA structure and proposition of the double helix model of this molecule and (2) the discovery of the mechanisms underlying DNA replication and processes involved in its functioning (i.e., transcription leading to formation of RNA from DNA and translation leading to synthesis of proteins from mRNA) have brought revolutionary changes in our understanding of the living cells. In the beginning of 1980s, the art of producing transgenic plants was crafted (Galun and Breiman 1997). The production of transgenic plants provides a way to modify the genetics of plants so that superior types can be bred in a relatively short term. The gene transfers take place between the sexually compatible individuals in conventional and molecular plant breeding approaches. As against this, gene transfer is practiced across a wider platform of individuals overcoming the barriers of sexual compatibility in the transgenic approach (Grover et al. 1999). For instance, transgenic plants have been produced for increased insect resistance by incorporating a gene from bacteria and for increased cold resistance incorporating a gene from fish using the methods of plant genetic engineering (Duman and Wisniewski 2014; Ibrahim and Shawer 2014). Since 1980, substantial progress has been made in transgenic plant production science. Transgenic plants with improved resistance to insect infestations using the most celebrated principle of Bt gene technology has been widely practised in a host of different crops (Peferon 1997). Significant success has also been obtained in breeding herbicide and virus resistance in crops by rDNA technology (Chen and Lin 2013). In recent years, transgenic plants have been produced to improve nutritional quality and to change the physiological and developmental aspects of plants (Zhu et al. 2007). To sum up, the approach of transgenic plant production is a new and, highly effective arsenal in the hands of agricultural scientists to enhance crop yields.

9.4 Production of Abiotic Stress Tolerant Transgenic Crops

Coming back to abiotic stresses, question we now address is how to engineer crops against abiotic stresses like salt, drought, and adverse temperatures. While much of the success in transgenic experiments has come for insect resistance and herbicide tolerance as mentioned above, several attempts have also been made to genetically engineer plants against abiotic stresses in the past 25 years. Scientists have explored a host of different transgenes for enhancing resistance of crops against salt, drought, and high and low temperatures. Several genes have enabled production of transgenic rice against drought, salt, flooding, and temperature extremes (Table 9.1). Likewise, host of plant species including model species like *Arabidopsis* and tobacco and crops like tomato and wheat have been genetically transformed for different abiotic stresses. It can be safely argued that results obtained from these experiments are of somewhat mixed nature. On the one hand, there are definite reports showing that plants have been successfully produced with enhanced abiotic stress tolerance by transgenic methods. On the other hand, there is poor success in field application of abiotic stress tolerant transgenic crops in spite of all the intensive efforts. Most of the success reported till this day is from laboratory-based experiments (Grover et al. 2013). It is therefore important to revisit these experiments and address what we possibly lack in our efforts in producing abiotic stress tolerant crops at the level of field cultivation.

The success in production of transgenic plants is principally governed by three critical inputs: (a) the availability of the effective gene, (b) relevant techniques for transferring the transgene inside the genome of the desired trans-host species, and (c) regulated expression of the transgene in the trans-host (Grover et al. 2001). The transfer of genes in genetic engineering experiments is largely a random event. Experiments have shown that the level of expression of the transcripts/proteins from the transgenes in the trans-hosts is highly variable. A great deal of effort is

Table 9.1 Protocol details involved in genetic engineering for increased abiotic stress tolerance in rice

Gene name	Encoding protein	Source species	Promoter	Phenotype of overexpression transgenic plants	References
Signal transduction genes					
SAPK4	Sucrose nonfermenting I-type serine threonine protein kinase	*Oryza sativa*	CaMV35S	Overexpression transgenic plants grew faster under salt stress conditions both at seedling and at mature plant stages	Diédhou et al. (2008)
DSM1	Mitogen activated protein kinase kinase kinase	*O. sativa*	Ubi-1 (*Zea mays* ubiquitin promoter)	Increased dehydration stress tolerance at seedling stage of overexpression transgenic plants	Ning et al. (2010)
OsSIK1	Receptor-like kinase	*O. sativa*	Double CaMV35S	Overexpression transgenic plants more tolerant to salt and drought stresses; enhanced peroxidase, superoxide dismutase, and catalase activities. Reduced stomatal density in overexpression transgenics	Ouyang et al. (2010)
OsCPK21	Calcium-dependent protein kinase	*O. sativa*	Ubi-1	Increased survival of overexpression transgenics under salt stress conditions. Growth inhibition of transgenic seedlings by abscisic acid (ABA) more than that of wild type (WT) seedlings	Asano et al. (2011)
OsITPK2	Inositol 1,3,4-triphosphate 5/6-kinase	*O. sativa*	CaMV35S	Hypersensitivity of overexpression transgenics to drought and salt stresses. Reduced levels of inositol triphosphate and genes related to osmoregulation and reactive oxygen species (ROS) homeostasis	Du et al. (2011)
OsCPK12	Calcium-dependent protein kinase	*O. sativa*	CaMV35S	Overexpression resulted in enhanced salinity tolerance, upregulation of ROS scavengers, greater ABA sensitivity and more susceptibility to blast disease resistance	Asano et al. (2012)
OsSIK2	S-domain receptor-like kinase	*O. sativa*	CaMV35S	Salt and drought stress tolerance resulting from overexpression. Early leaf development and delayed dark-induced senescence in transgenics	Chen et al. (2013)

