

Review paper

Salt Tolerance Breeding in Rice: A Review Article

Daniel Nadew

Ethiopian Institute of Agricultural Research (EIAR), Melkassa Agriculture Research Center, Adama, Ethiopia: P. O. Box: 436

Corresponding Author: Email: danielnadew35@gmail.com /danabeba2127@yahoo.com

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Abstract

Rice (*Oryza sativa* L.) is a major staple crop that feeds more than half of the world's population and is the monocotyledonous plant's model system. Rice, on the other hand, is extremely susceptible to salinity and is the saltiest grain crop. Salinity has a significant impact on plant growth and productivity, and is one of the leading causes of crop yield losses in agricultural soils around the world. Plants' salinity tolerance mechanisms are regulated by a combination of multi gene and environmental variables, which cause a slew of metabolic changes in every region of the plant. Stress-induced metabolic alterations in rice plants have been investigated extensively, particularly in plant components such as the stem, leaf, and root. Rice, which is classified as a typical glycophyte, faces a significant constraint in terms of salt tolerance. Soil salinity is one of the most significant restrictions to rice production around the world, particularly in coastal locations. Crop salt tolerance is a complicated trait that is influenced by a variety of genetic and non-genetic factors. Saline circumstances have a significant impact on the rice crop's survival, growth, and development. The survival date, plant height, root length, and stem and root weight are all affected by a higher salt content. The goal of this paper is to explore the problems that are preventing rice from improving its salinity stress tolerance, as well as potential solutions for improving salinity stress tolerance in this essential crop.

Key Words: Salt Tolerance, Rice, Salinity, Growth, Population Growth

INTRODUCTION

Rice (*Oryza sativa* L., $2n = 2x = 24$) is the world's second most extensively farmed cereal crop and the staple food for more than half of the world's population, accounting for two-thirds of calorie consumption in Asia and one-third of calorie intake in Africa and Latin America. Rice is an important staple crop that feeds more than half of the world's population. It belongs to the *Oryza* genus in the Poaceae family. There are several species in the *Oryza* genus, but only two are cultivated: *Oryza sativa* L. and *Oryza glaberrima* Steud. *Oryza sativa* is native to Asia, whereas *Oryza glaberrima* is native to West and Central Africa (Vaughan et al., 2008). Rice is grown in 114 of the world's 193 countries, over six continents: Asia, Africa, Australia, Latin America, and North America. Rice production must be enhanced in both quantity and quality to satisfy the needs of the world's rising population and to ensure global food security in the twenty-first century. It is one of the most widely produced grains, accounting for more than a third of the world's food supply (Munns, 2002; Wassmann et al., 2009). Global urbanization and climate change have had an impact on agricultural land in recent years, particularly drought and salt intrusion. As a result, producing high-yielding salt-tolerant rice cultivars could be the greatest approach for these places. Development of salinity tolerance in plants has been at the forefront of worldwide genetic technology programs. The development of a salt-tolerant rice breeding crop would likely be a cost-effective solution to the problem of salty soil. Molecular markers can now be employed in breeding because to advances in biotechnology. Breeders can discover genotypes for resistance and infection from an early stage using molecular markers associated with salt-resistant genes. Microsatellite marker analysis can be used to

determine the major gene position, which can be used to produce novel kinds (Mondal and Ganie, 2014). Salinity stress causes metabolite alterations, and numerous physiological mechanisms are thought to play a role in rice plants' overall ability to cope with excessive salts (Van et al., 2020; Ganie et al., 2019). Salt stress has been demonstrated to impact the Na^+/K^+ ratio, proline concentration, hydrogen peroxide, peroxidase activity, and sugars, among other things (Saini et al., 2018). As a result, by comparing changes in physiological and biochemical parameters in rice with and without salt treatment, it may be utilized to screen salt-tolerant rice types.

