

Genetic Improvement of Rice (*Oryza sativa*) For Salt Tolerance: A Review

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Abstract: Rice is one of the most important crops in the world. Salinity is a major problem limiting rice production in West Africa and the most serious abiotic constraint in Niger. It causes drastic yield losses and hence loss of income for rice farmers. Improvement of salt stress tolerance in rice varieties would increase grain yield of rice under slight salt-stress condition, and may also extend rice growing to regions with moderate salt content in soils. Many studies have been conducted in salt affected soils and rice genetic improvement in such hostile environment. The objective of this study was to provide a mini-review in genetic improvement of rice for salt tolerance under the following sections (1) characteristics of salt affected soils (2) scope of salt problem and the effect on plant growth (3) actors affecting salt tolerance of rice (4) screening methodologies for salt tolerance in rice (5) genetics of salt tolerance in rice (6) breeding methodology for tolerance to salinity in rice.

Keywords: Rice, salt tolerance, genetic, improvement

1. INTRODUCTION

Rice (*Oryza sativa* L.) has been cultivated as a major crop for over 10,000 years, and it sustains nearly one-half of the world population (Joseph *et al.*, 2010). Rice is the third most cultivated cereal after millet and sorghum in Niger. Three major types of rice cultivation occur in Niger, namely i) traditional rice growing under flooding in the Niger River edge and pond; ii) Small private rice growing; and iii) rice growing on irrigated perimeter with total control of water.

Rice production in Niger is limited by factors such as low soil fertility, weeds, diseases, extreme temperatures and salinity (Sido, 2010). Salinity is a major problem limiting rice production in West Africa, particularly within the Sahel where rainfed rice production is not feasible (Manneh *et al.*, 2007). It is the most serious abiotic constraint of rice production in Niger. Up to 20% of irrigated fields are affected by salt problems (Guero, 2000). Maas and Grattan (1999) have estimated that rice yields decrease by 12 % for every unit (dSm^{-1}) increase in EC above 3 dSm^{-1} . This accounts for drastic yield losses and hence loss of income for rice farmers (Gregorio *et al.*, 2002; El-Bably, 2002). Improvement of salt stress tolerance in rice varieties would increase grain yield of rice under slight salt-stress condition, and may also extend rice growing to regions with moderate salt content in soils (Ming-Zhe *et al.*, 2005).

The objective of this study was to do an overview of research on rice genetic improvement for salt tolerance.

Taxonomy, origin, biology and ecology of rice

Chang (1976) classified rice as follows

ORDER: Poales

FAMILY: Gramineae

TRIBE: Oryzeae

GENUS: *Oryza*

SPECIES:

⇒ *Oryza glaberrima*

⇒ *Oryza sativa*

There are 22 wild species of genus *Oryza*. Nine of the wild species are tetraploid. The remaining wild species and the two cultivated are diploid (Vaughan, 1994; Subudhi *et al.*, 2006). *O. sativa* is the most widely grown of the two cultivated species. *O. glaberrima* however, is grown solely in West African countries (Linares, 2002). It can be distinguished from *Oryza sativa* because of its short, roundish, tough ligules and the small number of secondary branches on its panicles (Morishima, 1984). *O. sativa* has a relatively small (430 million base pairs) diploid genome ($2n = 24$). This is the smallest genome of all crops and approximately 50% of the genome is composed of repetitive sequences (Chang, 2003).

The common rice, *Oryza sativa*, and the African rice *Oryza glaberima*, are thought to be examples of parallel evolution in crop plants (Khush, 1997). Asian rice (*Oryza sativa* L.) was domesticated around 10,000 years ago from the wild annual *O. rufipogon*. It includes two main subspecies: *indica* (adapted to tropical and subtropical floating, lowland and irrigated agrosystems) and *japonica* (adapted to temperate and tropical upland ecosystems) (Chang, 1984; Khush, 1997). In a parallel evolution path, *O. glaberrima* was domesticated from annual *O. breviligulata*, which in turn evolved from perennial *O. longistaminata* (Khush, 1997).

