

RICE IMPROVEMENT THROUGH MARKER-ASSISTED SELECTION FOR SALINITY TOLERANCE: A REVIEW

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ABSTRACT

Marker Assisted Selection (MAS) has recently advanced, resulting in the creation of more effective selection paraphernalia to replace the phenotype-based conventional selection. For MAS, some genes associated with unique traits with molecular markers have been discovered. In the last decade, molecular marker technologies have advanced rapidly, allowing for the development of linkage maps, marker-assisted breeding (MAB), and molecular dissection of complex agronomical traits. The MAB method involves transferring a single allele at a target locus from a donor variety or line to a recipient variety or line against donor introgressions around the genome when selecting. At each generation using a molecular marker allows for progenies genetic dissection, speeding up the selection process, resulting in greater genetic benefit per unit of time. Nowadays, MABC (Marker Assisted Backcross Breeding) is being used widely in plant breeding programs to develop new varieties/lines, especially in rice. This paper reviews recent literature on some examples of salt-tolerant rice variety/line development using the MAS technique in rice-growing countries within the shortest timeframe.

Key words: Conventional breeding, gene, marker-assisted selection, rice, salinity.

Introduction

Salinity in the soil is critical abiotic stress for rice improvement all over the world. The loss of rice production in coastal areas for salinity can be mitigated by improving salt-tolerant rice varieties. Since the expansion of saline soil continues, there is a need to develop salt-tolerant rice varieties with high yields to ensure food security (Mai and Hirai, 2021). The MABC (marker-assisted backcrossing) technique is the most effective strategy to overcome the limitations of traditional breeding and restore the parental genome in two to three generations (Ahmed *et al.*, 2016). It is also an unequivocal translational research tool for crop improvement in the genomics era (Singh *et al.*, 2018). It is a quick and effective molecular method for incorporating a desired stress tolerance QTL/gene into a high-yielding rice cultivar that has already been established and enhanced (Nair and Shylaraj, 2021). Traditional salt stress breeding takes a long time and is expensive. Because of its precision, time savings, and cost-effectiveness. SSR or microsatellite markers are effective in creating genetic maps, help in selection, and analyzing genetic variation in germplasm. SSR markers are being used to identify genes for salt tolerance, which will aid plant breeders in developing new cultivars (Moniruzzaman *et al.*, 2012). The potential application of MABC for the improvement of salt-tolerant rice is the topic of this review. This paper highlights updated information on rice, its economic importance, the effects of salt stress on rice, salinity tolerance mechanisms, marker-assisted selection, MAS for improving salinity tolerant rice, the backcross method, marker-assisted backcross method, some examples of marker-assisted backcross breeding in rice in various countries, DNA markers, marker-assisted gene pyramiding and marker-assisted recurrent selection (MARS). The latest improvements in breeding approaches for enhancing and generating salt-tolerant rice varieties through MAS are also discussed in this paper. Rice breeders will benefit from this revised information as they work to establish some salt-tolerant rice varieties.

Rice and its economic importance

Rice (*Oryza sativa* L.) is grown on more than 144 million farms worldwide, it is the highest than any other crop, with production distribution which varied in at least 114 countries. Around 154 million ha of rice

were harvested worldwide in 2010, with 137 million ha (88 percent of global rice harvested) in Asia, including 48 million ha (31 percent of global rice harvested) in Southeast Asia alone (FAOSTAT, 2012). The world's population is rising by the day, and by 2050 it is predicted to reach 9.1 billion people, yet agricultural productivity is not increased (Wani and Sah, 2014). It is a prominent cereal crop that is frequently consumed by people. It is a staple food for half of the world's population (Krishnamurthy *et al.*, 2020). Rice is the main source of energy and protein. The non-food rice parts are also used for different purposes. Rice straw is used as animal feed, fuel source, paper manufacturing, and in the cottage industry for the preparation of hats, mats, and ropes (ABSF, 2010).