Physiological and biochemical parameter techniques, on the other hand, lack defined assessment standards, and the measurement of these indices necessitates the use of appropriate instruments or kits, which can be time consuming to use. Overall vigor was reduced as a result of salinity stress, particularly in terms of the number of full grains and grain output per plant. This could be attributed to a significant drop in pollen viability as a result of the stress. Rice salt tolerance is a complicated genetic and physiological trait, with various levels of sensitivity at different stages of growth and development (Nam et al., 2015). The salt tolerance of rice throughout its life is a full reflection of salt tolerance at each growth and developmental stage, which is more practical and closer to production practice. Rice seedling sensitivity to salt stress varies with growth stage. The early seedling stage saw a greater reduction in relative seedling growth than the more established stage (Fig. 1). Young rice seedling tissues were shown to be more vulnerable to salinity, possibly because they were unable to avoid NaCl accumulation as well as more established seedlings. A variation in adaptive capacity

to Na⁺ toxicity suggested another hypothesis for differential sensitivity (Yousfi et al., 2007).

However, because of the variety of the soil, climatic and other external conditions may have an impact on rice physiological processes. Salt-tolerant rice varieties are difficult to screen in the field. As a result, screening in the lab is thought to be preferable to screening in the field. Because the salt types in saline-alkali fields are double salts, the salt tolerance determined in the lab does not always correspond to the salt tolerance found in the field (Kurotani et al., 2015). As a result, the most reliable technique to assess rice salt tolerance is to compare changes in morphological and physiological characteristics in different growth and developmental stages under salt treatment and normal conditions, both in the lab and in the field. The final goal of the review is to know the effect of salinity on rice growth, development and production as well as to have knowledge on breeding mechanisms for developing salt tolerance rice.

LITERATURE REVIEW

Rice's Salinity Stress Responsive Mechanisms

Salinity is an abiotic stress that has a negative impact on crop growth and yield. The response of cereal crops to salinity varies, with barley being the most tolerant and rice being the most vulnerable (Munns and Tester, 2008). Salinity promotes growth inhibition and yield drop in rice by causing difficulties in crop-area establishment, leaf-area development, and decreased dry matter (DM) output, as well as delayed seed sets and sterility. Salinity has been shown to have a significant impact on seedling development, seedling establishment, and grain yield components such as spikelet number and tiller number, resulting in a loss in grain production. Salt toxicity caused a decline in photosynthesis results in unfilled spikelets, according to Munns and Tester (2008). The number of tillers in rice is harmed by salt toxicity in the soil before heading, which affects the number of panicles and weight of each panicle during the three leaf stages until booting. It has been documented that in saline soils, symptoms similar to drought stress appear due to a decrease in water absorption due to the osmotic effect.

It is critical to first understand the basic molecular mechanisms of salt tolerance in order to raise or improve rice grain production under salinity. Salt tolerance is a complicated quantitative feature that is influenced by a number of genes (Chinnusamy et al., 2005). Rice has been classified as a salt-sensitive crop, particularly in the juvenile stage (Lutts et al., 1995), and salinity limits production efficiency at the mature stage (Todaka et al., 2012). Rice, as a transplanted crop, can reduce salinity at the seedling stage through management, such as

transferring older seedlings, but it cannot prevent stress at the blooming stage. Aside from seedling stage, blooming stage is another extremely sensitive growth stage that is impacted by salinity stress, while seedling salt tolerance is independent of flowering/reproductive stage (Singh et al., 2004). Salt tolerance can be measured over time by comparing the percentage of biomass produced in salinity vs control conditions (Munns et al., 2002).

Effects of salinity on chlorophyll content

Chlorophyll is a vital plant pigment that aids photosynthetic ability. Salinity levels can impact chlorophyll content, affecting plant growth and development. With increasing salt concentration, plant chlorophyll content decreased in all genotypes, but the drop was more pronounced in the salt-sensitive genotype than in the salt-tolerant genotype (Todaka et al., 2012).

