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Review

Improvement of Salinity Stress Tolerance in Rice: Challenges and Opportunities

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Abstract: Rice (*Oryza sativa* L.) is an important staple crop that feeds more than one half of the world's population and is the model system for monocotyledonous plants. However, rice is very sensitive to salinity and is the most salt sensitive cereal crop with a threshold of 3 dSm⁻¹ for most cultivated varieties. Despite many attempts using different strategies to improve salinity tolerance in rice, the achievements so far are quite modest. This review aims to discuss challenges that hinder the improvement of salinity stress tolerance in rice as well as potential opportunities for enhancing salinity stress tolerance in this important crop.

Keywords: salinity; rice; salt tolerance; wild rice; genetic engineering; conventional breeding; MAS

1. Introduction

Salinity is a general term used to describe the presence of elevated levels of different salts such as sodium chloride, magnesium and calcium sulphates and bicarbonates in soil and water [1]. With more than 830 million hectares (ha) of salt-affected land globally [2,3] (Figure 1) and approximately two million ha of land uncultivable due to excessive salinity added each year [4], salinity is a growing worldwide problem. Salinity can develop naturally. However, human intervention has disturbed natural ecosystems, changed the hydrology of the landscape and significantly accelerated the salinisation of waterways and land. Irrigation and extensive clearing of vegetation, which bring the groundwater with soluble salts to, or close to, the soil surface, are the two major human activities that accelerate salinity. Of the 230 million ha of the world's irrigated land, 45 million ha (20%) has been salt-affected [3]. When growing on salt-affected soils, crops must compete with salts in the soils for water as well as to cope with ion toxification, nutritional disorders and poor soil physical conditions to survive, therefore, their productivity was reduced [5,6]. By 2050, the world's population is predicted to reach 9.6 billion people and food production needs to increase approximately 70% by 2050 or 44 million metric tons annually to provide sufficient food for this population [7–9]. This is a challenge because there is very little potential for future expansion of arable lands, whereas environmental stresses affecting crop production are increasing [10–12]. Salinity and water deficit were listed as the two most critical factors that limit global crop production [13]. To help sustain the increasing population, crops with enhanced salinity tolerance must be developed to increase productivity on salt-affected lands.

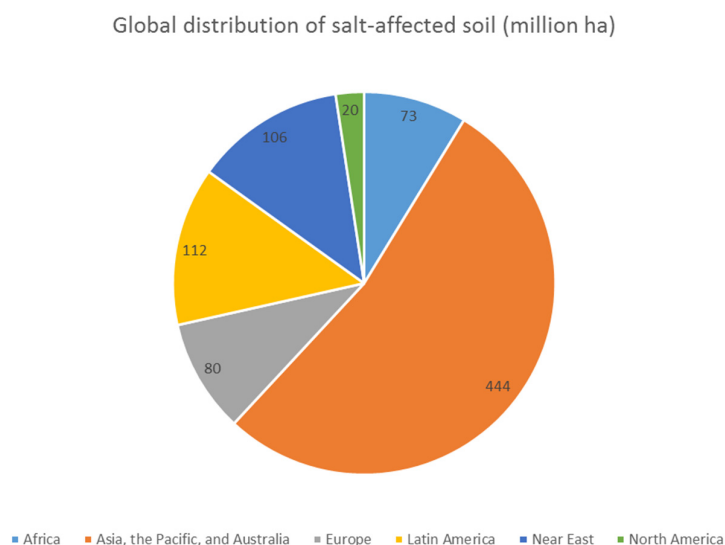


Figure 1. Global distribution of salt-affected soil (million ha).

Rice, one of the most important staple crops, feeds more than one half of the world's population. It is a member of the genus *Oryza* in the Family Poaceae. The *Oryza* genus has many species, of which two—*Oryza sativa* L. and *O. glaberrima* Steud—are cultivated. *Oryza sativa* is most commonly grown in Asia while *O. glaberrima* is native to West and central Africa [14,15]. Rice is cultivated in 114 of the 193 countries over the world in six continents, Asia, Africa, Australia, Europe, Latin America and North America [16]. In humid and sub-humid Asia, rice is the single most important source of employment and income for rural people, and it provides 50%–80% of the calories consumed for more than three billion Asians [17,18]. However, rice is very sensitive to salinity stress and is currently listed as the most salt sensitive cereal crop with a threshold of 3 dSm⁻¹ for most cultivated varieties [19], whereas, generally, a soil is only considered saline (salt-affected) if it has an EC_e (electrical conductivity of its saturation extract) above 4 dSm⁻¹ [20]. Even at EC_e as low as 3.5 dSm⁻¹, rice loses about 10% of its yield, and 50% yield loss was recorded for rice at EC_e 7.2 dSm⁻¹ [4]. Asia accounts for 90% of the world's production and consumption of rice [16,18] but “Sea-level rise is already increasing rice-killing salinity in the deltas of Asian rivers, the richest rice-growing areas of the world” [21]. Therefore, it is crucial to enhance rice tolerance to salinity stress to enable this staple crop to provide enough food for rice-consuming communities. Although some success has been reported for enhanced salinity stress tolerance in rice, the achievements so far are quite modest. This review will discuss challenges that hinder the success of the improvement of salinity stress tolerance in rice and identify potential opportunities for enhancing salinity stress tolerance in this important crop.

2. Rice Response to Salinity Stress

In terms of responses to salinity stress, plants can be divided into two groups—halophyte or glycophyte—based on their level of salinity tolerance. Halophytes are plants that tolerate relatively high salt concentrations (400 mM NaCl), while glycophytes tolerate low concentrations [22]. Most crops are glycophytes and their growth is retarded during salinity stress. Among the cereals, rye (*Secale cereale*) is the most tolerant (threshold of 11 dSm⁻¹) and rice is the most sensitive crop plant with a threshold of 3 dSm⁻¹ for most cultivated varieties [19].

Rice has been reported to be relatively tolerant to salinity stress during germination, active tillering and towards maturity, but sensitive during early seedling and reproductive stages [23,24]. An addition of as little as 50 mM NaCl in the soil can reduce rice yield significantly [25]. Generally, salinity causes two types of stresses on plants: osmotic and ionic stresses. Osmotic stress occurs after the concentration of salts around the roots of the plant increase beyond a threshold tolerance level while

ionic stress occurs when the concentration of salt in old leaves reaches a toxic level due to the influx of large amounts of Na^+ into the plant, resulting in increased Na^+ concentrations in the vacuole and cytoplasm leading to interruption of metabolic processes and cell death [6]. Like most plants, rice has evolved various mechanisms to cope with salinity stress including: (i) biosynthesis and accumulation of osmolytes for osmoprotection; (ii) ion homeostasis and compartmentation; (iii) antioxidants—ROS detoxification; and (iv) programmed cell death.