Effects of salt stress on rice and salinity tolerance mechanisms

Globally, soil salinity is anticipated to rise as a result of climate change and traditional irrigation techniques. About 800 million hectares of land in the world are affected by salt, accounting for more than 6% of the total land area (Krishnamurthy *et al.*, 2020). The accumulation of soluble salts in the soils of arid and semi-arid areas around the world has a significant impact on rice production (Ashraf *et al.*, 2008). Salinity is a problem of many irrigated, arid and semi-arid areas of the world where rainfall is insufficient to leach out salts from the root zone. Typical symptoms of salt injury in rice are stunted growth, leaf rolling, white leaf tip, white blotches in the leaf blade, drying of the older leaves, and poor root growth (Bekis, 2020). Salinity affects 21.5 million hectares of land throughout Asia, leading to the loss of up to 50% of fertile land by the mid-twentieth century (Nazar *et al.*, 2011).

Salt stress causes plant growth and development to be seriously affected, as well as membrane damage, ion imbalances due to Na⁺ and Cl⁻ accumulation, increased lipid peroxidation, and increased production of reactive oxygen species such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Kumar *et al.*, 2013). The key effect, which Munns and Tester called the "osmotic process," is in overall leaf production decreases and shoot growth reduction significantly (Munns and Tester, 2008). The leaves salt toxicity causes a slower impact. A genotype or salt-sensitive species is distinguished from a genotype that is salt tolerant by its inability to avoid salt accumulation to toxic levels in leaves (James *et al.*, 2011).

Osmotic adjustment, free radical scavenging, Stress signaling, vacuolar compartmentalization of ions, ion homeostasis, restoration of enzymatic activity, and quick responsiveness to external stimuli are only a few of the cascading and/or interacting events involved in salt tolerant (Golldack *et al.*, 2014). Salinity tolerance mechanisms work in a few different ways. To begin with, excess salt is not taken up by rice plants due to salt exclusion. Excess salt is consumed by resistant varieties, however, by the re-absorption process from the xylem it is reabsorbed and Na⁺ is not transported to the shoot. According to the root-shoot translocation process salt tolerance is connected to low electrolyte content in the shoot and a high content in the roots. Salt translocation is the ability of resistant rice plants to translocate a lower proportion of Na⁺ to the shoot. Osmotic adjustment: under high salinity, osmotic adjustment to external water potential is required, which gives a better nutrient acquisition and high ion selectively; synthesize organic solutes to adjust the osmotic potential of the cytoplasm and vacuole and compartmentalization of salt ions in the vacuoles (Bekis, 2020).

Marker-assisted selection

The most useful technique in terms of efficiency to boost production in salt-affected soils is a breeding approach augmented with molecular marker-assisted selection (Bizimana *et al.*, 2017). Marker-assisted selection (MAS), marker-assisted recurrent selection (MARS), genomic selection (GS) or genome-wide selection (GWS), marker-assisted pedigree selection (MAPS), and marker-assisted backcrossing are some of the terminologies used in modern breeding approaches (MABC). MABC is the most effective and extensively used method among the strategies outlined above (Golldack *et al.*, 2014). MABC can show a considerable improvement in such trait by transferring a specific region from a donor parent to the recipient parent (Oladosu *et al.*, 2019). To increase genetic diversity in MAS, segregating progeny, breeding species, exotic materials not suitable for the target environment, naturally occurring or induced mutations, transgenic events, large interspecific crosses, or a combination of these sources are all used (Moose and Mumm, 2008).

Depending on the characteristics and conditions, traditional phenotypic assays can be cheaper, quicker, and less accurate than genotypic assays based on molecular markers. As a consequence, molecular breeding can improve effectiveness and efficiency by saving time, energy, and effort. According to Mackill and Collard (Collard *et al.*, 2008), one reason for MAS's low impact is that not all markers are suitable for breeding. Non-breeder friendly markers like SCAR were developed to solve this issue (sequence characterized amplified region), RFLP, and RAPD could be converted to STS. Due to a lack of marker polymorphism and a credible marker-trait relationship, all markers cannot be used across populations (Heffner *et al.*, 2009). Recombination between QTLs of interest or markers and genes may also result in false selection. In this case, flanking or additional markers for the QTL/ target gene can be beneficial. Multiple factors that influence the effectiveness of QTL detection, including algorithms, techniques of mapping, polymorphic markers number used, and the form and size of the population are all factors to consider (Wang *et al.*, 2012).