The rice plant is an annual grass with round, hollow, jointed culms, flat leaves, and a terminal panicle. It is the only cultivated cereal plant adapted to growing in both flooded and non-flooded soils. Rice is grown under a wide range of climatic and geographical conditions on all five continents (Toriyama *et al.*, 2005). Rice is grown in widely diverse production environments. Five major rice growing environments can be broadly identified based on water regime: irrigated, rainfed lowland, tidal wetland, deepwater, and upland (Khush, 1984).

Characteristics of salt affected soils

Salinity refers to the increase in the soil surface of dissolved salts, mostly sodium chloride or common table salt, but calcium, magnesium, sulphates and bicarbonates are also implicated in soil contamination by salts (Mba *et al.*, 2007). A distinction can be made between primary salinization that involves salt accumulation through natural processes and secondary salinization processes caused by human interventions such as inappropriate irrigation practices (Gergely, 2012). There are two main categories of salt affected lands, the saline and sodic (alkali). This classification is based on the electrical conductivity (EC); soil pH; and exchangeable sodium percentage or sodium adsorption ratio (SAR) (Lamond and Whitney, 1992; Flowers and Flowers, 2005).

Saline soils are dominated by neutral soluble salts consisting of chlorides and sulphates of sodium, calcium and magnesium. The pH of saturated soil paste is less than 8.2 and an electrical conductivity (EC) of the saturated soil extract of more than 4 dS m⁻¹ at 25 °C. Sodium is generally the dominant soluble cation and the soil solution also contains appreciable quantities of divalent cations. In the presence of excess neutral soluble salts the clay fraction is flocculated and the soils have a stable structure. The permeability of soils to water and air and other physical characteristics are generally comparable to normal soils (FAO, 1988). Saline soils tend to dominate in arid and semi-arid regions.

Sodic soils tend to dominate in semi-arid and sub-humid regions. These soils contain sodium salts capable of alkaline hydrolysis, mainly Na₂CO₃. The pH of the saturated soil paste is more than 8.2. The exchangeable sodium percentage (ESP) is about 15 or more. The electrical conductivity of the saturated soil extract is generally less than 4 dSm⁻¹ at 25°C. Sodium is the dominant soluble cation and High pH of the soils results in precipitation of soluble Ca and Mg such that their concentration in the soil solution is very low (FAO, 1988). High sodium concentrations in soil generally cause soil dispersion. The later causes soil pore blockage resulting in the reduction of soil permeability (Frenkel *et al.*, 1978; Van De Graaff and Patterson, 2001; Pearson, 2004; Allotey *et al.*, 2008).

Scope of salt problem and the effect on plant growth

Oldeman *et al.* (1991) had estimated that 19.5 percent of irrigated land was salt-affected soils and that 2.1 percent of dryland agriculture, was salt-affected soils. The estimate of Flowers (2004) puts the figure of salt polluted soils at 900×10^6 ha. All estimates indicate a disturbing trend for this major constraint to irrigated and dry land agriculture.

Most crops are adversely affected by salinity. In general, cereals are more tolerant than legumes (Reynolds *et al.*, 2005). Many wild relatives of crop plants show greater tolerance than their domesticated descendents (Blum, 2004). Salt has both osmotic and salt-specific effects on plants, (Munns, 2002; Munns, 2005), impacting at different times (Shannon, 1997). Rapid changes can occur in field salinity (Richards, 1984). The effects of these changes are increased by plants preferentially extracting water from less saline areas of the root zone, by drought in rainfed environments (Srivastava and Jana, 1984), and by water logging in irrigated environments. This can be compounded by additional stresses which vary with time (Gregorio *et al.*, 2003). Among cereals, rice (*Oryza sativa*) is the most sensitive (Munns and Tester, 2008).

The general effect of salinity is to reduce the growth rate resulting in smaller leaves, shorter stature, and sometimes fewer leaves (Munns and Termaat, 1987; Jacoby, 1994). Rajendran *et al.* (2009) reported that salinity stress affects crop growth, yield and productivity. The reduction in shoot growth occurs in two phases: a rapid response to the increase in external osmotic pressure, and a slower response due to the accumulation of Na^+ in leaves (Munns and Tester, 2008).