Effects of Salinity on Rice Grain Composition

Carbohydrates

Rice grain is mostly made up of carbohydrates, the majority of which are starches. Carbohydrates make up to 87 percent of the total caloric content of rice grains (Mahender, Anandan, Pradhan, & Pandit, 2016). The endosperm, which accounts for 50 percent to 90 percent of the dry weight of rice grains, is one of the most important components (Chen, Wang, & Ouwerkerk, 2012). Grain texture, taste, and visual qualities are determinants of rice grain quality, which are further influenced by the composition and structure of starch molecules (Calingacion et al., 2014; Yang et al., 2007).

Amylose and amylopectin are two types of starch deposited in the endosperm. Sucrose synthase catalyzes the conversion of sucrose into glucose and fructose in the developing rice grain, which is the first step in starch biosynthesis (Chan et al., 1990).

Amylose

The effects of salt stress on rice grain starch or amylose variation vary depending on the genotypes tested or the salt level utilized, as evidenced by the literature. For example, in research of assessing the amylose concentration in the grains of a salt resistant and salt susceptible cultivar of rice produced under controlled conditions, it was shown that at 8 dS/m or greater salt levels, amylose content dropped (Rao, Mishra, & Gupta, 2013).

Furthermore, the rice plant's salinity threshold is 3.0 dS/m, hence both salt levels employed in the study (Thitisaksakul et al., 2015) are quite close to the salinity threshold. This shows that the rice grain composition was affected by the

low salt levels.

Amylopectin

Amylopectin is important for starch functionality because it comprises branch chains that impact starch's crystalline structure and pasting properties (Jane et al., 1999). Thitisaksakul et al. (2015) found that salinity had no effect on the branch chain length profile of amylopectin, but that when glucan was classified into different classes (A class, DP 612; B1 class, DP 1324; B2 class, DP 2536; and B3 class, DP _37), the salt treatments applied at the anthesis stage resulted in a reduction in chain length (Thitisaksakul et al., 2015).

Reducing Sugars

When 2 and 4 dS/m of salt were treated to a rice cultivar Nippon bare at the seedling and anther appearance stages, there was no discernible change in sugar content in the grain (Thitisaksakul et al., 2015). This could be owing to the fact that the salt levels utilized in this investigation were not sufficiently high to have any effect on rice composition.

Proteins and Amino Acids

a material that contributes 5% to 12% of the total dry weight of the grain (Chen et al., 2012). Rice sensory quality is thought to be determined by proteins (Calingacion et al., 2014). Different salinity levels have been reported to affect rice grain proteins (Wani et al., 2012). For example, in a study conducted by (Ahmed, Bhuiya, & Wadud, 1982), high salinity levels (6 and 8 dS/m) resulted in a considerable increase in protein content in brown rice in cv. Pokkali, but protein content remained identical to control at lower salinity levels. Amino acids are essential components of human nutrition. Some necessary amino acids are missing from regularly ingested cereals such as rice and wheat (Gay et al., 2010). For instance, during 2006 and 2007, researchers looked at the effect of salt stress on proline content in three rice varieties: Aychade, Fidji, and Giano. They discovered that proline levels in rice grain were 10 to 50 times greater than 2AP (2-acetyl-1-pyrroline).

Minerals and Vitamins

Some micro- and macro-mineral components found in rice grain, such as Zn, Fe, Cu, N, K, P, and Mg, are needed for human nutrition in addition to protein and carbohydrate (Heinemann et al., 2005). However, environmental conditions such as salinity stress modify their absorption and uptake (Turan, Turkmen, & Taban, 2007). For example, when comparing the contents of macronutrients and micronutrients in the grains of two rice varieties (Pokkali

salt tolerant and KDML105 salt sensitive) with different salt tolerances, (Saleethong, Sanitchon, Kong-Ngern, & Theerakulpisut, 2013) discovered that Mn, Cu, and Zn were stored more in Pokkali than in KDML105 under saline conditions (25mMNaCl). The concentrations of N, P, K, and Mg in both cultivars were found to be lower, whereas those of Na and Cu rose.

Fats and Fatty Acids

other elements, it is essential for human body metabolic functioning (Thitisaksakul et al., 2015).