(continued)

Table 9.1 (continued)

Gene name	Encoding protein	Source species	Promoter	Phenotype of overexpression transgenic plants	References
OsCPK9	Calcium-dependent protein kinase	*O. sativa*	CaMV35S	Increased drought tolerance through enhanced stomatal closure and better osmoregulation in transgenics. Higher pollen viability leading to increased spikelet fertility	Wei et al. (2014)
OsCPK4	Calcium-dependent protein kinase	*O. sativa*	Ubi-1	Salt and drought stress tolerance by prevention of stress-induced membrane lipid peroxidation in the transgenics. Overexpression transgenics exhibited higher water holding capacity and reduced electrolyte leakage under stress	Campo et al. (2014)
Transcription factor genes					
JERF3	Ethylene response factor protein	*O. sativa*	CaMV35S	Increased drought and osmotic stress tolerance; higher accumulation of soluble sugars and proline, upregulation of stress-responsive genes like *WCOR413-like*, *OsEnol* and *OsSPDS2* in overexpression transgenics	Zhang et al. (2010)
OsNAC10	NAC protein (acronym for *NAM* [no apical meristem], *ATAF1*-2, *CUC2*[cup-shaped cotyledon])	*O. sativa*	Constitutive GOS2 promoter and root specific RCc3 promoter	Increased tolerance to drought, salt, and low temperature at vegetative stage. Enlarged roots, greater tolerance, and improved grain yield under field level drought stress	Jeong et al. (2010)
OsWRKY45-1 and OsWRKY45-2	WRKY protein	*O. sativa*	Ubi-1	Against salt stress, OsWRKY45-1 allele overexpression showed no difference, OsWRKY45-2 allele overexpression showed increased tolerance. ABA signaling negatively regulated by OsWRKY45-1 allele and positively regulated by OsWRKY45-2 allele	Tao et al. (2011)

(continued)

Table 9.1 (continued)

Gene name	Encoding protein	Source species	Promoter	Phenotype of overexpression transgenic plants	References
ZmCBF3	C-repeat binding transcription factor	*Z. mays*	Ubi-1	Increased tolerance to drought, salt and cold stresses; no growth retardation and yield penalty under normal conditions	Xu et al. (2011)
OsDREB2A	Dehydration response element binding factor	*O. sativa*	4ABRC(stress-inducible promoter)	Induced overexpression enhanced survival of transgenic plants under severe drought and salt conditions	Cui et al. (2011)
OsNAC9	NAC protein	*O. sativa*	GOS2 and RCc3	Increased grain yield under normal conditions; under drought stress, increased yield in RCc3:OsNAC9 transgenics. Changed root architecture possibly imparting increased drought stress tolerance	Redillas et al. (2012)
OsbZIP46	Basic leucine zipper transcription factor	*O. sativa*	Ubi-1	Overexpression of native gene showed no effect on drought tolerance whereas overexpression of a constitutively active form of OsbZIP46 (named OsbZIP46CA1) enhanced drought and salt stress tolerance	Tang et al. (2012)
OsbZIP16	Basic leucine zipper transcription factor	*O. sativa*	Act-1(rice actin 1 promoter)	Increased tolerance to drought stress at seedling and tillering stages; increased sensitivity to ABA	Chen et al. (2012)
ZFP182	TFIII-A type zinc finger transcription factor	*O. sativa*	CaMV35S	Increased tolerance to salt, cold, and drought stresses; accumulation of proline and soluble sugars in transgenic plants	Huang et al. (2012)
EDT1/HDG11	Homeodomain-leucine zipper transcription factor	*Arabidopsis thaliana*	Act-1	Drought stress tolerance assigned to the extensively developed root system, lesser stomatal density, greater water use efficiency, accumulation of compatible osmolytes and higher antioxidant enzyme activities. Increased seed setting, bigger panicles, more tillers, and enhanced photosynthesis activity leading to higher grain yield of transgenic plants	Yu et al. (2013)

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Table 9.1 (continued)