Roots are also reduced in length and mass but may become thinner or thicker. Maturity rate may be delayed or advanced depending on species (Shannon and Grieve, 1999). Salinity effects on plants are complex (Greenway and Munns, 1980). The initial and primary effect of salinity, especially at moderate salinity concentrations, is due to its osmotic effects (Munns and Termaat, 1986). At the whole plant level, ion concentrations in plant tissues increase as a result of salinity stress. The measurable or visible effects of salinity on plants can include reduced growth rate, damage of meristems in growing shoots, reductions in yield components, or typical symptoms of nutritional disorders under osmotic and ionic stress. Grain yield reduction of rice under stress of root zone salinity can be caused by injuries at both seedling and maturity stages. In most commonly cultivated rice cultivars, young seedlings were very sensitive to root zone salinity (Zeng and Shannon, 2000).

The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties within a species. The severity of salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al.*, 1994).

Salt affected soil can be improved using many techniques. These are: physical and hydro-technical amelioration, biological amelioration, and chemical amelioration (FAO, 1988).

Factors affecting salt tolerance of rice

Temperature and relative humidity are the most important climatic factors affecting salt tolerance (Singh *et al.*, 2005). Many agronomic factors besides genetic potential affect the salt tolerance of plants. These include water regime, water quality, fertility level, land leveling, sowing/transplanting methods, crop rotation and ontogenic drift (Maas and Hoffman, 1977). Thus rice sensitivity to salt stress varies with plant growth stage (Makihara *et al.*, 1999; Rao *et al.*, 2008; Singh *et al.*, 2010b).

Soil texture and structure greatly affect the expression of plant traits. A genotype can behave differently with its inherent salt tolerance in different soil textural classes at a particular stress level. Heavy clay soils impose more stress on a plant than sandy soils (Singh *et al.*, 2010b).

Screening methodologies for salt tolerance in rice

Several methods were used for rice screening. These are:

Field screening: It is the most ideal method for identifying adapted and tolerant genotypes because salt tolerance is a complex phenomenon. However, spatial variability (Singh *et al.*, 2010a) and Rapid changes (Richards, 1984) in the field makes escape possible. Hence, field screening becomes less reliable (Singh *et al.*, 2010a) because of stress heterogeneity, the presence of other soil-related stresses and the significant influence of climatic factors (Xie *et al.*, 2001; Gregorio *et al.*, 2002).

Screening in microplots: This method is used to screen mostly early segregating populations and also stabilized populations in addition to genetic studies (Singh *et al.*, 2010a).

Salinity screening in solution culture: The solution culture technique is used in two ways: first, for screening up to seedling stage; second, for screening up to maturity.

Screening in trays: This method is employed for large-scale screening of varieties at germination/seedling stage (Singh *et al.*, 2010a).

Screening in pots: For more precise studies of individual plant response under constant stress, round porcelain or plastic pots of 20–30 cm diameter, with a capacity of 4 or 16 kg soil and a provision to allow or prevent leaching from the bottom portion, are used. Genetic and physiological studies on salt tolerance that require precision are mostly done in pots (Singh *et al.*, 2010a).

Genetics of salt tolerance in rice

The first reports on inheritance of salt tolerance came from Akbar and Yabuno (1972; 1975) in which it was inferred that inheritance of panicle sterility under salinity stress is controlled by a small number of dominant genes, but their studies were not extended to later segregating generations. Akbar and Yabuno (1977) reported that rice sterility in saline conditions, is determined by at least three genes. Most inheritance studies indicated a normal distribution of the trait in different populations, indicating its polygenic inheritance. Moeljopawiro and Ikehashi (1981) did one of the earliest studies in rice using two crosses between two moderately tolerant parents and between two tolerant parents rather than the contrasting parents. They found a low genetic response to selection and a high degree of environmental fluctuations. Shannon (1985) suggested that there is evidence of a genetically complex trait, showing heterosis, dominance and additive effects. In diallel analysis the effects of salinity on the seedling stage and on sterility suggested both additive and dominance effects, some with high heritability (Moeljopawiro and Ikehashi, 1981; Akbar *et al.*, 1985). Another inheritance study for salinity tolerance in rice by Mishra *et al.* (1998) also inferred polygenic inheritance. It was also inferred that the salinity tolerance trait also lacked maternal influence. A similar inheritance study for sodicity tolerance was conducted involving the same populations. Results indicated that sodicity tolerance is also a polygenic trait acting both additively and with interactions between the alleles at some loci (Singh *et al.*, 2001).