2.1. Biosynthesis and Accumulation of Organic Osmolytes

Organic osmolytes are small solutes that are used by cells to maintain cell volume under water stress. There are four major chemical categories of organic osmolytes: (i) small carbohydrates including sugars (e.g., trehalose), polyols (glycerol, inositols, sorbitol, etc.) and derivatives; (ii) amino acids (such as glycine, proline, taurine, etc.) and derivatives; (iii) methylamines (such as glycine betaine) and methylsulphonium solutes including dimethylsulphonopropionate (DMSF); and (iv) urea [26]. In an environment that has osmotic concentration changes, these osmolytes can be up- or down-regulated to prevent osmotic shrinkage in many species [26]. The first phase of salinity stress on plants is osmotic stress caused by excess salts outside the roots which affects plant growth immediately [6,27]. In low salt environments, plant cells can take up water and nutrients from the soil solution to support higher osmotic pressures compared to that of the soil solution. However, in high salt environments, the osmotic pressure of the soil exceeds that of plant cells (osmotic stress) and reduces the ability of plants cells to take up soil water and minerals [28]. To cope with osmotic stress, plant cells need to adjust their osmotic potential by accumulation of organic and/or compatible osmolytes. Garcia et al. [29] found that under salinity stress rice accumulates trehalose and that at low to moderate concentrations, trehalose reduces Na^+ accumulation, *salt* (an osmotically regulated gene) expression, and growth inhibition. At higher concentrations, trehalose can prevent NaCl -induced loss of chlorophyll in blades, preserve root integrity, and enhance rice growth. A salt tolerant rice cultivar was reported to accumulate more proline during salinity stress compared to salt sensitive rice cultivar [30,31]. Proline is thought to play an essential role as an osmoregulator in plants exposed to hyperosmotic stresses such as drought and salinity [32]. Under stress condition, proline appears to be the most widely distributed osmolyte accumulated in organisms ranging from eubacteria to plants [32]. Glycine betaine, a quaternary ammonium compound that occurs naturally in a wide range of plants, animals and microorganisms [33] is also a very effective compatible solute [34,35]. Although rice plants do not accumulate glycine betaine endogenously, Harinasut and co-workers [36] found that rice plants can uptake exogenous glycine betaine and accumulate it in leaves to help the plants to maintain quantum yield of PSII during salinity stress.

2.2. Ion Homeostasis and Compartmentation

During salinity stress, increased extracellular Na^+ concentrations create a large electrochemical gradient that favours the passive transport of Na^+ into the cell through K^+ transporters, resulting in high cytosolic Na^+ concentration [37]. The influx of large amounts of Na^+ into the plant, when exposed to salinity stress, increases the intracellular Na^+ content, which results in deleterious effects by competing with K^+ in enzyme activation and protein biosynthesis [38,39]. One of the mechanisms that plants employ to tolerate salinity stress is to exclude Na^+ uptake by roots thereby maintaining low concentrations of Na^+ in the leaves. A failure in Na^+ exclusion manifests its toxic effect after days or weeks and causes premature death of older leaves [6]. Na^+ concentration in the leaves of rice is correlated to the level of salinity stress tolerance in both Indica and Japonica varieties [40]. Several studies have suggested that a low cytosolic Na^+/K^+ ratio is required to maintain a favourable ionic homeostasis in plant cells under salinity stress condition [41,42] and that a low Na^+/K^+ ratio improves photosynthesis and overall plant growth [43]. Wang et al. [44] reported that under salinity stress, genes related to sodium transport from roots to shoot such as *OsHKT1;1*, *OsHAK10* and *OsHAK16* were upregulated in the old leaves of rice. Meanwhile genes involved in facilitating Na^+

exclusion from root xylem vessels to reduce shoot accumulation, like *OsHKT1;5* and *OsSOS1*, were downregulated, leading to high concentrations of Na^+ accumulated in old leaves compared to young leaves. During salinity stress, salinity tolerant rice cultivars accumulate less Na^+ in leaves and shoots compared to salinity sensitive rice cultivars [40,45–47]. Salt tolerant rice cultivar Pokkali was reported to be able to reduce the uptake of Na^+ into cytosol and maintain a low cytosolic Na^+ by transient uptake of Na^+ into cytoplasm and fast extrusion of Na^+ into vacuoles, while the salt sensitive rice cultivar BRRI Dhan29 failed to do so [48].

2.3. Antioxidants—ROS Detoxification

Reactive oxygen species (ROS) are versatile molecules playing dual roles as both toxic compounds and signal transduction molecules that mediate responses to environmental stresses, pathogen infection, developmental stimuli and even programmed cell death in plants [49,50]. If left unchecked, copious ROS production can cause protein denaturation, mutation of DNA and peroxidation of lipids [51]. ROS levels have been reported to increase, causing significant injury and eventual death in plants during salinity stress [52–54]. In order to protect the cell from oxidative injury, plants have evolved a complex antioxidant defence system to scavenge and detoxify ROS [55–57]. Salt tolerant rice cultivar Pokkali was reported to have higher activity of ROS scavenging enzymes such as catalase (CAT) and enhanced levels of antioxidants including ascorbate (ASC) and glutathione (GSH), compared to the salt sensitive rice cultivar (Pusa Basmati) during salinity stress. A concerted action of both enzymatic and non-enzymatic ROS scavenging machineries is vital to overcome salinity-induced oxidative stress in rice [51]. Dionisio-sese et al. [58] reported that salt tolerant rice varieties display protection mechanisms against increased radical production during salinity stress by maintaining the specific activity of antioxidant enzymes.

2.4. Programmed Cell Death (PCD)

Being sessile, plants are particularly vulnerable to aberrant environmental conditions including saline soils and water deficit. To mitigate salinity stress, plants implement a range of strategies. However, if these mechanisms are unable to cope with the increased stress, the plant will implement PCD as a last ditch effort to survive [59,60]. Programmed cell death is a physiological and genetically controlled process that is evolutionarily conserved across kingdoms and allows multicellular organisms to eliminate excessive or damaged cells which arise during development and in response to abiotic and biotic stress [61,62]. Alongside cell division and cell migration, PCD enables the organism to strictly control cell numbers and tissue size and to protect itself from unwanted cells that threaten cellular homeostasis [63]. Liu et al. [64] found that cell death progressed in a well-regulated manner in rice roots during salinity stress. This suggested a possible function of the dead cells in preventing the influx of excess Na^+ ions into the inner parts of roots and into shoots, leading to salt exclusion. It could also be possible that the plant sacrificed cells to prevent uncontrolled death and the release of toxins to protect and keep other cells growing. PCD is also reported to occur in the root tip of rice as a response to salinity stress [65].