Because environmental pressures have a significant impact on the nutritional value of rice grains, it's likely that environmental cues like salinity stress have a negative impact on the fat content of rice grains as well. The total phenol content, total phenolic acids, saturated fatty acids, and mono-unsaturated fatty acids of the rice grain increased as the NaCl concentration and the soaking temperature increased, but the content of alpha-tocopherol and poly-unsaturated fatty acids decreased.

Quantitative Trait Loci (QTL) and Candidate Genes for Salt Stress

The discovery of molecular markers that are intimately related to salt-tolerant genes can serve as a landmark for the physical location of these genes, making marker assisted selection easier (MAS). RFLP markers were used to locate a key gene for salt tolerance in rice to chromosome 7. (Zhang et al., 1995). Salt tolerance has also been connected to random amplified polymorphic DNA markers (Ding et al., 1998). In addition, several QTLs for salinity tolerance have been discovered (Prasad et al. 2000).

At the vegetative stage, Lang et al., 2001 discovered one microsatellite marker, RM 223, which was linked to salt tolerance. Koyama et al. (2001) used RFLP markers to find QTLs for Na and K uptake as well as the Na:K ratio. On chromosomes 6, 4, 1, and 9, major QTLs were discovered.

Genetics of Salt Tolerance

Inheritance of practically all traits linked with salt tolerance is influenced by both additive and dominant effects, according to genetic studies at IRRI (Lee et al., 1996). Characters linked with salinity tolerance, such as shoot length, low Na and high K content in shoots, and large dry weights of shoots and roots, demonstrated extremely significant additive effects at the seedling stage under saline conditions, despite the low heredity of these characters (Lee et al., 2003). Characters at the maturing stage, such as plant height and yield per plant, revealed highly significant. Additive effects, implying that additive gene action is more important in the inheritance of these

traits (Mishra et al., 1990). It was also deduced that the salinity tolerance trait is polygenic in origin and is not influenced by maternal factors (Mishra et al., 1998).

Transgenic Rice for Salt Tolerance

Breeders all across the world are attempting to develop transgenic plants to address a variety of issues, including increased yield, stress tolerance, and shelf life. Rice transgenic plants with improved salt tolerance have been tried. Hoshida et al. (2000) found that transgenic rice over expressing the chloroplast glutamine synthetase gene was more resistant to salt stress. Huizhong Wang et al., 2000,

investigated the salt tolerance of transgenic rice with the *mtlD* and *gutD* genes. Transgenic rice plants could create and accumulate mannitol and sorbitol, according to sugar alcohol analysis. Transgenic plants had a substantially higher salt tolerance than their non-transgenic counterparts. The expression of the yeast Na^+/H^+ antiporter *SOD2* in transgenic rice was studied by Zhao et al. (2006). In comparison to non-transformed controls, these transgenic plants accumulated more K^+ , Ca^{2+} , Mg^{2+} , and less Na^+ in their shoots. Tobacco *OPBP1* improves transgenic rice salt tolerance and disease resistance, according to Xujun Chen and Zejian Guo (2008).