According to Lin *et al.* (2004) and Hu *et al.* (2012), salt tolerance of rice is the genetics of quantitative characters, which is controlled by multiple genes, with the additive and dominant effects, the former playing a major role (Moeljopawiro and Ikehashi, 1981; Gregorio and Senadhira, 1993; Gu *et al.*, 1999). Akbar *et al.* (1985) reported that the dry matter weight of rice seedling under salt stress was affected by at least two groups of genes with additive effect, and no epistatic effect was detected. Thi Lang *et al.* (2010) reported that a dominant gene controls resistance to salt stress in the allelism test. Gregorio and Senadhira (1993) observed that there were two groups of genes involved in the sodium and potassium uptake in rice, one group for sodium exclusion and the other for potassium absorption.

Breeding Methodology for tolerance to salinity in rice

✓ Conventional Approaches

These approaches include: mass selection, pureline selection, pedigree, bulk pedigree, modified bulk pedigree, shuttle breeding and backcross methods.

Mass selection method selects and bulks a few hundred to a few thousand superior plants on the basis of phenotype. It is used to improve old local varieties or purify existing varieties. Varieties are maintained through mass selection. Only those varieties that show genetic variation can be improved through mass selection (IRRI, 2007).

Pureline is the progeny of a single, homozygous, self pollinated plant. With pureline selection method you select a large number of plants whose individual progenies are tested. The best progeny is then released as a variety. It is used to develop a variety from local selections, introductions and old pureline varieties (IRRI, 2007).

The pedigree method has been the most widely used and successful in rice improvement. The method requires much time to periodically evaluate lines throughout the growing season and to keep records

on which selection at maturity is based (Jennings *et al.*, 1979). This is a classical method in which the lineage of the plant selection in the segregating generation is maintained until it is stabilized in the F7 or F8 generation. But, due to cumbersome procedures and the involvement of more resources, breeders are modifying this method and not adhering to it strictly (Singh *et al.*, 2010b).

The bulk pedigree method seems particularly suitable to breeding for resistance to abiotic stresses (Grando and Ceccarelli, 2009). It has also proved to be ideal for use in participatory breeding programmes with self-pollinated crops (Ceccarelli and Grando, 2007). After producing the F1 and the F2 on station, three years of multi-location yield testing and selection of the bulks are carried out in the target environment(s). Selection is done between bulks by identifying the best populations for either yield or other characters. In parallel with the field testing of the bulks, a within-bulks selection is conducted only in those bulks that are selected for ext level of field testing. The families deriving from the populations that maintained their superiority for three cropping seasons will enter yield testing (Grando and Ceccarelli, 2009).

The modified bulk pedigree method is combination of pedigree and bulk breeding methods; this is almost as effective as the pedigree method, with relatively less use of resources. It has flexibility and is useful for less heritable traits, with the individual F2 plants harvested in bulk up to the F4 or F5 generations, followed by panicle selection and handling of the population as in the pedigree method. However, for highly heritable traits, the individual plants are selected in the earlier generations (F2 or F3), followed by bulking for a few generations and ultimately single-plant or panicle selection in the F5 or F6 generation (Singh *et al.*, 2010b).

In the shuttle breeding approach, pre-breeding or advanced breeding materials are evaluated at different locations for their adaptability and the best adapted materials are again crossed and evaluated at different target sites in replicated trials. The latter step functions like the multi-location testing of advanced breeding materials (Mishra, 1994; Singh *et al.*, 2010b).

In the backcross method the F1 generation and the progenies in the subsequent generations are repeatedly backcrossed to one of the original parents used in the cross. The objective of backcrosses method is to improve one or two specific defects of a high yielding variety. The backcross method has not been used extensively because of a lack of suitable recurrent parents (Khush, 1978).