3. Approaches to Improve Salinity Stress Tolerance in Rice—Progress and Challenges

To minimize the effect of salinity stress on rice, a number of strategies have been employed. Among these, water and soil management practices have facilitated agricultural production on soils marginalized by salinity but achieving additional gain by these approaches seems problematic. More recently, crop improvement strategies that are based on the use of molecular marker techniques and biotechnology are being used in conjunction with traditional breeding efforts to develop salt tolerant crop species [50,60,61]. Based on the known mechanisms that rice has to cope with salinity stress, the three major approaches of conventional breeding, marker assisted selection and genetic engineering have been used to enhance salt tolerance in rice. Progress to date shows some success but many challenges.

3.1. Conventional Breeding

Conventional breeding has been used for production of salt tolerant rice varieties through two basic steps [66]. The first is to generate/obtain a breeding population that is highly variable for salt tolerance; and the second involves selection among the segregating progeny for individuals that combine most of the parent's useful traits and high degree of salt tolerance. There are two important factors for determining salinity tolerance, threshold and slope. Threshold indicates the highest permissible salt level without reduction in yield. Slope indicates per cent reduction in yield per unit rise in salt level above the threshold. The threshold of rice is 3.0 dSm^{-1} and slope is 12% per dSm^{-1} [67]. Rice plants respond differently to salinity depending on the stage of development, type and concentration of salt, duration of exposure, water regime, soil pH, humidity, solar radiation and temperature [68].

By using several methods such as introduction, hybridization and mutagenesis for generation of highly variable salt tolerant breeding populations, considerable success has been obtained in raising rice genotypes with improved tolerance towards salinity [69–76]. Attempts to improve salt tolerance in rice by conventional breeding, however, have experienced difficulties. One of the reasons is that it is difficult to establish the physiological characters which best define salt tolerant genotypes. These traits are controlled by numerous genes that generate a continuous variation, the so-called “quantitative trait loci” (QTL) [77]. In addition, limited parental resources for breeding also contributes to limited success of breeding programs for enhanced rice salinity stress tolerance.

The idea of using physiological criteria for screening salt tolerance has been embraced by many researchers [78–82]. Basically, it could be possible to increase resistance to salinity beyond the existing phenotypic range by selecting individual physiological traits that contribute to salt resistance and combining them in a breeding programme [69,70]. However, no salt tolerant varieties have been developed and released by using this approach to date. Thus, the use of yield loss under stress to measure salt tolerance in the controlled environment has been considered more reliable and efficient method than using the physiological traits or conducting trials in field conditions [83].

Another complication that needs to be considered when breeding for salinity tolerance in rice is that different rice varieties have varied levels of salinity tolerance at different growth stages. Rice is comparatively tolerant to salinity stress during germination, active tillering, and towards maturity, but is sensitive during early seedling and reproductive stages. Moreover, salinity tolerance at the seedling and reproductive stages are only weakly, if at all, associated [84]. It has been reported that salinity tolerance at the seedling stage is independent of salinity tolerance at flowering/reproductive stage [85], for example, CN499-160-13-6 genotype has been confirmed as susceptible at seedling stage, but tolerant at the flowering stage [86]. Due to the variation in sensitivity to salinity during the life cycle, evaluation of salinity tolerance in rice is complex [87]. Some varieties have been found to be comparatively tolerant at the flowering stage due to better viability of pollen and higher grain yield (up to 40%) under salinity stress. This occurred in varieties such as Karalata, Pokkali, IR72046-B-R-7-3-1-2, etc., but other genotypes such as IR66946-3R-178-1-1 and IR65858-4B-11-1-2 have been categorized as sensitive at the flowering stage due to reduced grain yield, although high pollen viability occurred [88]. Therefore, screening of salinity tolerance in breeding programs has been suggested in two steps: (i) screening seedling salinity tolerance for large segregating populations (early generation screening) under controlled conditions; and (ii) testing salinity tolerance of promising lines from the first round screening at the reproductive stage, preferably under the field conditions [83].

Despite some variation in the gene pool for salinity tolerance in rice, those with good salinity tolerance have poor agronomic characteristics, and thus are not potential salinity tolerant donors in breeding programs for improvement of salinity tolerance. Comparison of the relative tolerance (measured as the survival of seedlings in a salinized hydroponic system) of seven wild species of rice and the two cultivated species, *O. sativa* and *O. glaberrima*, found that none of the wild species were as tolerant as the most tolerant of the cultivated lines of *O. sativa* tested, such as Nona Bokra [89]. Of the remaining ten or so species within genus *Oryza*, there is no information concerning their tolerance to

salinity, except for *Porteresia coarctata* Takeoka which is a native of salt-marshes around the coasts of the Bay of Bengal, and can grow in as high as 20% seawater without any adverse effect on growth [90]. Between the two main subspecies of rice, it is observed that Indica is more tolerant than Japonica. Tolerant Indica varieties are good Na^+ excluders, absorb high amounts of K^+ , and maintain a low Na^+/K^+ ratio in the shoot [46]. This also demonstrates that there is a great variation in salinity tolerance among rice varieties [91–94].

Although many successes in breeding salinity tolerant rice varieties have been reported, the fact is that the use of traditional salt tolerant parents such as Nona Bokra, Pokkali SR26B, and Kalarata do not possess the level of tolerance found [67]. Most salt tolerant rice landraces and cultivars have poor agronomic characteristics such as tall plant stature, poor grain quality, low yield, and photosensitivity [67,68]. Pokkali has been recognized for providing more salt tolerance when compared to other tolerant cultivars and thus it is used as a highly potential salt tolerant donor [87]. In this cultivar, a major QTL (Saltol) was identified which is located on chromosome 1 and involved in salt tolerance at the vegetative stage [95]. This QTL caused 64–80% of the phenotypic variation under saline conditions which has also been confirmed in other varieties of rice such as Nona Bokra [85,88,96]. Another rice variety FL478 (IR 66946-3R-178-1-1, derived from crossing between Pokkali and IR 29), has been promoted as an improved donor for breeding programs, as it has a high level of seedling stage salinity tolerance and is photoperiod insensitive, shorter and flowers earlier than the original Pokkali. FL478 also maintains a lower Na^+/K^+ ratio than both the parent lines [97,98]. Based on its ability to tiller well and maintain high potassium content under salinity stress, FL478 appears to be a good candidate for salinity stress tolerance in rice, particularly at the vegetative stage of growth.