MAS for improvement of salinity tolerant rice

To recognize and confirm a QTL, congenic strains differ in a small chromosome region flanking the target QTL are used. To assess the presence of parental alleles at the QTL region, the consistency of an effect, and its breeding value; test crosses are made by crossing different tester lines with pairs of close isogenic lines (NIL) (Landi *et al.*, 2007). These are necessary lines for the systematic and high-throughput cloning of QTLs for salinity tolerance (Huang *et al.*, 2006). To understand the salinity polygenic traits, it is often necessary to generate thousands of introgressed rice lines (Jian-Long *et al.*, 2005). Introgress lines, which are supposed to include a large percentage of the allelic variety in a respective crop's primary gene pool, influence certain complex phenotypes after trait introgression. When researchers discovered that management methods were failing to reduce salinity stress on crop growth due to a lot of environmental factors, they turned to breed to investigate salt tolerance in rice. But where does the salinity tolerance genetic trait come from? Then researchers discovered crop landraces that local farmers grew in saline climate-prone areas around the world, these landraces, however, did not produce a lot of yields. The creation of molecular DNA markers ushered in a novel age of plant breeding, enabling scientists to rapidly classify salinity-tolerant landraces in the laboratory (Bonilla *et al.*, 2002).

The backcross method: Breeders can visually select which progeny most closely resembles the RP in early backcross generations. However, it may be impossible to distinguish between backcross progeny and the RP based on individual plants in future generations (i.e. after BC2). Additional backcrosses must be done to keep the favorable traits of the RPs, with the idea that backcrossing until at least BC6 will restore the RP as much as possible (Hasan *et al.*, 2015).

Marker-assisted Backcross Method: The MABC (Marker-assisted Backcross Method) approach is essential for fundamental research applications in rice, as it allows for significantly more precise development of new and advanced varieties than traditional backcrossing. MABC is utilized to alleviate abiotic stresses like salinity, submergence, and so on. MABC's primary job is to shrink a donor parent's chromosomal size. It also cuts down on linkage drag. This will contribute to the development of rice variety. As a result, MABC should be employed to improve rice variety quality and production (Bishwas *et al.*, 2016). MABB is a reliable and successful approach for inserting the desired trait into elite varieties without affecting their basic characteristics (Nair and Shylaraj, 2021).

Success with marker-assisted backcrossing (MABC): MABC contains a set of backcrossings to remove the donor's genetic background while retrieving as many of the RPs' genetic traits as possible (Hasan *et al.*, 2015). With MABC and marker-based genome scanning, most recurrent genomes were quickly recovered in just a few crossings (Melchinger, 1999). By limiting carried-over donor segments flanking the target locus, MABC can also be employed to create near-isogenic lines (ILs), allowing for precision introgression of specific genes and extensive analysis of QTLs. Several researchers have discussed methods for maximizing sample sizes and selection approaches in marker-assisted selection (Thi *et al.*, 2013). The following are the three selecting steps (Collard *et al.*, 2008):

Selection of the foreground: In foreground selection, the breeder chose plants with the donor parent's marker allele at the target gene. The aim is to keep the target locus heterozygous (one donor allele and one RP allele) until the ultimate backcross is completed (Hasan *et al.*, 2015).

Selection of recombinants: Recombinant selection is the second level, which entails choosing BC progeny with the target gene and recombination events between the target locus and associated flanking markers (Collard *et al.*, 2008). The goal of recombinant selection is to make the donor chromosomal segment that contains the target region smaller (i.e. size of the introgression). This is significant because the rate of decrease of this donor fragment is slower than for unlinked areas, and numerous unwanted genes that harm crop performance may be linked to the target gene from the donor parent (i.e. linkage drag, Figure3) (Hospital and Charcosset, 1997).