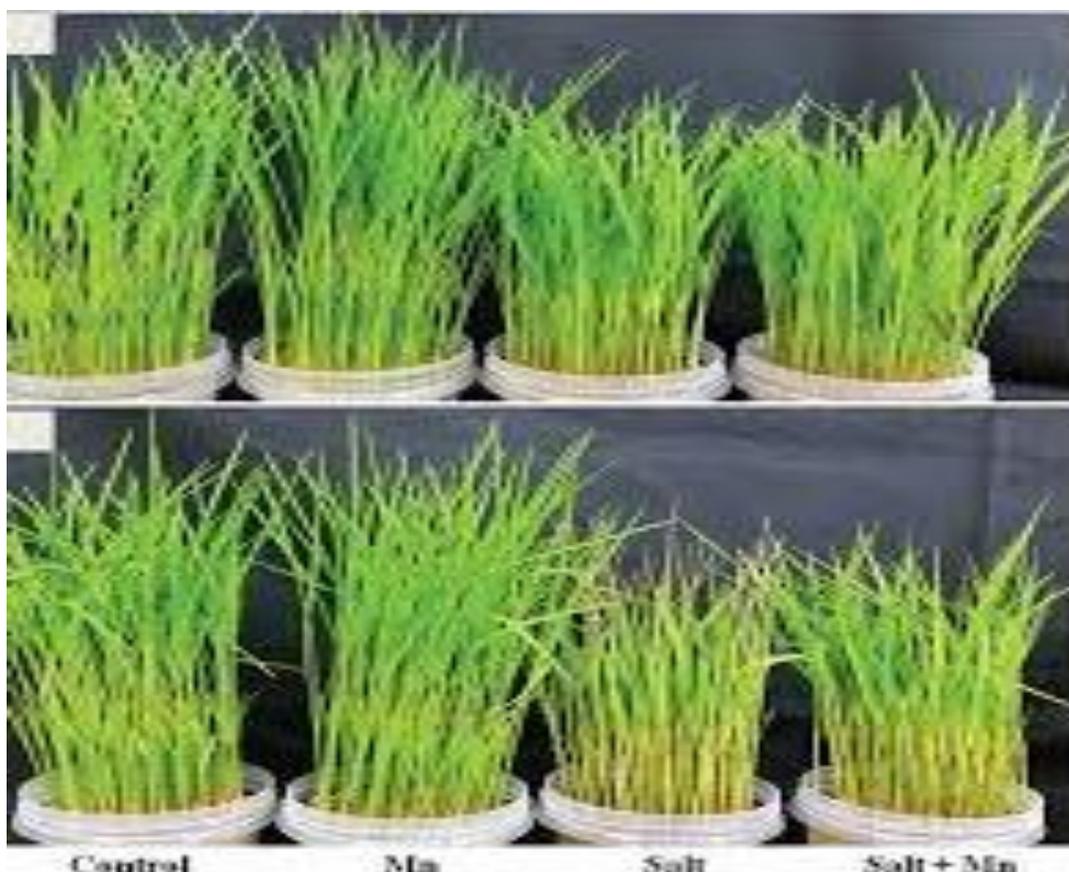


Figure 1: Salt Stress Tolerance in Rice

Because it permits the introduction of gene(s) influencing phenotypes without altering the desirable qualities of an elite genotype, genetic modification has become a valuable technique in plant breeding efforts (Bhatnagar et al., 2008). Rice genetic improvement has a lot of potential now that an efficient *Agrobacterium*-mediated transformation technology has been developed.

To date, genes encoding compatible organic solutes, antioxidants (ROS detoxification), ion transport, heat-shock and late embryogenesis abundant proteins, programmed cell death, signal transduction, and transcription factors have been targeted for genetic engineering for salinity tolerance in plants.

To produce salinity tolerance in rice, all of the known

mechanisms that rice utilizes to cope with salinity have been incorporated into genetic engineering programs (Hiei et al., 1994).

Approaches to Improve Salinity Stress Tolerance in Rice

A variety of measures have been used to reduce the impact of salt stress on rice. Water and soil management measures have aided agricultural productivity on salinity-affected soils, but gaining more benefit from these methods appears to be difficult. Crop improvement technologies based on molecular marker techniques and biotechnology have lately been utilized in tandem with traditional breeding attempts to generate salt-tolerant crop varieties (Vaidyanathan et al., 2003). The three basic approaches of traditional breeding, marker assisted selection, and genetic engineering have been utilized to improve salt tolerance in rice based on the known mechanisms that rice possesses to cope with salinity stress (Williams et al., 2008).

Conventional Breeding

Through two main procedures, conventional breeding has been employed to produce salt resistant rice types (Bresseghele et al., 2013). The first is to create/obtain a breeding population with a high degree of salt tolerance variability, and the second is to choose individuals from the segregating progeny that have most of the parent's important features as well as a high degree of salt tolerance. The threshold and slope are two significant parameters in determining salinity tolerance.