✓ Non-conventional Approaches

F1 anther culture has the twin advantages of increasing speed and improving breeding efficiency. Thus, it has become an effective tool to attain homozygosity of recombinants within the shortest possible time (Singh *et al.*, 2010b). Following a conventional cross, it takes a minimum of four to five years before complete or 100% homozygosity is reached, as only two generations can usually be advanced in a single year. The use of anther culture overcomes this problem by regenerating F1 pollen into homozygous plants (Singh *et al.*, 1992; Singh and Mishra, 1995).

The development of gene identification technologies using the tools of biotechnology provides ample opportunities for scientists to further improve modern cultivars (Jena and Mackill, 2008). Genomics tools and approaches, particularly the QTL mapping and comparative mapping offer new possibilities for crop improvement (Paterson, 2012; Saito *et al.*, 2012). Significant progress in rice functional genomics is being made since the completion of the international rice genome sequencing project (Chen *et al.*, 2011) which offers tremendous opportunities for breeders to improve this important crop by molecular breeding. Many molecular and genetic analyses have been performed on rice different traits such as: heading date (Izawa *et al.*, 2003; Izawa, 2007; Shibaya *et al.*, 2011), spikelet sterility (Ikehashi and Araki, 1986; Sawamura and Sano, 1996; Zhang and Lu, 1996; Wan *et al.*, 1998; Wang *et al.*, 1998; Yan *et al.*, 2000; Ji *et al.*, 2005; Zhao *et al.*, 2006; Zhang *et al.*, 2011), leaf size (Li *et al.*, 1998; Shen *et al.*, 2003; Kobayashi *et al.*, 2006; Yue *et al.*, 2006; Tong *et al.*, 2007; Farooq *et al.*, 2010) and yield components traits (Li *et al.*, 1998; Erik *et al.*, 2002; Ishimaru, 2003; Thomson *et al.*, 2003; Yoon *et al.*, 2006).

A major QTL (Saltol) derived from the salt-tolerant cultivar Pokkali has been located on chromosome 1. This QTL confers salinity tolerance at the vegetative stage and explains 64 to 80% of the phenotypic variance (Bonilla *et al.*, 2002); it has also been detected in other varieties (Takehisa *et al.*, 2004). A gene for salt tolerance at the vegetative stage has been identified in a similar position in the cultivar Nona Bokra and positionally cloned (Ren *et al.*, 2005).

Markers associated with tolerance for a variety of environmental stresses rank as important targets for molecular MAS in cereal breeding because these complex traits are often prohibitively difficult to screen using classical selection techniques. Efforts to identify QTL associated with tolerance to drought, salt and mineral deficiencies or toxicities (Champoux *et al.*, 1995; Flowers *et al.*, 2000; Gregorio, 2002; Kamoshita *et al.*, 2002; Nguyen *et al.*, 2002; Price *et al.*, 2002) in a number of genetic backgrounds represent an important first step towards achieving this goal. Additional studies have specifically addressed the problems associated with G x G and G x E (Hittalmani *et al.*, 1995; Zheng *et al.*, 2000; Hittalmani *et al.*, 2003; Li *et al.*, 2003). In the area of biotic stress, several genes have been cloned and characterized for resistance to major diseases such as bacterial blight and blast (Song *et al.*, 1995; Yoshimura *et al.*, 1998; Bryan *et al.*, 2000; Sun *et al.*, 2004) and many other genes for disease resistance have been tagged with linked markers (Valent *et al.*, 2001).

2. CONCLUSION

There are 24 species of rice including 22 wild species and two (2) cultivated (*O. sativa* and *O. glaberrima*). Rice is grown in widely diverse production environments: irrigated, rainfed lowland, tidal wetland, deepwater, and upland. Rice production is being constrained by salt problem. There are two main categories of salt affected lands, the saline and sodic (alkali). This classification is based on the electrical conductivity (EC); soil pH; and exchangeable sodium percentage or sodium adsorption ratio (SAR). It is estimated that 19.5 percent of irrigated land was salt-affected soils and that 2.1 percent of dryland agriculture, was salt-affected soils.

Several methods were used for rice screening including; field screening, screening in microplots, screening in solution culture, screening in trays and screening in Pots. Many breeding methodologies are also employed to improved rice tolerance under salinity conditions. These include conventional and non conventional techniques.

Salt tolerance of rice is the genetics of quantitative characters, which is controlled by multiple genes, with the additive and dominant effects.

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