3.2. Marker Assisted Selection

Recently, success in improving salt tolerance in rice relied on a remarkable effort to identify a major quantitative trait locus (QTL) contributing to salt tolerance in rice. A major QTL designed “Saltol” was mapped on chromosome 1 using a population generated from a cross between the sensitive variety IR29 and a tolerant landrace, Pokkali. This QTL accounts for more than 70% of the variation in salt uptake in this population and marker assisted backcrossing is being used to incorporate this QTL into popular varieties sensitive to salt stress [84].

Salt tolerance is a complex trait, so the QTLs related with salt tolerance have a significant role in understanding the stress-response and generating stress-tolerant plants [86]. As mentioned above, remarkable efforts have been made in identifying QTLs and their components affecting salt tolerance. There are many methods for identifying gene/QTLs such as traditional map-based cloning [99], new approaches like microarray based transcriptional profiling of differential gene expression [100] or combination of genetic mapping and expression profiling [101,102]. Several QTLs involved in salt tolerance have been reported. For example twenty-five major QTLs, each explaining more than 10% of the phenotypic variance, were mapped on chromosomes 1, 2, 3 and 8 from a mapping population F2 between salt tolerance variety CSR27 and MI48, using 79 SSR and EST markers distributed over the twelve rice chromosomes at an average interval of 20.7 cM and total map distance of 1634.5 cM. These included one QTL for seedling salt injury score, nine for Na^+ concentration, three for K^+ concentration and four for Cl^- concentration in leaf and stem tissues at vegetative and reproductive stages [103]. Using the same population, a combination of genetic mapping and bulked transcriptome profiling was used to narrow down the number of differentially expressed salt-responsive genes. Eight significant QTL intervals were mapped on chromosomes 1, 8, and 12 for the salt ion concentrations and a QTL controlling stress susceptibility index (SSI) for spikelet fertility was co-located in one of these intervals on chromosome 8. However, a total 2681 genes in these QTL intervals, made it difficult to pinpoint the genes responsible for the functional differences for the traits [102]. Another study analyzing 100 SSR markers on 140 IR29/Pokkali recombinant inbred lines (RILs) confirmed the location of the Saltol QTL on chromosome 1 and identified additional QTLs associated with tolerance. Analysis of a series of backcross lines and near-isogenic lines [104]

developed to better characterize the effect of the Saltol locus revealed that Saltol mainly acted to control shoot Na^+/K^+ homeostasis [105,106].

With a better understanding of the mechanisms and genetics of salt tolerance, more precise breeding approaches have been used for developing higher salt tolerance. Marker assisted selection (MAS) has been seen as a means of improving the speed and efficiency of breeding programs because it is growth stage independent, is unaffected by environment, has no dominance effect and is efficient to use in early generations. Marker assisted backcrossing (MABC) is another useful method for introgression or substitution of a target gene/QTL from salt tolerant parent to recipient by applying molecular markers. There are several successful examples where Saltol QTL has been precisely introgressed into elite rice varieties through MABC such as PB1121 and PB6 [107], AS996 [108], Bac Thom 7 [109,110] and BRRI Dhan-49 [111].

The drawbacks in using marker assisted selection are, besides a relatively high cost, “linkage drag” of undesirable traits due to the large size of regions of chromosome identified by QTL [112] and the fact that environment and genetic background have a significant influence on the QTL that are identified [113]. Thus, challenges still remain for further improvement to make MAS and MABC more affordable and reliable tools for breeding salt tolerance in rice.

3.3. Genetic Engineering

Genetic modification has become a powerful tool in plant breeding programs since it allows the introduction of gene(s) controlling traits without affecting the desirable characteristics of an elite genotype [56]. The advent of an efficient *Agrobacterium*-mediated transformation method for rice [114] displays great potential from the genetic improvement of this plant. To date, genetic engineering for salinity tolerance in plants has focused on genes that encode compatible organic solutes, antioxidants (detoxification of ROS), ion transport, heat-shock and late embryogenesis abundant proteins, programmed cell death, signal transduction and transcription factor (Table 1). Generally, all of the known mechanisms that rice uses to cope with salinity have been brought into genetic engineering programs to generate salinity tolerance in rice.

Table 1. Summary of genetic engineering approach for salinity stress tolerance in rice.

Gene	Protein	Gene Sources	DS	Comments	Reference
Machinery: Antioxidants and ROS detoxification					
<i>CAT1 and GST</i>	Catalase and Glutathione S-transferase	<i>Suaeda salsa</i>	G, V	Enhanced salt tolerance. Increased GST, CAT and SOD activities; Decreased H_2O_2 and Electrolytes leakage.	[115]
<i>GlyII</i>	Glyoxalase II	<i>Oryza sativa</i>	V	Enhanced salt tolerance. Increase Glyoxalase (1, 2, 3 and 4) activities. Increased shoot/root dry weight; accumulate less Na^+ , more K^+ .	[116]
<i>GS2</i>	Chloroplastic Glutamine synthetase	<i>Oryza sativa</i>	V	Enhanced salt tolerance. Higher quantum yield of PSII, less Na^+ accumulation.	[117]
<i>katE</i>	Catalase	<i>Escherichia coli</i>	V	Enhanced salt tolerance. Increase catalase activities. Less damage by NaCl at both developmental stages.	[118,119]
<i>Mn-SOD</i>	Mitochondrial manganese superoxide dismutase	<i>Saccharomyces cerevisiae</i>	V	Enhanced salt tolerance. Increase SOD activities. Higher quantum yield of PSII.	[120]
<i>Sod1</i> dismutase	Cytosolic copper zinc superoxide	<i>Avicennia marina</i>	V	Enhanced salt tolerance. Higher fresh weight and dry weight in transgenic plants than non-transgenics.	[121]

Table 1. Cont.

