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Transgenic Crops Targeting Ion Homeostasis Machinery: Bangladesh Perspective for Adaptation to Climate Change to Ensure Food Security

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Abstract

Salinity stress is one of the major adversarial impacts of climate change that limits crop productivity worldwide, especially in developing countries. To overcome this situation it is necessary to understand the cellular basis of salt stress tolerance mechanisms. Various genes involved in ion exclusion, osmotic tolerance, Reactive Oxygen Species (ROS) scavenging and other regulation mechanisms influence salinity tolerance in crops. Function of these candidate genes/sequences may vary in different plants and within different tissues. For the last two decades, in Bangladesh several approaches have been taken to develop transgenic rice, the staple crop. Work is in progress to monitor stable incorporation of these transgenes. Further evaluation is needed to check their adaptation/resistance in natural environments. Good performance in field conditions will lead to acceptance of these varieties for commercial productions in order to ensure food security.

Introduction

Climate change is a reality, which is currently evident in many parts of the world. Increased temperatures, increased salinity, irregular water precipitation, fluctuation of previously known weather patterns have become common phenomenon. Implications of these on agriculture are clear, direct, and very significant. Climate change leads to constrained agricultural production, which in turn results in reduced yield of food crops, thus causing threat to worldwide food security. Developing countries, like Bangladesh, are especially vulnerable to climate change because they depend heavily on agriculture.

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Among these climate change phenomenon, salinity is the most alarming as it is likely to be further enhanced by sea-level rise. Asian countries like, Bangladesh, India, Philippines are facing major risks from saline infusion (FAO 2014). Among these countries, Bangladesh is considered to be one of the most vulnerable countries of the world to CCSLR (the Climate Change and Sea Level Rise) as a number of environmental issues are working together in hindering crop production. For example, productivity of rice, the staple crop is falling alarmingly and it is likely to fall nearly 10% by 2050 (IPCC 2007). Therefore, development of saline tolerant rice varieties is an urgent need at present to ensure the food security for more than 160 million people of Bangladesh.

In the current global environmental scenario invention of saline-tolerant, high-yielding genotypes has become key research. With the advent of recent biotechnological tools, like knowledge of genomics and genetic mechanisms, precision crop improvement has become a reachable goal. Merging of biotechnological approaches with traditional breeding methods has also accelerated the increase in crop production.

In this review the major adaptive mechanisms of plants towards salt tolerance at the physiological and molecular levels has been discussed and potential tolerance genes identified for transformation. Transgene expression through constitutive, tissue specific or induction through different promoters has also been discussed. Finally, a few case studies in Bangladesh to develop transgenic rice have been described.

Salinity: Global threat towards food security

Globally, irrigated lands cover some 230 million hectares, of which nearly 62 million hectares land is salt-affected (FAO 2014). The inflation-adjusted cost of salt-induced land degradation in 2013 was estimated at US$441 per hectare, yielding an estimate of global economic losses at US$27.3 billion per year. Every day for more than 20 years, an average of 2,000 hectares of irrigated land in arid and semi-arid areas across 75 countries have been degraded by salt (UNU-INWEH 2014).

Due to the enormous uncertainties surrounding global climate change, salinity is likely to decrease production of rice, wheat, corn, soybeans, and potatoes, the staples for billions of people and major food crops in Asia, North America and Africa. Increased salinity may lead to average per-person reductions in food availability of 3.2% (99 kcal per day), in fruit and vegetable intake of 4.0% (14.9g per day), which could cause loss in lives of more than 5 million adults worldwide in 2050 due to changes in diet from reduced crop productivity (FAO 2014).

In the Indo-Gangetic Basin, crop yield losses for wheat, rice, sugarcane and cotton grown on salt-affected lands could be 40 per cent, 45 per cent, 48 per cent, and 63 per cent, respectively. This will lead to employment losses approximately 50–80 man-days per hectare, with an estimated 20–40 per cent increase in human health problems and 15–50 per cent increase in animal health problems (UNU-INWEH 2014).

The reality is that world agriculture needs to produce about 70% more food crop compared to present for an additional 2.3 billion people by 2050. Engineering of salinity tolerance in agronomically important crop plants is therefore required to increase their productivity by enabling them to grow in saline soils, which are otherwise left uncultivated.