Gene name	Encoding protein	Source species	Promoter	Phenotype of overexpression transgenic plants	References
AtDREB1A	Dehydration response element binding factor	*A. thaliana*	rd29-A (stress-inducible promoter)	Higher drought stress tolerance associated with osmolyte accumulation, chlorophyll maintenance, higher relative water content, and reduced ion leakage. Increased spikelet fertility and grain yield under normal and stressful conditions	Ravikumar et al. (2014)
Antioxidant genes					
katE	Catalase	*Escherichia coli*	CaMV35S	Overexpression transgenics were tolerant to salt stress throughout the life cycle. Higher catalase activity was observed in the transgenics	Nagamiya et al. (2007)
glyII	Glyoxylase II	*O. sativa*	CaMV35S	Higher tolerance to toxic levels of methylglyoxal and NaCl in overexpression transgenics with sustained growth under salt stress conditions	Singla-Pareek et al. (2008)
Sod1	Copper Zinc superoxide dismutase	*Avicennia marina*	Ubi-1	Increased tolerance to salt and drought stress and to oxidative stress induced by methyl viologen	Prashanth et al. (2008)
GST and *CAT1*	Glutathione-*S*-transferase (GST) and catalase	*O. sativa*	CaMV35S	Increased tolerance to cadmium alone and a combination of cadmium and heat stress. Lesser oxidative damage under stressful conditions mediated by coexpression of GST and catalase enzymes	Zhao et al. (2009)
AeMDHAR	Monodehydroascorbate reductase	*Acanthus ebracteatus*	Double CaMV358	Salt tolerance at germination and seedling stages. Higher number of tillers and 1,000-grain weight of transgenic plants	Sultana et al. (2012)
OsMIOX	Myo-inositol oxygenase	*O. sativa*	CaMV35S	Increased tolerance to polyethylene glycol induced drought stress. Higher expression of ROS scavenging genes and higher ROS scavenging enzyme activity and proline content in transgenic plants	Duan et al. (2012)

(continued)

Table 9.1 (continued)

Gene name	Encoding protein	Source species	Promoter	Phenotype of overexpression transgenic plants	References
γ-ECS	γ-glutamylcysteine synthetase	*O. sativa*	Rab21 (stress-inducible promoter)	Higher salt tolerance concurrent with efficient redox homeostasis and 1.5-fold higher germination rate; lesser ion leakage and higher chlorophyll-fluorescence upon methyl viologen treatment. Higher grain yield and biomass of field grown transgenic plants	Choe et al. (2013)
Osmotic homeostasis genes					
SAMDC	*S*-adenosylmethionine decarboxylase	*Tritordeum*	ABA-inducible promoter	Higher biomass and increased spermidine and spermine accumulation under salt stress	Roy and Wu (2002)
codA	Choline oxidase	*Arthrobacter globiformis*	CaMV35S	Glycine betaine accumulation; more than 50 % transgenic plants tolerant to salt stress	Mohanty et al. (2002)
otsA and *otsB* fusion	Trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP)	*E. coli*	ABA-inducible promoter and rbcS (Rice rubisco small subunit) promoter	Tolerance to salt, drought, and low-temperature stresses. Greater trehalose accumulation, increase photosynthesis, maintained plant growth, reduced photo-oxidation, and better ion homeostasis under stressful conditions	Garg et al. (2002)
otsA and *otsB* fusion	TPS and TPP	*E. coli*	Ubi-1	High accumulation of trehalose in leaves and seeds of transgenic plants. Greater tolerance to cold, drought, and salt stresses without stunting of growth	Jang et al. (2003)
p5cs	Δ^1-pyrroline-5-carboxylate synthetase	*Vigna aconitifolia*	Act-1 and an ABA-inducible promoter	Higher accumulation of proline both under constitutive and stress-induced production of *p5cs* in transgenic plants. Faster growth under salt and water deficiency stress. Transgenic plants with stress-inducible synthesis of *p5cs* showed greater biomass than constitutively synthesizing transgenic plants	Su and Wu (2004)

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Table 9.1 (continued)

Gene name	Encoding protein	Source species	Promoter	Phenotype of overexpression transgenic plants	References
adc	Arginine decarboxylase	*Datura stramonium*	Ubi-1	Higher putrescine production in transgenic plants under drought stress leading to greater accumulation of spermidine and spermine and stress tolerance	Capell et al. (2004)
COX	Choline oxidase	*Arthrobacter pascens*	SIP (ABA-inducible promoter) and Ubi-1	Greater salt stress tolerance in SIP transgenics than Ubi-1 transgenics in spite of lower glycine betaine accumulation in the former. Greater biomass production in SIP transgenics grown under salt stress conditions	Su et al. (2006)
codA	Choline oxidase	*A. globiformis*	CaMV35S	Tolerance to water stress and better yield; higher photo system II activity, increased detoxification of ROS. Survival rate and agronomic performance of transgenics is better under prolonged water stress	Kathuria et al. (2009)
OsTPS1	Trehalose-6-phosphate synthase	*O. sativa*	Act-1	Transgenic plants tolerant to cold, salt, and drought stresses; higher trehalose and proline accumulation, upregulation of several stress-responsive genes	Li et al. (2011)
Ion homeostasis genes					
AgNHX1	Vacuolar-type Na^+/H^+ antiporter	*Atriplex gmelini*	CaMV35S plus first intron of catalase from *Ricinus communis* L.	Overexpression in salt sensitive rice cultivar *Kinuhikari* resulted in increased salt stress tolerance. As against WT rice, transgenic plants showed survival under 3 day treatment of 300 mM NaCl	Ohta et al. (2002)
OsNHX1	Vacuolar Na^+/H^+ antiporter	*O. sativa*	CaMV35S	Increased tolerance to salt stress in overexpression transgenics plants	Fukuda et al. (2004)