Gene	Protein	Gene Sources	DS	Comments	Reference
Machinery: Ion homeostasis and compartmentation					
<i>nhaA</i>	Na ⁺ /H ⁺ antiporter	<i>Escherichia coli</i>	G, V, R	Improved salt tolerance. Increase germination, shoot height. Transport more Na ⁺ to vacuoles. Better yield.	[122]
<i>AgNHX1</i>	Vacuolar-type Na ⁺ /H ⁺ antiporter	<i>Atriplex gmelini</i>	V	Improved salt tolerance.	[123]
<i>OsNHX1</i>	Vacuolar (Na ⁺ , K ⁺)/H ⁺ antiporter	<i>Oryza sativa</i>	V, R	Improved salt tolerance. Transgenics had better growth, decrease osmotic potential under NaCl stress.	[124–126]
<i>SOS2</i>	Plasma membrane Na ⁺ /H ⁺ antiporter	<i>Schizosaccharomyces pombe</i>	V	Enhanced salt tolerance. Transgenic plants had higher photosynthesis, low concentration of ROS, less Na ⁺ accumulation, low Na ⁺ /K ⁺ ratio, better yield component compare to non-transgenic.	[127]
<i>PgNHX1</i>	Vacuolar Na ⁺ /H ⁺ antiporter	<i>Pennisetum glaucum</i>	V, R	Enhanced salt tolerance. Delay senescence, shoot and root lengths are better than non-transgenic under NaCl stress.	[128]
<i>OsKAT1</i>	Shaker K ⁺ channel	<i>Oryza sativa</i>	cell	Cell culture line increase growth and cell K ⁺ content during NaCl stress.	[129]
Machinery: Osmotic adjustment					
<i>ADC</i>	Arginine decarboxylase	<i>Avena sativa</i>	V	Enhanced salt tolerance. Increase in biomass under salinity–stress conditions.	[130]
<i>codA</i>	Choline oxidase	<i>Arthrobacter globiformis</i>	V	Enhanced salt tolerance. Transgenic plants accumulate glycinebetaine. Better yield under NaCl compare to non-transgenics.	[131,132]
<i>COX</i>	choline oxidase	<i>Arthrobacter pascens</i>	V	Enhanced salt tolerance. Transgenic plants accumulate glycinebetaine. increase dry weight under NaCl compare to non-transgenics.	[133]
<i>P5SC</i>	Δ1-pyrroline-5 carboxylate synthetase	<i>Vigna aconitifolia</i>	V	Enhanced salt tolerance. Transgenic plants maintain shoot height and fresh and dry weight under NaCl stress. Accumulate more proline under NaCl stress.	[134]
<i>P5CSF129A</i>	Δ1-pyrroline-5 carboxylate synthetase	<i>Vigna aconitifolia</i>	V	Enhanced salt tolerance. Transgenic plants maintain shoot height and fresh and dry weight under NaCl stress. Accumulate more proline, less lipid peroxidation.	[135]
<i>SAMDC</i>	S-adenosylmethionine decarboxylase	<i>Tritordeum</i>	V	Enhanced salt tolerance. Transgenic plants had better shoot length and better fresh weight under NaCl stress.	[136]
<i>TPS and TPP</i>	Trehalose-6-phosphate synthase and Trehalose-6-phosphate phosphatase	<i>Escherichia coli</i>	V	Enhanced salt tolerance. Transgenic plants accumulate more trehalose. Better dry weight, better shoot height, maintain maximum quantum yield of PSII. Accumulate less Na ⁺ , maintain Na ⁺ /K ⁺ ratio.	[137,138]
Machinery: Programmed cell death					
<i>AtBAG4</i>	AtBAG4	<i>Arabidopsis</i>		Enhanced salt tolerance. Transgenic plants maintain cell membrane integrity, photosynthesis, low concentration of ROS, inhibit cell death, less Na ⁺ accumulation, maintain low Na ⁺ /K ⁺ ratio, better yield component compare to non-transgenic.	[1,139]
<i>p35</i>	p35	<i>Baculovirus</i>			
<i>Hsp70</i>	Hsp70	<i>Citrus tristeza virus</i>	V R		
<i>SflAP</i>	IAP	<i>Spodoptera frugiperda</i>			

Table 1. Cont.

Gene	Protein	Gene Sources	DS	Comments	Reference
Machinery: Signal transduction					
<i>HVA1</i>	LEA	<i>Hordeum vulgare</i>	V	Enhanced salt tolerance. Transgenic plants maintain better growth, plant height, root fresh weight under NaCl stress.	[140,141]
<i>OsCDPK7</i>	CDPK	<i>Oryza sativa</i>	V	Enhanced salt tolerance. Transgenic plants survived better under NaCl stress.	[142]
<i>OsMAPK5</i>	MAPK	<i>Oryza sativa</i>	V	Enhanced salt tolerance	[143]
<i>CNAtr</i>	Calcineurin	Mouse	V	Enhanced salt tolerance. Less Na ⁺ accumulated in roots. Root growth was less inhibited than shoot growth under NaCl stress.	[144]
Machinery: Transcription factor					
<i>OPBP1</i> AP2/ERF	transcription factor	Tobacco	V	Enhanced salt tolerance. Transgenic plants survived salt shock while non-transgenic die off.	[145]
<i>CBF3/DREB1A</i> (<i>CBF3</i>) and <i>ABF3</i>	transcription factor	<i>Arabidopsis</i>	V	Enhanced salt tolerance. Transgenic improved growth and maximum quantum yield of PSII.	[146]

Note: DS: Developmental stage; G: germination; V: vegetative stage; R: reproductive stage.

Despite some promising reports, the development of a salt-tolerant cultivar by way of transgenics still awaits more investigation. To date, no genetic engineered rice for enhanced salinity tolerance has been commercialized. Despite all the genes that have been introgressed into rice improve salinity tolerance (Table 1), a transgenic salt tolerant variety still has not reached the farmer. There are two major challenges that may hinder the success of this approach.

Firstly, there are a number of genes involved in salinity tolerance in rice but the gene that determines the major response to salinity stress has not yet been confirmed. Currently, the genetic engineering approach for salinity stress tolerance in rice has mostly transformed single genes in one of the four categories of salt stress response into rice (i.e., osmoprotectant, antioxidants, ion transporter or programmed cell death). Existing evidence suggests that members of protein families involved in ion transport, osmoregulation, and protection from oxidative damage are essential in governing high salt tolerance [100]. The response to salinity stress of Pokkali, the natural salt tolerant rice variety, showed that this variety may employ several strategies to cope with salt stress such as transient uptake Na⁺ into cytoplasm and fast extrusion Na⁺ into vacuoles [48] and increase activities of ROS scavenging enzyme such as catalase (CAT) and enhanced levels of antioxidants like ascorbate (ASC) and glutathione (GSH) during salt stress [51]. No dominant salt tolerance strategy has been determined for this variety. In addition, there was a weak link between tolerance to salinity stress at seedling and reproductive stages in some genotypes within the species *Oryza sativa* L., which also makes it difficult to ensure that a single transgene can strongly enhance salt tolerance in transgenic rice for the whole life cycle. Therefore, it would be worth trying to introgress a locus consisting of genes from different salt response categories into rice for enhanced salt tolerant in this important crop.