Salinity: Bangladesh at a high risk

Bangladesh is a densely populated country of the world and here the probability of increasing cultivable land is non-existent. At present, around 1.056 million hectares in the coastal regions are affected by soil salinity of various degrees, which has increased from 0.833 million hectares in about the last four decades (SRDI 2010). Therefore, it is one of the most serious environmental factors limiting the productivity of rice.

According to the Soil Resource and Development Institute (SRDI 2010, Siddiky et al. 2012), around 0.74 million hectares of land are being cultivated in the coastal region, out of which around 0.38 million hectares are now salt-affected. Out of 0.38 million hectares of salt-affected land, a survey indicates that around 0.33 million hectares are affected by salt up to 12 dSm^{-1}, in the dry season, whereas some areas worse. Existing rice cultivars produced using traditional breeding methods and released by BRRI can grow only up to 8 dS/m with some yield loss (Saleque et al. 2015). Climate experts have predicted that the increasing amount of salt in these crop lands will cause the area's landscape to become barren in the near future (Haque 2006). The salinity level may rise up to 15/16 dSm^{-1} in coastal regions but even if 50% of the moderate salinity-prone farmland could be covered under transgenic rice, the country's crop productivity would have a significant boost.

Plants response towards saline condition

Salinity is determined by the total amount of dissolved salts present in it. Two most common ways to measure salinity are: (a) Total dissolved solids (TDS), and (b) Electrical conductivity (EC). The meaning of salinity is having electrical conductivity (ECe) equal to or more than 4 dSm^{-1} in irrigation water samples at temperature 25°C (Bahmani et al. 2015). Cations and anions that plays most important role in salinity are sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), chloride (Cl), sulfate (SO_4^{2-}) and bicarbonate (HCO_3). Among these the most prevalent are firstly Na (in almost plants) and secondly Cl (especially in trees like citrus). Therefore, the plants salinity tolerance research deals with NaCl as the salinizing salt.

Based on the capacity to grow in a saline environment, plants can be classified into two categories. These are glycophyte and halophyte. Most crop plant species are glycophytes, which are salt sensitive. In contrast, halophytes are able to grow in habitats excessively rich in salts, such as, salt marshes, sea coasts, and saline or alkaline semi-deserts and steppes. The salt tolerance in halophytes involves a range of adaptations, including ion compartmentalization, osmolyte production, osmotic adjustment, succulence, selective transport, salt exclusion, enzyme responses, salt excretion, and genetic control (Zhang et al. 2001). While in absence of these adaptations, salinity has several deleterious effects on salt sensitive plants, such as:

i. Osmotic stress (resulted in loss of cell turgor),
ii. Ion toxicity (mainly due to the Na^+ and Cl^- and SO_4^{2-}),
iii. Mineral deficiency (specially Potassium, Iron and Zinc),
iv. Ion imbalance,
v. Oxidative stress,
vi. Stomatal blockade (resulted in reduction of carbon dioxide availability),
vii. Photosynthesis inhibition,
viii. Cell division inhibition,
ix. Increase of sensitivity to diseases and a combination of these.

Some glycophytes such as traditional cultivars or landraces have adapted to the environment, e.g. coastal salinity, and may have evolved with 1-2 of the above defense mechanisms against salinity stress (Lisa et al. 2004 and Rahman et al. 2016). The physiological responses of plants towards salinity and their relation to salinity resistance have been much studied and frequently reviewed for many crop species, including rice, wheat, bean, tomato, onion, pepper, corn, potato etc. All these research established that these salt sensitive plant faces severe reduction in crop productivity in presence of salinity (Ashraf et al. 2014).

Molecular mechanisms of plant salinity tolerance

In general, plant adaptation to environmental stresses depends on the activation of molecular networks involved in stress perception, signal transduction, and the expression of specific stress-related genes and metabolites. *In silico* research showed that the sequences that are involved in abiotic stress resistance mechanism are common for many of the stresses, like, drought, cold, salinity etc. (Razzaque et al. 2014 and Naika et al. 2013). Thus, they share similar network and are highly correlated in a regulatory manner. The sequences that are involved in these tolerance mechanisms include stress responsive genes, transcription factors, regulatory genes and enzymes. One of the major perturbations during salinity stress is the overload of the Na^+ ions at the expense of K^+, which if left un-managed can cause severe plant damage and death (Ismail et al. 2014). So a major defense pathway for many glycophytes and halophytes is reduction of the Na^+ load, accomplished by the following mechanisms:

i. Salt Overly Sensitive 1 (*SOS1*) pathway
ii. Sodium influx and Na^+/K^+ balance
iii. Sodium efflux
iv. Sodium compartmentalization