(continued)

Table 9.1 (continued)

Gene name	Encoding protein	Source species	Promoter	Phenotype of overexpression transgenic plants	References
nhaA	Na^+/H^+ antiporter	*E. coli*	CaMV35S	Enhanced growth of transgenic plants compared to WT under salt stress conditions; higher sodium and proline contents under salt or drought stress	Wu et al. (2005)
SOD2	Plasma membrane Na^+/H^+antiporter	*Saccharomyces pombe*	CaMV35S	Increased salt stress tolerance in transgenic plants associated with higher P-ATPase activity, enhanced photosynthesis and H^+ exchange capacity in roots and reduced ROS generation	Zhao et al. (2006)
PgNHX1	Vacuolar Na^+/H^+ antiporter	*Pennisetum glaucam*	ABA-inducible promoter	Overexpression resulted in enhanced tolerance to salinity with extensive root development as compared to WT plants	Verma et al. (2007)
OsKAT1	Shaker family Potassium channel	*O. sativa*	Ubi-1	Higher salt tolerance and increased cellular K^+ content; lower Na^+ to K^+ ratios in overexpression cells	Obata et al. (2007)
OsHAK5	Potassium transporter	*O. sativa*	CaMV35S	Tolerance to salt stress; increased uptake of K^+ by roots and root to shoot transport of K^+, higher K^+/Na^+ ratio in the shoots	Yang et al. (2014)

therefore needed to select the progenies that optimally express the trans-protein. To do so, it is important that methods of genetic transformation must yield larger populations of the transformed progenies. The progenies need to be adequately screened to select transgenic types with the optimal levels of gene expression. For this input, it is critical that gene transfer technologies are suitably optimized for effective biotechnological applications.

9.5 Methods of Genetic Transformation of Crops

The transfer of genes in destined plant cells is a critical step in rDNA technology. Since 1970s, several techniques have been optimized to deliver the desired genes to plant cells (Anami et al. 2013). Among these, *Agrobacterium tumefaciens* has been the major workhorse for plant scientists for the gene transfer. For a large number of plant species, this is the most-promising method of gene delivery. When *Agrobacterium* infects a wound site, a portion of its DNA (called transfer/T-DNA) is mobilized and gets integrated into the chromosome of the host cells which begin to proliferate, leading to the formation of tumor-like growth. The genes transferred from the bacterium to the plant are carried on the extrachromosomal circular plasmids called tumor-inducing (Ti) plasmids (Tzfira and Citovsky 2006). For the *Agrobacterium* to be an effective vehicle for DNA transfer, the tumor-inducing gene of Ti plasmids is removed through a process called ‘disarming’ (Simpson et al. 1986). The bulk of the genetic transformations with *Agrobacterium* were initially shown to work with dicot plant species. In recent years, there has been significant success in genetic transformation of monocot species like rice, corn, and wheat by *Agrobacterium* (Ji et al. 2013).

Apart from *Agrobacterium*, several other methods for genetic transformation have been devised. One such method is PEG-mediated DNA uptake where PEG (polyethylene glycol) and $CaCl_2$ are used for stimulation of the DNA uptake process as well as its integration in the isolated protoplasts (Datta et al. 1992). Another method is a related technique of ‘electroporation’ where the isolated protoplasts are given a shock treatment electrically by giving a short, high voltage pulse, to make transient pores in the cell membranes. This enables the plasmid DNA molecules to pass through the pores and enter from culture medium to inside plant cells (Ji et al. 2013). Microprojectile bombardment or biolistics is another innovative way for DNA transfer (Ji et al. 2013). In this method, a microprojectile gun or DNA particle gun is used to deliver DNA directly into plant cells by shooting it through the cell. Microscopic particles of tungsten or gold are coated with plasmid DNA molecules and then shot into the target cells. In the process, DNA is transferred to the nucleus which then integrates into the plant genome. Among the various methods mentioned above, *Agrobacterium*-based approach stands out as the most favored technique for genetic transformation of crops because of its simplicity, reliability, and wider applicability.