Another reason that may contribute to the limited outcome of the genetic engineering approach for enhanced salinity stress tolerance in rice is gene silencing. Despite significant scientific and technical advances in gene expression systems and plant transformation technologies, many transgenic plants do not display the expected transgene expression. Furthermore, numerous reports demonstrated considerable differences in transgene expression between transgenic lines harbouring the same cassette as well as within the same line over multiple generations. Whilst many of these differences were most probably due to influences of the adjacent host chromosome sequences, it is increasingly obvious that factors other than the integration site and strength of the transgene promoter were also involved [147]. It is now known that epigenetic effects and gene silencing have large influences on gene expression either by transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS). Unfortunately, gene silencing is notoriously unpredictable and represents a significant bottleneck to

the success of transgenic systems. Gene silencing can be triggered by the presence of foreign genes or additional copies of endogenous genes; interestingly, it has also been observed against endogenous genes in non-transgenic plants [148,149].

Silencing may occur at the transcriptional or post-transcriptional level. Transcriptional gene silencing is an irreversible process that occurs when dsRNA molecules target promoter regions resulting in methylation and termination of transcription [150,151]. Post-transcriptional silencing on the other hand occurs when dsRNA molecules derived from transcribed regions are processed by silencing machinery and the subsequent short RNAs target and degrade homologous transcript sequences. Unlike TGS however, PTGS is often reversible and used as a quick acting regulator of transcript half-life and subsequent gene expression [152]. The molecular processes of both TGS and PTGS have both been well characterized and are not in the scope of this review, and for further information please see the review by Rajee Kumar et al. [153]. Numerous studies have also reported transgene silencing in rice [154–156]. To obtain the desired results from transgenic technologies, it is imperative to mitigate the levels of transgene silencing and control gene expression at anticipated levels.

To overcome transgene silencing, researchers have developed a range of strategies including extrachromosomal episomal expression, artificial chromosomes, incorporation of introns into transgene coding regions and expression of viral silencing suppressors. Over the last three decades, significant progress has been made towards the mechanisms underpinning gene silencing and the advent of transgenics has significantly accelerated this progress. With this understanding is an increased knowledge of the factors surrounding transgene expression stability. The emerging technologies of genome editing allow scientists to control the transformation process to either be integration free or at least direct the integration location. This control will no doubt lead to further discoveries and possibly lead to new platforms that alleviate transgene silencing to increase the stability of transgene expression.

4. Opportunity

We have already highlighted the challenges that hinder the improvement of salinity stress tolerance in rice including: (i) limited parental resources for conventional breeding; and (ii) the complexity of salinity tolerance leading to modest success in both marker assisted selection and genetic engineered approaches for enhanced salinity tolerance in rice. Here, we will discuss: (i) the potential of using wild rice as resources for desalinization in irrigated land as well as genetic resources for both conventional breeding and genetic engineering; and (ii) potential of using genome editing to improve salinity tolerance in rice (Table 2).

4.1. Wild Rice—A Potential Candidate for Desalinization Crop

Irrigation leads to salinization. Each year approximately two million ha of irrigated land becomes uncultivable due to excessive salts [4]. A common method used to reclaim salt-affected soils is leaching, a process in which an excess amount of water is applied to dissolve and remove salts in soils, particularly from root zones by passaging the water through soil profiles [157]. The standing water in a rice paddy can leach the salts in the soil surface to a reduced level, making the salt-affected soils more suitable for subsequent crops. Rice is probably the only crop that can grow well in flooded conditions. Therefore rice is recommended as a desalinization crop for reclamation of salt-affected soils [158], if the soil salinity is sufficiently reduced.

O. rufipogon, the progenitor of cultivated rice *O. sativa*, harbours QTLs that determine salt tolerance [159]. Tian et al. [159] identified 15 putative quantitative trait loci (QTLs) associated with four traits including salt tolerance score (STS), relative root dry weight (RRW), relative shoot dry weight (RSW) and relative total dry weight RTW in 87 introgression lines (ILs), which were derived from a cross between an elite indica cultivar Teqing and an accession of common wild rice (*O. rufipogon* Griff.) in China. The authors found that STS was significantly positively correlated with all other three traits. *O. rufipogon*-derived alleles at 13 QTLs (86.7%) could improve the salt tolerance in the Teqing background.

Further, *O. rufipogon* also possesses high quality grain. In a study of Australian wild rice growing in the natural floodplain environment, Wurm et al. [160] found that the protein content of *O. meridionalis* and *O. rufipogon* was similar at 10% protein and higher than the 7% protein reported for a large number of pooled samples of *O. sativa* [160]. Concentrations of zinc, calcium, manganese and phosphorus were also higher in the grains of the wild rices than the pooled values for *O. sativa*. Cheng et al. [161] investigated the nutritional components of *O. rufipogon*, *O. officinalis*, and *O. granulata* and determined the protein content in the husked rice grains to be 14.5%, 16.3% and 15.3%, respectively, significantly higher than for six *O. sativa* cultivars. One sample of *O. officinalis* had a protein content as high as 19.3%. The same study found that the sulphur, phosphorus, magnesium, zinc, and ferrite content in the three wild rice species was 30%–158% higher than that of the six *O. sativa* cultivars. Variability in genetic traits responsible for the accumulation of micro- and macro-nutrients in wild rice is greater than in *O. sativa* [162].

4.2. Wild Rice—An Invaluable Genetic Resource

The International Rice Research Institute (IRRI) recognises 25 wild *Oryza* species, in addition to the two cultigens (*O. sativa* and *O. glaberrima*) (IRRI, n.d.). Wild relatives of *Oryza*, are a genetic resource underpinning the future of a major food crop, at a time when agricultural systems will be required to accommodate adaptation to climate change, while feeding an increasing population [163]. Wild *Oryza* species in the A-genome group are of most interest, as the A-genome group includes the two cultivated species, *O. sativa* and *O. glaberrima*. The wild A-genome species of African origin are perennial *O. longistaminata* and annual *O. barthii*, of South America origin is the perennial *O. glumaepatula*, of Austral-Asia origin are perennial *O. rufipogon* and annuals *O. nivara* and *O. meridionalis* [15].

Sequencing of the genomes of the following wild rice species has already been completed: *O. brachyantha* [164] *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. nivara* [165], *O. barthii*, *O. glumaepatula*, *O. longistaminata*, *O. meridionalis*, *O. nivara*, *O. punctata* and *O. rufipogon* [166] in addition to African and Asian cultivated rice, *O. glaberrima* [165] and *O. sativa*. These studies reveal very high levels of genomic structural variation leading to considerable morphological and reproductive diversification [165]. Lineage-specific, protein-coding genes under positive selection included those involved in flower development, reproduction and biotic and abiotic defences. The *O. meridionalis* from northern Australia had great divergence in protein-coding genes, non-coding RNA genes, structural variation and specific functional enrichment of miRNA target genes. It was 2.5-fold more distantly related to the other AA taxa than the genomes of the other species were to each other [165].