i. *Salt Overly Sensitive 1 (SOS1) pathway:* Understanding of plant responses to excessive Na^+ concentration has progressed enormously since the discovery of the Salt Overly Sensitive 1 (*SOS1*) pathway in *Arabidopsis* (Shi et al. 2000). The prominent genes working in this pathway include *SOS1*, a plasma membrane Na^+/H^+ antiporter (Shi et al. 2000), *SOS2*, a serine/threonine protein kinase (Liu et al. 2000), *SOS3*, a Ca^{2+} sensor (Liu et al. 2000), High-affinity K^+ Transporter (*HKT1*), a transporter that facilitates K^+ or Na^+ uptake into the cell and *NHX1*, a Na^+/H^+ antiporter localized on vacuolar membrane for Na^+ compartmentation in the vacuole. Upon salt stress perception at the plant cell membrane, coordinated signaling conducted through the *SOS1* pathway components results in the up-regulation of both the *SOS1* and NHX1 transcripts that resulting in lower Na^+ accumulation in root, xylem stream and shoot cells (Juan et al. 2014).

ii. *Sodium influx and Na^+/K^+ balance:* Under normal physiological conditions, plants maintain a high potassium and sodium (K^+/Na^+) ratio in the cytosol (Khan 2011). Accumulation of high Na^+ concentration in the extracellular spaces generates a very high electrical membrane potential difference, which facilitate a passive movement of Na^+ ions into the cytosol.

This passive movement of Na^+ ions into root cells is facilitated by ion transporters or channels. These different transporters function in parallel, mediating Na^+ uptake into the roots and are dependent upon species and growth conditions.

Several members of the High-affinity K^+ Transporter (*HKT1*) family have been identified that play a significant role in the Na^+ influx (Khan 2011). The tissue specific activity of *HKT1* has recently been shown to correlate with lower overall Na^+ movement from root to shoot and the subsequent salt tolerance.

Transgenic *Arabidopsis* expressing the *HKT1* gene in the mature root stele cells showed a drastic decrease of Na^+ accumulation in the shoot by 37 to 64% (Khan 2011). This decrease in shoot Na^+ content was mediated by the increased Na^+ influx into the root stele cells, which in turn, decreased the flow of Na^+ from root to shoot and increased salt tolerance.

iii. *Sodium efflux:* If soil salinity is high, sodium enters into the plant through roots. But accumulation of Na^+ in the cytosol of root cell at toxic levels is prevented by transporting Na^+ to the shoot cell. As a result Na^+ accumulates in high concentration both outside and inside of the shoot cell plasma membrane. This generated electrochemical gradient across the membrane, in turn activates ion transport to remove excess Na^+ from the cell (Blumwald 2000). For this purpose, specific Na^+-ATPases are present in the plasma membrane. The only channels through which Na^+ is excluded of the cell are Na^+/H^+ antiporters. These Na^+/H^+ antiporters play a crucial role in maintaining cellular sodium level, cytoplasmic pH and cell turgor (Zhang and Blumwald 2001).

iv. *Sodium compartmentalization:* Besides excluding excess Na^+ from cytosol, the vacuolar compartmentalization of Na^+ is another very important mechanism to cope with salinity stress (Apse and Blumwald 2002). Excess Na^+ in the cytosol is taken into the vacuole by cation/H^+ antiporters localized on vacuolar membranes (Liu et al. 2010). These antiporters are driven by electrochemical gradient of protons generated by the vacuolar H^+-translocating enzymes such as H^+-ATPase and H^+-PPase (Apse and Blumwald 2002).

Transformation techniques to introduce foreign gene(s) into plants

The emphasis for plant transformation research has been encouraged for: (*a*) generation of plants with useful phenotypes unattainable by conventional plant breeding and (*b*) overcoming the limitations in varieties more efficiently than conventional breeding (Brich 1997).

The capacity to introduce and express diverse foreign genes in plants has been extended to over 120 species in at least 35 families. Successes include most major economic crops, vegetables, ornamental, medicinal, fruit, tree, and fodder plants.