9.6 Nanotechnology in Gene Transfer Experiments

Of late, principles of nanotechnology have been extensively applied in physical, biological, agricultural, and medical sciences in a highly beneficial manner. In textile industry, the use of nanotechnology includes minimizing loss of cellulose during processing of cotton into end product, coming out with 100 nm diameter fibers capable of being good absorbent of chemical input in agriculture and boosting the efficacy of enzymes required in cellulose–ethanol conversion (Lang 2013). The application of nanotechnology to produce nanosilica from rice husk has not only addressed the problem of rice husk disposal but also has come handy in making substances like glass and concrete (Awizar et al. 2013). Delivery systems for pests, nutrients, and plant hormones have been manufactured using nanotechnology tools (Sekhon 2014; Nair et al. 2010). Nanotechnology facilitates controlled release of agrochemicals allowing direct delivery of various macromolecules at the preconceived sites for enhancing plant disease resistance, nutrient utilization, and plant growth (Nair et al. 2010). Nanoencapsulation increases efficiency in terms of usage and safe handling of pesticides (Nair et al. 2010).

Lately, researchers have started applying nanotechnology in plant genetic engineering with noticeable results. Torney et al. (2007) demonstrated that a honeycomb mesoporous silica nanoparticle (MSN) system with 3 nm pores can act as an effective conduit for DNA and chemicals for their onward journey to segregated plant cells and intact leaves. Using MSN particles coated with a plasmid expressing green fluorescent protein (GFP) under CaMV35S promoter, the researchers showed transient expression in tobacco mesophyll protoplasts. Intact plant cells of tobacco cotyledons were also successfully transformed using gene gun after the mesopores of the DNA-coated MSN particles were capped by gold nanoparticles. Efficacy of this system for generating stable transgenic plants was established by raising callus from GFP bombarded immature maize embryos. Subsequently, the researchers generated transgenic tobacco plants harboring GFP gene driven by an inducible promoter which could be chemically triggered by addition of β-oestradiol. Transgenic plantlets were bombarded with β-oestradiol-loaded MSN particles having their pores capped by gold nanoparticles to prevent leaching. Presence of DTT as an uncapping agent in the germination medium allowed for the controlled release of the chemical inducer followed by expression of the GFP transgene. As the gold nanoparticles can prevent the molecules from moving out, the group, utilizing this trait, coated the β-oestradiol containing MSN particles with inducible GFP gene, capped the extremes with gold nanoparticles, and bombarded this system into wild type plants. Undoing the capping in presence of DTT resulted into controlled release of chemicals triggering gene expression in plants. Further diversification like increasing the probe size and multifunctionalization of these MSNs can help achieve target-specific delivery of proteins, nucleotides, and chemicals in plant cells. In addition, several biogenic species can be delivered together and released in a controlled fashion at the target sites by the use of MSN technology. This group proposed that nanotechnology can be applied to plant science to aid further investigation of plant genomics and gene function as well as improvement of crop species.

9.7 Future Generation Stress Tolerant Transgenic Plants

As discussed above, the production of abiotic stress tolerant transgenic plants is a highly desired commercial goal in present-day agriculture. To realize this goal, newer and more effective methods of genetic transformations are needed. The new discovery that using nanotechnology tools, gene transfers can be achieved in a controlled manner opens up new directions. Using this new method, it should be possible to transform wider range of species. Further, it should be possible to apply nanotechnology-based approach for genetically transforming those species which are not amenable to genetic transformation using *Agrobacterium*. By combining biotechnology and nanotechnology sciences, it should be possible to more effectively achieve goals like production of abiotic stress tolerant crops and plants with other desired agronomic traits.

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References

Anami S, Njuguna E, Coussens G et al (2013) Higher plant transformation: principles and molecular tools. Int J Dev Biol 57:483–494. doi:10.1387/ijdb.130232mv

Asano T, Hakata M, Nakamura H et al (2011) Functional characterisation of OsCPK21, a calcium-dependant protein kinase that confers salt tolerance in rice. Plant Mol Biol 75:179–191. doi:10.1007/s11103-010-9717-1

Asano T, Hayashi N, Kobayashi M et al (2012) A rice calcium-dependant protein kinase OsCPK12 oppositely modulates salt-stress tolerance and blast disease resistance. Plant J 69:26–36. doi:10.1111/j.1365-313X.2011.04766.x

Awizar DA, Othman NK, Jalar A et al (2013) Nanosilicate extraction from rice husk ash as green corrosion inhibitor. Int J Electrochem Sci 8:1759–1769

Campo S, Baldrich P, Messeguer J et al (2014) Overexpression of a calcium-dependant protein kinase confers salt and drought tolerance in rice by preventing membrane lipid peroxidation. Plant Physiol 165:688–704. doi:10.1104/pp.113.230268