Unlike the densely populated rice growing areas in Asia where there is a tradition of rice cultivation spanning millennia, in northern Australia wild populations of A-genome *O. meridionalis* and *O. rufipogon* occur in vast swaths on the monsoonal floodplains of largely unmodified river catchments, and largely in the absence of cultigens. Recent work on these under-researched and possibly under-valued northern Australian populations of native *Oryza* have revealed new taxa [166–168]. These taxa were identified within perennial populations previously referred to as *O. rufipogon*. Waters et al. [167] focused on the chloroplast genome, using samples from just four individuals at a single site in each of Vietnam and northern Australia for *O. rufipogon*, and four individuals of *O. meridionalis* from a site in northern Australia. They found that the Australian *O. rufipogon* was more similar to the Australian *O. meridionalis* than it was to the Asian *O. rufipogon*. Following on from this work, a more extensive study was undertaken involving a larger number of accessions of *O. rufipogon* from in Asia and *O. rufipogon* and *O. meridionalis* from accessions and new field samples in Australia, about which morphological and ecological observations could be made in situ. Assessment of both nuclear and maternal genomes indicated that the taxon identified in Australia as *O. rufipogon* actually includes two types. One shares morphology and loci in the chloroplast genome with *O. meridionalis*. The other is more closely related to Asian *O. rufipogon* in the chloroplast genome, morphology and nuclear genome. This situation may be interpreted as indicating a common ancestor for Australian *O. rufipogon* and *O. meridionalis* and a later arrival of Asian *O. rufipogon*. Further work on this group of

A-genome *Oryza* species, using whole chloroplast genomes found that these two perennial Australian taxa and *O. meridionalis* were more similar to each other than to Asian *O. rufipogon* [166,168]. The genetic distance between Australian perennial *Oryza* Taxon A and both Asian *O. sativa* and *O. rufipogon*, was found to be similar to that between the Asian cultivated and wild species. This was interpreted as indicating that divergence of *O. rufipogon* in Asia and *Oryza* Taxon A in Australia was likely to have happened over a much longer period and been driven by allopatric evolution [166,168]. These authors point to opportunities for further investigations of nuclear genetic diversity for rice breeding resources.

During the processes of domestication and cultivation, useful genes for disease and pest resistance may introgress from wild relatives, and enable rice to adapt to new environments [15]. This has been recorded for bacterial blight (*Xa21* from *O. longistaminata*, *Xa23* from *O. rufipogon* and *Xa27* from *O. minuta*) and blast disease (*Pi9* from *O. minuta*) (see [169]). Ballini et al. [169] found that for *O. sativa* varieties IR64 and IR36 the blast resistance gene *Pi33* introgressed from *O. rufipogon*, and that the large chromosome fragment associated with this introgression (25% of chromosome 8) indicates it has been preserved due to selection pressure.

However, introgression occurs in both directions. In most areas where wild rices grow, introgression from cultivated paddy fields nearby has occurred [15,165,170–172]. As a result, many accessions of wild rices are not necessarily representative of the wild genome [15,165]. Most populations of wild *Oryza* are under threat from habitat destruction and/or introgression from cultigens from paddy fields into nearby wild populations. For example in China, *O. rufipogon* is currently threatened due to habitat fragmentation and reduction, in addition to introgression from cultivated rice [165,171,173]. Rates of gene flow from cultivated rice to *O. rufipogon* have been measured as 1.1% to 2.2% [170] and up to 2.94% [173]. These high rates have led some to recommend actions such as the establishment of exclusion zones or pollen traps to be established between wild and cultivated populations to prevent ecological consequences, including if GM crops are being grown [170], in order to protect the biosafety of wild rice populations.

Gao [171] found that Chinese *O. rufipogon* demonstrated greater microsite variation within than among populations, and attributed this to pollen movement being restricted to less than 6–9 km, or seed adapted to gravitational dispersal and immediate burial [174]. Pusadee et al. [172] found genetic distance among populations on the Indo-China Peninsula correlated with geographic distance, which specifically correlated with soil and temperature, possibly demonstrating the effects of environment on divergence.

Allele mining in wild rice populations for functionally understood genes may provide new resources for crop development. *Porteresia coarctata* (*O. coarctata*), occurring in coastal saline coastal wetlands has been identified as a model for understanding salinity tolerance in rice [175]. Investigation of diversity in *OsDREB1F* gene in *O. nivara* and *O. rufipogon* has found an association with drought tolerance. Some work has been undertaken to explore possible genetic and phenotypic adaptations of wild Australian rice to low soil P [176]. However, in that case, the environment from which the accessions were collected was not known, such that no link could be made back to adaptation to P deficient soils. Collection of accessions could be targeted according to habitat gradients across the range of wild populations. Australian A-genome wild rice occurs in expansive floodplains across monsoonal northern Australia [177]. The processes that have led to the development of these floodplains began approximately 6000 years ago when sea level rise meant estuarine and then mangrove sediments were deposited in the river valley floors [178,179]. By 4000 years ago, mangrove substrates began to be overlain by freshwater deposition and then these have been reasonably stable at their present level for some 1500 years [178] and current floodwater sediment loads are low [180]. In some places, the saline estuarine sediments are less than 1 m below the flood plain soil [178,179], and fossil shells of mangrove gastropods make their way to the floodplain surface far from extant mangroves (Penelope Wurm personal observation). The floodplain landform includes expansive gentle slopes, back swamps along the landward edge and below sea level in places, relict ox-bow lakes, and tidal creeks and, more recently, expanses of salt water intrusion. Soils are predominantly heavy grey cracking clay

vertisols that are chemically highly variable [181]. Soil pH ranges from acidic to alkaline, and salinity to some degree is common [181,182]. Soil fertility appears to be very variable with extractable P ranging from 5 to 34 ppm an extractable K from 40 to 1015 ppm and total N from 0.14% to 0.86% [181]. How this habitat variability is reflected in the allele diversity of A-genome Australian wild rice is yet to be investigated.