To develop saline tolerant plant following categories of genes has been identified for transformation,

i. Ion homeostasis: Na^+/H^+ antiporter (with its different family members), Na^+ ATPase etc.

ii. Salt Overly Sensitive: *SOS1* gene, vacuolar Na^+/H^+ antiporter, vacuolar pyrophosphatase etc.

iii. Osmotic tolerance: genes including Trehalose-6-phosphate synthase (TPS), Trehalose-6-phosphate phosphatase (TPP), Mannitol-1-phosphate dehydrogenase (mt1D), Glutathione S-transferase (GST), Catalase etc.

iv. Transcription factors: genes involved in sensing and signaling mechanisms like Calcineurin-B, interacting protein kinases (CIPK), Sucrose nonfermenting1-1 type protein kinase and transcription factors involve in salt-tolerance, like, DREB, AP2, ERF, SNAC1, SNAC10 etc.

Attempts to introduce salt tolerance in crop species

Several genes have been introduced into plant genome to attain enhanced salinity tolerance as discussed below.

i. *Targeting ion homeostasis machinery through vacuolar Na^+/H^+ antiporter genes* (NHX): Importance of ion transporter like vacuolar Na^+/H^+ antiporter genes (NHX) in abiotic stress signaling is well documented (Razzaque et al. 2014). Transformation of several plant species with ion transporters, particularly the vacuolar Na^+/H^+ antiporter genes, have resulted in enhanced tolerance to elevated salt concentrations. The *Arabidopsis* vacuolar membrane Na^+/H^+ antiporters have been introduced in several crop plants to enhance their salt tolerance. Introduction of *AtNHX1* in *Brassica* conferred salt stress tolerance and transgenic plants showed 2.3% high plant fresh weight and 2.34% high grain yield under (100 mM) NaCl (Zhang et al. 2001). Transgenic cotton that expressed *Arabidopsis AtNHX1* showed enhanced salt stress tolerance (He et al. 2005). Transgenic tobacco transformed with *BnNHX1* (*Brassica napus*) showed improved plant growth and better seed production under 200 mM NaCl stress, while wild-type plants died (Wang et al. 2004).

The expression of *AgNHX1* (*Atriplex gmelini*) in transgenic rice plants exhibited enhanced survival of seedlings (81 to 100%) under salt stress (Ohta et al. 2002). Similarly, transformation of rice with *OsNHX1* and *PgNHX1* (*Pennisetum glaucum*) conferred salt tolerance and transgenic plants showed higher shoot and root growth (Fukuda et al. 2004, Chen et al. 2007, Verma et al. 2007). Transformation of wheat and maize with *AtNHX1* showed tolerance to salt stress (Xue et al. 2004 and Yin et al. 2004).

ii. *Targeting salt overly sensitive signaling pathway through Salt Overly Sensitive (SOS) genes: SOS1* was first identified in *Arabidopsis* mutant that showed altered root growth on saline media (Wu et al. 1996). Another two genes (*SOS2* and *SOS3*) were also identified that function as regulators for the activity of *SOS1* and enhances the *SOS1* mediated Na^+/H^+ exchange across plasma membrane (Qiu et al. 2003). Transforming this gene into *Brassica juncea* and *Brassica* *campestris* exhibited salt tolerance upto 25 and 50 mM, respectively (Chakraborty et al. 2012).

Yang et al. (2009) generated several transgenic *Arabidopsis* lines that expressed *SOS1*, *SOS2*, *SOS3* and *AtNHX1*, either individually or in combination. They reported high salt stress tolerance for those transgenic plants reaching resistance upto 50 mM of NaCl.

iii. Targeting osmotic homeostasis machinery through osmolytes genes: Mannitol and trehalose function as osmolytes that accumulate during stress and eliminates cell dehydration (Loescher et al. 1992). Accumulations of carbohydrates, such as, sugars (e.g. glucose, fructose, fructans, and trehalose) and starch occur under salt stress. Sucrose content was found to increase in tomato (*Solanum lycopersicum*) under salinity due to increased activity of sucrose phosphate synthase (Gao et al. 1998). Engineered mannitol expression has been reported to enhance seed germination during salt stress in transformed *Arabidopsis* (Thomas et al. 1995). But these molecules may have varied effect on physiology and reproductive characteristics. For example, in potato abnormal morphology was observed when trehalose-6-phosphate synthase (TPSI) was introduced (Yeo et al. 2000). However, introduction of 1-pyrroline-5-carboxylate synthetase reported to give better tolerance to salinity but cost yield penalty and weight loss (Hmida-Sayari et al. 2005).

iv. Targeting transcription factor machinery: Through regulation of target genes, transcription factors also play roles in stress responses (Tran et al. 2004). Stress responsive *NAC1*, belonging to the NAC family, was reported to be induced under salt stress (Balazadeh et al. 2010). *SNAC1* gene over expression was observed to increase stomatal closure, preventing plants to go through excessive water loss (Hu et al. 2006).