Selection of the Background: Except for the target locus, all genomic areas can be chosen in the background using RP marker alleles, and the target locus is chosen based on the phenotype. This selection is crucial to limit the number of unwanted genes introduced by the donor. It is simple to eliminate undesirable donor alleles in the same genomic area as the target locus using molecular markers. The third phase of MABC is choosing BC progeny with the highest proportion of RP genome using markers unrelated to the target locus, a process known as "background selection." Back-ground selection is defined as the use of firmly connected flanking markers for recombinant selection and unlinked markers for RP selection in the literature (Geetha *et al.*, 2017).

Various advantages of MABC over the traditional backcrossing

Increase the speed of recovery of the recurrent parent genome: Six backcrosses are required in traditional backcross breeding to recover the RP genome. However, by employing the MABC method, the RP genome might be recovered by BC4 or BC3 or even BC2, saving two to four BC generations (Babu *et al.*, 2005).

Reduce linkage drag : To minimize the huge number of donor chromosomes, at least six backcross generations are required, however, MABC may only require two or three. Linkage drag necessitates many more backcross generations, and if the unwanted genes are extremely firmly linked to the target locus, ordinary backcrossing may be ineffective (Collard *et al.*, 2008).

More accurate selection: It is extremely difficult to identify polygenic traits using traditional breeding procedures. However, in the case of MABC, markers can be used to select based on gene expression (Hasan *et al.*, 2015).

DNA markers: DNA markers, in particular, can be actively promoted in rice breeding to enhance salinity tolerance (Ashraf and Foolad., 2012). A new age of plant breeding started after the discovery of the DNA's double helix model, which was a gene revolution. Many types of DNA markers have been promising to regulate a certain character since 1980 (Xu, 2010). Rice breeders often exploit natural allelic variation to increase crop yield. Cloned QTLs numbers are essential for developing salinity tolerance is very low since finding main QTLs that are appropriate for replicating is difficult. Furthermore, phenotypic effects of QTLs and correct characterization in the saline condition are ineffective in the majority of cases (Salvi and Tuberosa, 2005).

Marker-assisted recurrent selection (MARS): Marker-assisted recurrent selection (MARS) can be distinguished by the following characteristics: (i) relatively large populations exposed to selection at every cycle; (ii) lower prices for marker data points; (iii) selection before flowering; (iv) flanking versus single markers; (v) increased number of generations per year from one to four. Developing salt-tolerant crops necessitates the detection and application of genes from a variety of sources. Genetic variation between and within crop germplasm, as well as uniformity in released varieties, could be measured by molecular markers (Steele *et al.*, 2006). To incorporate stress-tolerant genes into rice, researchers combined marker-assisted selection and marker-assisted backcrossing and with participatory plant breeding (Steele *et al.*, 2006).

Conclusion

MABC has raised a lot of aspirations, which has over-optimism in some situations and disappointment in others since many of the expectations have yet to be met. While MABC has bright future possibilities and potential results, it also has some limitations, such as facilities, infrastructure, qualified manpower, lack of private sector participation, supplies, and consumables. The development priorities of many developing countries do not include genetic enrichment programs by utilizing molecular methods when considering financial help in the agricultural sector. The MABC production and implementation process was deemed to be expensive at various times. The most important costs at the beginning of MABC are the improvement of a genetic linkage map for the interest species, as well as the detection of associations between QTLs or genes, and economically relevant characters. For developing countries, such a cost might be prohibitively costly. Breeders must develop a cost-effective MABC technology that allows them to recombine agronomically relevant genes from different sources and determine genotypes across the entire genome. It can be concluded from previous marker-assisted selection research on rice that this selection is a technique that can be used to generate new rice varieties with salt tolerance and improved quality. This paves the way for new salt-tolerant rice varieties to be established in saline-prone areas around the world.

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