Capell T, Bassie L, Christou P et al (2004) Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proc Natl Acad Sci USA 101:9909–9914. doi:10.1073/pnas.0306974101

Chen H, Lin Y (2013) Promise and issues of genetically modified crops. Curr Opin Plant Biol 16:255–260. doi:10.1016/j.pbi.2013.03.007

Chen H, Chen W, Zhou J et al (2012) Basic leucine zipper transcription factor OsbZIP16 positively regulates drought resistance in rice. Plant Sci 193:8–17. doi:10.1016/j.plantsci.2012.05.003

Chen LJ, Wuriyanghan H, Zhang YQ et al (2013) An S-domain receptor-like kinase, OsSIK2, confers abiotic stress tolerance and delays dark-induced leaf senescence in rice. Plant Physiol 163:1752–1765. doi:10.1104/pp.113.224881

Choe YH, Kim YS, Kim IS et al (2013) Homologous expression of γ-glutamylcysteine synthetase increases grain yield and tolerance of transgenic rice plants to environmental stresses. J Plant Physiol 170:610–618. doi:10.1016/j.jplph.2012.12.002

Cui M, Zhang W, Zhang Q et al (2011) Induced over-expression of the transcription factor *OsDREB2A* improves drought tolerance in rice. Plant Physiol Biochem 49:1384–1391. doi:10.1016/j.plaphy.2011.09.012

Datta SK, Datta K, Soltanifar N et al (1992) Herbicide-resistant Indica rice plants from IRRI breeding line IR72 after PEG-mediated transformation of protoplasts. Plant Mol Biol 20:619–629

Diédhou CJ, Popova OV, Dietz KJ et al (2008) The SNFI-type serine-threonine protein kinase *SAPK4* regulates stress-responsive gene expression in rice. BMC Plant Biol 8:1–13. doi:10.1186/1471-2229/8/49

Du H, Liu L, You L et al (2011) Characterization of an inositol 1,3,4-triphosphate 5/6-kinase gene that is essential for drought and salt stress response in rice. Plant Mol Biol 77:547–563. doi:10.1007/s11103-011-9830-9

Duan J, Zhang M, Zhang H et al (2012) *OsMIOX*, a *myo*-inositol oxygenase gene, improves drought tolerance through scavenging of reactive oxygen species in rice (*Oryza sativa* L.). Plant Sci 196:143–151. doi:10.1016/j.plantsci.2012.08.003

Duman JG, Wisniewski MJ (2014) The use of antifreeze proteins for frost protection in sensitive crop plants. Environ Exp Bot 106:60–69. doi:10.1016/j.envexpbot.2014.01.001

Ekanayake IJ, De Datta SK, Steponkus (1989) Spikelet sterility and flowering response of rice to water stress at anthesis. Ann Bot 63:257–264

El-Soda M, Malosetti M, Zwaan BJ et al (2014) Genotype X environment interaction QTL mapping in plants: lessons from *Arabidopsis*. Trends Plant Sci 19:390–398. doi:10.1016/j.tplants.2014.01.001

Fukuda A, Nakamura A, Tagiri A et al (2004) Function, intracellular localization and the importance in salt tolerance of a vascular Na^+/H^+ antiporter from rice. Plant Cell Physiol 45:146–159

Galun E, Breiman A (1997) Tools for genetic transformation. In: Selectable genes, transgenic plants. Imperial College Press, London, pp 62–64

Garg AK, Kim JK, Owens TG et al (2002) Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci USA 99:15898–15903. doi:10.1073/pnas.252637799

Grover A, Sahi C, Sanan N et al (1999) Taming abiotic stresses in plants through genetic engineering: current strategies and perspective. Plant Sci 143:101–111

Grover A, Kapoor A, Lakshmi OS et al (2001) Understanding molecular alphabets of the plant abiotic stress responses. Curr Sci 80:206–216

Grover A, Aggarwal PK, Kapoor A et al (2003) Addressing abiotic stresses in agriculture through transgenic technology. Curr Sci 84:355–368

Grover A, Mittal D, Negi M et al (2013) Generating high temperature tolerant transgenic plants: achievements and challenges. Plant Sci 205–206:38–47

Huang J, Sun S, Xu D et al (2012) A TFIIIA-type zinc finger protein confers multiple abiotic stress tolerance in transgenic rice (*Oryza sativa* L.). Plant Mol Biol 80:337–350. doi:10.1007/s11103-012-9955-5

Ibrahim RA, Shawer DM (2014) Transgenic *Bt*-plants and the future of crop protection. Int J Agri Food Res 3:14–40

Jang IC, Oh SJ, Seo JS et al (2003) Expression of a biofunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. Plant Physiol 131:516–524. doi:10.1104/pp.007237