Threshold is the maximum amount of salt that can be used without reducing yield. Per unit increase in salt level above the threshold, the slope implies a 1% drop in yield. Rice has a threshold of 3.0 dSm⁻¹ and a slope of 12 percent per dS⁻¹ (Reddy et al., 2014). Rice plants respond to salinity in different ways depending on their stage of development, the type and concentration of salt they are exposed to, the length of time they are exposed to it, the water regime they are in, the soil pH, humidity, sun radiation, and temperature (Ismail et al., 2007).

Despite significant heterogeneity in the salt tolerance gene pool in rice, individuals with strong salinity tolerance have low agronomic qualities and hence are not suitable salinity tolerant donors in breeding programs for salinity tolerance enhancement. When the relative tolerance (measured as seedling survival in a salinized hydroponic system) of seven wild rice species and the two cultivated species, *O.*

sativa and *O. glaberrima*, were compared, it was discovered 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 (Prasad et al. 2000).

Marker Assisted Selection

Rice salt tolerance has recently improved thanks to a spectacular effort to uncover a significant quantitative trait locus (QTL) involved in salt tolerance. A population derived from a hybrid between the sensitive variety IR29 and a tolerant landrace, Pokkali, was used to locate a significant QTL named "Saltol" on chromosome 1. This QTL is responsible for more than 70% of the variance in salt intake in this population, and it is being utilized to incorporate this QTL into popular salt-sensitive cultivars using marker assisted backcrossing (Lafitte et al., 2004). Because salt tolerance is a complex characteristic, QTLs associated with it play an important role in understanding stress responses and developing stress-tolerant plants (Gorantla et al., 2005). As previously stated, significant progress has been achieved in identifying QTLs and their components that affect salt tolerance. Traditional map-based cloning, innovative approaches like microarray-based transcriptional profiling of differential gene expression, or a combination of genetic mapping and expression profiling are all options for discovering gene/QTLs (Marino et al., 2009).

A number of QTLs linked to salt tolerance have been discovered.

For example, using 79 SSR and EST markers distributed over the twelve rice chromosomes at an average interval of 20.7 cM and a total map distance of 1634.5 cM, twenty-five major QTLs were mapped on chromosomes 1, 2, 3, and 8 from a mapping population F2 between salt tolerance variety CSR27 and MI48, each explaining more than 10% of the phenotypic variance. More precise breeding procedures for creating increased salt tolerance have been adopted as a result of a better understanding of the mechanics and genetics of salt tolerance. Because it is development stage independent, unaffected by the environment, has no dominant effect, and is efficient to utilize in early generations, marker assisted selection (MAS) has been considered as a way to improve the speed and efficiency of breeding programs. By using molecular markers, another helpful strategy for introgression or substitution of a target gene/QTL from a salt tolerant parent to a recipient is known as marker assisted backcrossing (MABC) (Salvi et al., 2005).