In the face of the threats of both habitat destruction and introgression from cultigens, the wild rice populations of northern Australia comprise a globally significant, intact biodiversity resource. The extensive Australian populations of *O. meridionalis*, *O. rufipogon* and *O. australiensis* (E-genome) have been largely isolated from cultigens. However, cultivation of rice in tropical Australia is expanding. Cultivated rice has been trialled in the Northern Territory on the Adelaide River floodplain at Fogg Dam between 1950s and 1970s [183] and then currently at Tortilla Flats (Northern Territory Government, n.d.), in Western Australia in the Ord River region in the 1970s and 1980s and then currently since 2009 (Western Australian Government, n.d.), and in Queensland at Proserpine, Burdekin and Mackay [184]. Rice is also grown in temperate Australia in the Murray-Darling River Basin. However, unlike temperate growing areas, many of the rice sites where rice is being trialled (or may have escaped) in tropical northern Australia are likely to be adjacent to wild populations. Given the as yet unexplored significance of Australian native rice to global food production, their conservation and management should be considered in equal weight to the cultivation of *O. sativa* in northern Australia.

4.3. The Next Frontier—The Use of Genome Editing for Integration Free Improvement of Crop Traits

The advent of plant transformation technologies allowing the regeneration of transgenic plants from singularly transformed cells in the 1980s introduced a powerful tool to plant molecular biologists and enabled the discovery of fundamental concepts of plant biology [185]. Since these initial discoveries, significant progress has been made in the field of transgenics including improved control over integration, targeted modification to DNA sequences and marker free transformation systems. Recently, more sophisticated approaches that enable integration free “editing” of specific host DNA sequences have been developed. These next generation strategies are based on the formation of specific double stranded DNA breaks (DSBs) at desired genome targets and the use of host DNA repair systems to generate the desired changes using non-homologous end joining (NHEJ) or less commonly homologous recombination (HR). A critical breakthrough enabling the development of genome editing was the identification of sequence specific nucleases (SSNs) that efficiently make specific DSBs in complex genomes.

4.3.1. Genome Editing Technologies

Currently, several approaches are available for the creation of sequence specific DSBs that are used for genome editing. Meganucleases, TAL effector nucleases (TALENs) and zinc finger nucleases (ZFNs) all use synthetic DNA-binding proteins that recognize specific DNA targets. Although effective, these systems required significant modification depending on the desired target. The recent advent of the clustered regularly interspersed short palindromic repeats (CRISPR)/Cas system has dramatically improved the simplicity of genome editing and made it accessible to researchers worldwide. The CRISPR/Cas systems take advantage of prokaryote defence systems designed to remove foreign DNA. Essentially, the foreign DNA is flanked by host repeat sequences and transcribed as a CRISPR RNA. This CRISPR RNA directs a sequence specific nuclease Cas9 to create double stranded nicks in the genome [186]. The use of the guide RNA in the CRISPR/Cas9 system separates it from the three other methods which require extensive protein engineering. In CRISPR/Cas9 only the 20 bp guide sequence is required to be modified thus providing added flexibility of the target sequence. Other advantages of the CRIPR/Cas system include: (i) increased number of potential recombination sites compared to ZFNs; (ii) improved delivery due to the small size of the guide RNAs compared to TALEN and ZFN vector systems; (iii) target specificity of CRISPR/Cas enables multiplexing potential. Accordingly, CRISPR/Cas has been the technology of choice in recent years and has been utilized

for the editing of a range of plants including, *Arabidopsis*, tobacco, wheat, rice, corn and sorghum. As a global staple with a relatively small genome size with efficient transformation systems already established. Rice is an ideal target for use within genome editing strategies.

Recent studies have shown improved resistance to rice blast by mutating the *OsERF922* gene using the CRISPR/Cas system [187]. Genome edited plants containing the mutant *OsERF922* gene but not DNA integration were selected in the T1 population following segregation and demonstrated a significant reduction in the number of lesions upon challenge with rice blast [187]. In other studies by Xu et al. [188], it was shown that CRISPR/Cas derived mutations are inheritable and integration-free in genome-modified rice in as early as the T1 generation. It is becoming apparent that genome modification of rice will be a powerful tool for the improvement of rice in future applications.

It is often desired to incorporate specific mutations in the host genome sequence without additional integrations. In contrast to transgenic approaches, genome editing does not involve transgene integration but rather incorporation of DNA modifications which are directed by homology-dependent repair. Despite the advantages of the CRISPR/Cas9 and other genome modification strategies, many are still hindered by the presence of integrated sequences that must be removed by segregation in future generations. Thus, to realize the full potential of genome modification strategies for the improvement of rice research, this issue needs to be addressed. Recent studies therefore have focused on eliminating DNA integration in combination with genome editing.

4.3.2. Strategies to Minimize Transgene Integration

Studies have shown that *Agrobacterium* strains containing dysfunctional VirD2 protein display significantly reduced T-DNA integration. Importantly, transient expression directed by the mutant *Agrobacterium* was only mildly affected and thus these strains demonstrate potential utilization in genome editing applications. Alternatively, research has been performed on the chemical modification of the DNA coating of gold particles used for microprojectile bombardment that prevents DNA introduction into the nucleus, thus allowing transient expression but not stable integration. The bombardment of single stranded DNA that cannot be integrated is another potential strategy to avoid template integration for genome editing approaches. Although these technologies are not fully developed, these encouraging results suggest that efficient genome editing systems within DNA integration will be developed and utilized for the improvement of rice. Finally, although the jury is still out on whether genome edited crops will undergo the same regulatory requirements as their transgenic siblings, at the very least, the power that genome editing provides for research scientists to target DNA regions provides exceptional power for the characterization of specific plant genes and improved rice varieties.

Table 2. Summary of challenges and opportunities for improvement of rice salinity stress tolerance.

Challenges		
- Increased global salinisation	Limited parental resources for conventional breeding	Complexity of salinity tolerance in rice.
- Rapid increase of the world's population		- Many genes involve in salinity stress tolerance in rice. - Transgene silencing
Opportunities		
Wild rice—an excellent candidate for desalinization crop	Wild rice—an invaluable genetic resource for conventional breeding and other approaches	The next frontier—the use of genome editing for integration free improvement of crop traits

5. Conclusions

Rice is the staple food for more than one half of the world's population and is grown widely in 114 countries across the world. Hence, an improvement in rice production would have a significant impact on communities. Rice is also recommended as a desalinization crop due to its ability to grow

well in flooded conditions and the standing water in rice paddy can leach the salts out of the topsoil to make salt-affected land suitable for the subsequent crops [158]. Although many challenges impede attempts to improve salinity stress tolerance in rice, there are also a number of opportunities. Wild rice is a potential option for both: (i) a genetic resource for novel genes and mechanisms for salt tolerance; and (ii) a candidate for growing in salt-affected flooding areas subject to good salinity tolerance and high quality grain. Advanced technologies such as genome editing provide an excellent tool for reducing gene silencing in the genetic engineering approach for enhanced salinity tolerance in rice. It would also be the next frontier for integration-free improvement of crop traits with salinity stress tolerance as a target.

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