Jenks MA, Hasegawa PM, Jain SM (eds) (2007) Advances in molecular breeding toward drought and salt tolerant crops. Springer, Dordrecht

Jeong JS, Kim YS, Baek KH et al (2010) Root-specific expression of *OsNAC10* improves drought tolerance and grain yield in rice under field drought conditions. Plant Physiol 153:185–197. doi:10.1104/pp.110.154773

Ji Q, Xu X, Wang K (2013) Genetic transformation of major cereal crops. Int J Dev Biol 57:495–508. doi:10.1387/ijdb.130244kw

Kallarackal J, Millburn JA, Baker DA (1990) Water relations of the banana. III. effects of controlled water stress on water potential, transpiration, photosynthesis and leaf growth. Aust J Plant Pathol 17:79–90. doi:10.1071/PP9900079

Kathuria H, Giri J, Nataraja KN et al (2009) Glycinebetaine-induced water-stress tolerance in *codA*-expressing transgenic *indica* rice is associated with up-regulation of several stress responsive genes. Plant Biotechnol J 7:512–526. doi:10.1111/j.1467-7652.2009.00420.x

Kaya C, Ashraf M, Dikilitas M et al (2013) Alleviation of salt stress-induced adverse effects on maize plants by exogenous application of indoleacetic acid (IAA) and inorganic nutrients-A field trial. Aust J Crop Sci 7:249–254

Lang SS (2013) Waste fiber can be recycled into valuable products using new technique of electrospinning, Cornell researchers report. http://www.news.cornell.edu/stories/2003/09/electrospinning-cellulose-waste-fiber. Accessed 26 Sept 2014

Li HW, Zang BS, Deng XW et al (2011) Overexpression of the trehalose-6-phosphate synthase gene *OsTPS1* enhances abiotic stress tolerance in rice. Planta 234:1007–1018. doi:10.1007/s00425-011-1458-0

Mohanty A, Kathuria H, Ferjani A et al (2002) Transgenics of an elite *indica* rice variety Pusa Basmati 1 harbouring the *codA* gene are highly tolerant to salt stress. Theor Appl Genet 106:51–57. doi:10.1007/s00122-002-1063-5

Nagamiya K, Motohashi T, Nakao K et al (2007) Enhancement of salt tolerance in transgenic rice expressing an *Escherichia coli* catalase gene, *katE*. Plant Biotechnol Rep 1:49–55. doi:10.1007/s11816-007-0007-6

Nair R, Varghese SH, Nair BG (2010) Nanoparticulate material delivery to plants. Plant Sci 179:154–163. doi:10.1016/j.plantsci.2010.04.012

Ning J, Li X, Hicks LM et al (2010) A Raf-like MAPKKK gene *DSM1* mediates drought resistance through reactive oxygen species scavenging in rice. Plant Physiol 152:876–890. doi:10.1104/pp.109.149856

Obata T, Kitamoto HK, Nakamura A et al (2007) Rice shaker potassium channel OsKAT1 confers tolerance to salinity stress on yeast and rice cells. Plant Physiol 144:1978–1985. doi:10.1104/pp.107.101154

Ohta M, Hayashi Y, Nakashima A et al (2002) Introduction of a Na^+/H^+ antiporter gene from *Atriplex gmelini* confers salt tolerance to rice. FEBS Lett 532:279–282

Ouyang SQ, Liu YF, Liu P et al (2010) Receptor-like OsSIK1 improves drought and salt stress tolerance in rice (*Oryza sativa*) plants. Plant J 62:316–329. doi:10.1111/j.1365-313X.2010.04146.x

Perferoen M (1997) Progress and prospects for field use of *Bt* genes in crops. TIBTECH 15:173–177

Prashanth SR, Sadhasivam V, Parida A (2008) Over expression of cytosolic copper/zinc superoxide dismutase from a mangrove plant *Avicennia marina* in *indica* rice var Pusa Basmati-1 confers abiotic stress tolerance. Transgenic Res 17:281–291

Ravikumar G, Manimaran P, Voleti SR et al (2014) Stress-inducible expression of *AtDREB1A* transcription factor greatly improves drought stress tolerance in transgenic *indica* rice. Transgenic Res 23:421–439

Redillas MCFR, Jin JS, Kim YS et al (2012) The overexpression of *OsNAC9* alters the root architecture of rice plants enhancing drought resistance and grain yield under field conditions. Plant Biotechnol J 10:792–805

Roy M, Wu R (2002) Overexpression of *S*-adenosylmethionine decarboxylase gene in rice increases polyamine level and enhances sodium chloride-stress tolerance. Plant Sci 163:987–992

Sahi C, Singh A, Blumwald E et al (2006) Beyond osmolytes and transporters: novel plant salt-stress tolerance-related genes from transcriptional profiling data. Physiol Plant 127:1–9