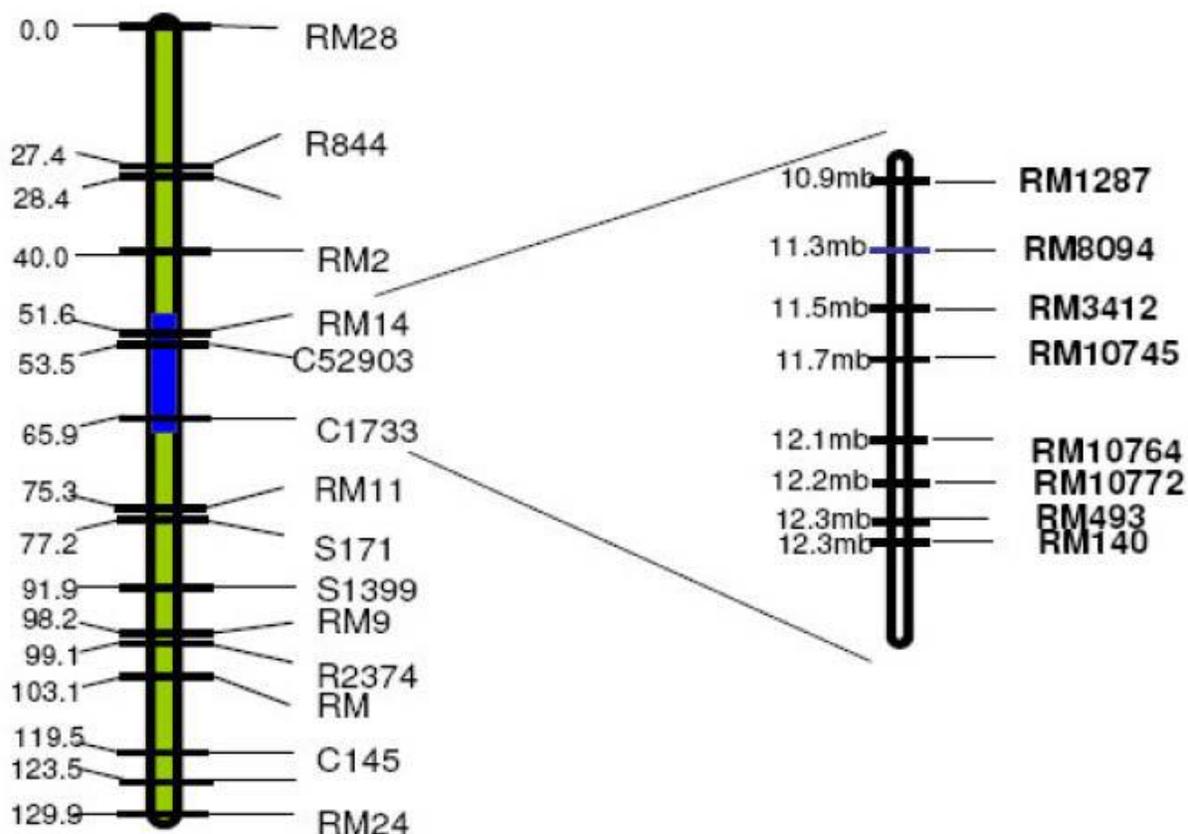


Figure 2: Saltol segment on chromosome 1 of rice

SUMMARY AND CONCLUSION

Rice is tolerant of a wide range of climate conditions. It can be grown in both dry and wetland environments, at both low and high altitudes. Rainfed rice is grown on almost 45 percent of the world's rice land and produces around 25% of total rice yield, whereas irrigated rice is grown on 55 percent of the world's rice land and yields 76 percent of the world's rice. Salinity tolerance is a complicated phenomenon that necessitates the interaction of several features. To produce a salt-tolerant germplasm, we must concentrate on investigating each characteristic separately. In rice, salt tolerance is primarily linked to the preservation of ion homeostasis, particularly the K^+/Na^+ ratio, through sodium exclusion, compartmentation, and partitioning. The K^+/Na^+ ratio has a positive connection with salt tolerance, making it the best indicator of growth and yield under salt stress. This Na-K ratio, which is the balance of Na^+ and K^+ in the shoot, could potentially be a useful criterion for determining rice salinity tolerance. Under saline conditions, the reproductive stage is one of the most vulnerable growth stages. This is the most crucial stage in terms of grain yield since good fertilization at this stage translates to grain yield in the end.

Although there are numerous obstacles to improving rice's salinity stress tolerance, there are also numerous potentials. Wild rice could be used as a genetic resource for novel salt resistance genes and processes, as well as a contender for cultivating in salt-affected flooding areas with good salinity tolerance and high-quality grain. In the genetic engineering strategy for better salinity tolerance in rice, advanced technologies such as genome editing provide an ideal tool for lowering gene silence. It would also be the next step in the development of integration-free crop characteristics with salinity stress tolerance as a goal. These features have a significant positive link with salinity tolerance (survival days, plant height, root length, plant dry weight, and root dry weight).

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