SNAC1 controls over 80 genes, among which 40 of them are involved in drought stress response, such as, signal transduction, production of osmolyte, detoxification, protection of macromolecules and stomatal closure etc. (Khong et al. 2008). Over expression of a NAC transcription factor in both rice and wheat conferred salt tolerance, thereby predicting their role in stress mitigation (Nakashima et al. 2007). NAC is also reported to alter Na^+ accumulation in chrysanthemum (Roy et al. 2014). There are other important transcription factors have been noticed to improve salt-tolerance after transformation, such as, DREB improved germination in tobacco, MYB improved chlorophyll retention in tomato, AP2 and ERF improved biomass content in wheat (Roy et al. 2014).

v. Targeting promoter performance: Numerous genes have potential to have to produce improved salinity tolerance. Use of stress inducible promoters to express genes conferring salt tolerance may be preferable than constitutive promoters. Because when the genes are controlled by a promoter that is either cell type specific and/or stress inducible or have a tag on the protein that directs it to the correct cellular organelle, then the desired salt tolerant phenotype is more easily obtained (Roy et al. 2014).

Approaches carried out in Bangladesh to develop salinity tolerant rice

Bangladesh is the 7th most populated country of the world with current estimates of 160 million people. As rice is the staple food several attempts has been made for its improvement. Several attempts have been made to transfer the gene(s) described above into crop plants in order to improve tolerance.

i. *Targeting ion homeostasis machinery through vacuolar Na^+/H^+ antiporter genes* (NHX): Binnatoa rice was transformed to improve salt tolerance by targeting ion homeostasis machinery by over-expression of *OsNHX1* under the constitutive promoter Actin1D and CaMV35S. Degree of salt tolerance and overall performance of the two transgenic rice bearing Actin1D and CaMV35S promoters displayed no significant difference, suggesting similitude of role in gene regulation of the two constitutive promoters (Islam et al. 2009).

ii. Targeting salt overly sensitive signaling pathway: In a study, the coding sequence of *SOS1* gene (3447 bp) was cloned (Razzaque et al. 2013) and transformed into farmer popular rice variety BRRI dhan28. The *SOS1* gene encodes a plasma membrane Na^+/H^+ antiporter that play an important role in germination and growth in saline environments. This study showed an improved salinity tolerance level of 150 to 200 mM in the high yielding but salt sensitive BRRI dhan28 (Yasmin et al. 2015).

iii. Targeting transcription factor machinery: To improve salt tolerance in rice by targeting transcription factor machinery, the *SNAC1* gene (from the salt tolerant rice landrace Pokkali) was cloned and transformed into a salt sensitive rice variety Binnatoa (Abdullah-Al-Emran et al. 2010) and BRRI dhan55 using the *in planta* method. The transgenic lines exhibited better tolerance compared to the non-transgenic plants at 120 mM salt (Parvin et al. 2015).

Other attempts made to develop salt tolerance rice

i. *Targeting replication machinery:* To improve the salinity tolerance of rice a DEAD-box helicase (PDH45) gene from pea was introduced into Binnatoa through *Agrobacterium*-mediated transformation (Amin et al. 2012). Higher chlorophyll content, decreased root length and higher leaf K^+/Na^+ ratio was found in transgenic plants compared to wild type plants under salt stress, which indicated induced ion homeostasis. Moreover, PDH45 expressing transgenic rice plants showed better fertility at the reproductive stage and produced much higher grain yield compared to WT plants under continuous salt stress.

ii. *Pyramiding of antioxidant genes:* To develop transgenic salt tolerant rice by targeting antioxidant machinery, Singla-Pareek and her colleagues utilized two genes that encodes for Glyoxalase I (isolated from a *Brassica juncea*) and Glyoxalase II (isolated from *Oryza sativa* L.cv IRBB10) (Singla-Pareek et al. 2008). These genes are involved in salt tolerance mechanism through glutathione-based detoxification of methylglyoxal in plant. The transgenic plants showed higher constitutive activity of glyoxalase II that increased further upon salt stress, reflecting the upregulation of endogenous glyoxalase II. The transgenic rice showed higher tolerance to toxic concentrations of methylglyoxal (MG) and NaCl.