Sekhon BS (2014) Nanotechnology in agri-food production: an overview. Nanotechnol Sci Appl 7:31–53. doi:10.2147/NSA.S39406

Simpson RB, Spielmann A, Margosian L et al (1986) A disarmed binary vector from *Agrobacterium tumefaciens* functions in *Agrobacterium rhizogenes*. Plant Mol Biol 6:403–415

Singh A, Grover A (2008) Genetic engineering for heat tolerance in plants. Physiol Mol Biol Plants 14:155–166

Singla-Pareek SL, Yadav SK, Pareek A et al (2008) Enhancing salt tolerance in a crop plant by overexpression of glyoxalase II. Transgenic Res 17:171–180. doi:10.1007/s11248-007-9082-2

Su J, Wu R (2004) Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis. Plant Sci 166:941–948. doi:10.1016/j.plantsci.2003.12.004

Su J, Hirji R, Zhang L et al (2006) Evaluation of the stress-inducible production of choline oxidase in transgenic rice as a strategy for producing the stress-protectant glycine betaine. J Exp Bot 57:1129–1135. doi:10.1093/jxb/erj133

Sultana S, Khew CY, Morshed MM et al (2012) Overexpression of monodehydroascorbate reductase from a mangrove plant (AeMDHAR) confers salt tolerance on rice. J Plant Physiol 169:311–318. doi:10.1016/j.jplph.2011.09.004

Tang N, Zhang H, Li X et al (2012) Constitutive activation of transcription factor OsZIP46 improves drought tolerance in rice. Plant Physiol 158:1755–1768. doi:10.1104/pp.111.190389

Tao Z, Kou Y, Liu H et al (2011) *OsWRKY45* alleles play different roles in abscisic acid signalling and salt tolerance but similar roles in drought and cold tolerance in rice. J Exp Bot 62:4863–4873. doi:10.1093/jxb/err144

Torney F, Trewyn BG, Lin VSY et al (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat Nanotechnol 2:295–300. doi:10.1038/nnano.2007.108

Tzfira T, Citovisky V (2006) *Agrobacterium*-mediated genetic transformation of plants: biology and biotechnology. Curr Opin Biotechnol 17:147–154. doi:10.1016/j.copbio.2006.01.009

Verma D, Singla-Pareek SL, Rajagopal D et al (2007) Functional validation of a novel isoform of Na^+/H^+ antiporter from *Pennisetum glaucum* for enhancing salinity tolerance in rice. J Biosci 32:621–628

Wei S, Hu W, Deng X et al (2014) A rice calcium-dependant protein kinase OsCPK9 positively regulates drought stress tolerance and spikelet fertility. BMC Plant Biol 14:1–13. doi:10.1186/1471-2229-14-133

Wu L, Fan Z, Guo L et al (2005) Over-expression of the bacterial *nhaA* gene in rice enhances salt and drought tolerance. Plant Sci 168:297–302. doi:10.1016/j.plantsci.2004.05.033

Xu M, Li L, Fan Y et al (2011) ZmCBF3 overexpression improves tolerance to abiotic stress in transgenic rice (*Oryza sativa*) without yield penalty. Plant Cell Rep 30:1949–1957. doi:10.1007/s0029-011-1103-1

Yang T, Zhang S, Hu Y et al (2014) The role of OsHAKS5 in potassium acquisition and transport from roots to shoots in rice at low potassium supply levels. Plant Physiol. doi:10.1104/pp.246520

Yoshida S (1981) Fundamentals of rice crop science. International Rice Research Institute, Philippines

Yu L, Chen X, Wang Z et al (2013) Arabidopsis *Enhanced Drought Tolernace1/HOMEODOMAIN GLABROUS11* confers drought tolerance in transgenic rice without yield penalty. Plant Physiol 162:1378–1391. doi:10.1104/pp.113.217576

Zhang Z, Li F, Li D et al (2010) Functional analyses of ethylene response factor JERF3 with the amin of improving tolerance to drought and osmotic stress in transgenic rice. Transgenic Res 19:809–818. doi:10.1007/s11248-009-9357-x

Zhao F, Guo S, Zhang H et al (2006) Expression of yeast *SOD2* in transgenic rice results in increased salt tolerance. Plant Sci 170:216–224. doi:10.1016/j.plantsci.2005.08.017

Zhao FY, Liu W, Zhang SH (2009) Different responses of plant growth and antioxidant system to the combination of cadmium and heat stress in the transgenic and non-transgenic rice. J Integr Plant Biol 51:942–950. doi:10.1111/j.1744.2009.00865.x

Zhu C, Naqvi S, Gomez-Galera et al (2007) Transgenic strategies for the nutritional enhancement of plants. TRENDS Plant Sci 12:548-555. doi:10.1016/j.tplants.2007.09.007