Prodhan et al. (2010) reported successful introduction of *kat*E gene in three rice cultivars, Nipponbare, Kasalath and BR-5 successfully. Introduction of *kat*E gene significantly improved the salt tolerance of the transgenic *indica* lines which could mature and set seed under stress at 100mM salt. Particularly, these transgenic plants could survive for one month in 150 mM and for 20 days in 200 and 250 mM NaCl (Prodhan et al. 2010).

iii. *Targeting different transcript sizes of anti-porter genes:* The rice *OsNHX1* antiporter gene produces three different transcripts where transcript 1 and 2 produce proteins of equal size, while transcript 3 produces a truncated protein at the C terminus region. However, the 5' Un-Translated region (UTR) is variable in all three transcripts of the *OsNHX1* gene (196, 325, and 186 bp in transcript 1, 2, and 3, respectively), (LOC_Os07g47100, gramene). This is in contrast to *Arabidopsis* (a model plant and not a crop species like rice) which has only a single transcript. The properties of a transcript are controlled by some features of their UTRs as the UTR of a gene has important biological roles which can influence the half-life, intracellular localization, and differential translational efficiency of the corresponding mRNA, particularly for regulatory genes responsive to stress. Over-expression of vacuolar Na^+/H^+ antiporter from the CDS and 5' UTR (1.9 kb) and complete cDNA (2.3 kb) that a higher level of reproductive level salt tolerance was reported for the 2.3 kb construct compared to the moderate level of stress obtained from the 1.9 kb construct. The result of the experiment showed increased expression of the *OsNHX1* gene under 100 mM salt stress (NaCl) only in case of the 2.3 kb transgenic line along with higher K^+ content (Biswas et al. 2016).

iv. *Combining genes to improve salt tolerance:* Integration of multiple copies of transgene at the same locus has reported to be associated with the instability of transgene expression in plants (Hu et al. 2006). However, insertion of two genes is also reported to be acceptable (Hu et al. 2006). In this study, the double copies of the transgene actually showed considerably better salinity tolerance compared to the non-transformed wildtype BRRI dhan28 (Biswas et al. 2016).

Promoters to assist salt tolerance

Over-expression of foreign genes under constitutive promoter can be detrimental to host plant and result in sterility, growth retardation, loss of yield, abnormal morphology and transgene silencing (Xu et al. 2006). Therefore, inducible promoters are often preferred over constitutive ones for the expression of foreign genes only when they are required under stress.

RD29A is one such stress-inducible promoter that is quickly and strongly induced by salt stresses. At both 100 and 200 mM salt exposure, *RD29A-GUS* expression was observed to be maximum in leaves and seeds at 24 hours without any expression in roots. Therefore, maximal expression of the *GUS* gene at 24 hours indicates the suitability of the *RD29A* promoter for driving transgenes suitable in conferring salt tolerance. This promoter was also found suitable for driving transgenes in leaves under drought stress as well (Sarker et al. 2016).

In contrast to the RD29A promoter, Adh promoter that controls the expression of alcohol dehydrogenase gene under abiotic stresses is reported to be root specific (Hossain et al. 2006). *Adh-GUS* activity was found to be two-fold higher in 10 hrs salt stress as well as submergence compared to control calli (Ashraf et al. 2014).

Future of attaining stable transgenic Bangladeshi rice variety

Generally, integration of the foreign gene confirmed through PCR amplification, RT-PCR and Southern hybridization. While performing transformation with *SOS1*, two copies of integration were observed (Yasmin et al. 2015). RT-PCR helped selection of the best line with higher expression of the desired transgene. Stable integration of the incorporated gene or promoter is extremely essential in order to be inherited. Therefore, the transgene has to stably integrated, has to show Mendelian inheritance and shown to be inherited in future generation up to $F_{4\text{-}5}$ produced through selfing for attaining homozygosity (Biswas et al. 2015).

Conclusion

One of the alarming issues of the present day and also for the forthcoming future is ensuring food security. One approach to address these challenges, has been to include saline soil as arable area. However, we need successful development of more transgenic salt-tolerant crops which will effectively survive in saline-stresses field conditions. Moreover, we need functional greenhouses for contained trials and field facilities to test them under confined conditions. The regulatory system also needs to be made free of bureaucratic delays. Therefore, there needs to be more investment in research training as well as infrastructure. The promise of genome-editing technologies like Crispr-Cas9, which is not only technically friendly but also may need less regulatory oversight, should be adopted with urgent research funding.

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