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Improvement in Salinity Tolerance in Rice Using Biotechnology

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Abstract: Rice has been amongst the most important cereal crop on earth, or more correctly the second most staple diet of people after wheat. The statement is self-explanatory to the demand of rice worldwide and the supply. But with increasing population, the demand of rice is also undoubtedly increasing. The increase in supply is though held back by many factors that adversely affect the growth of a healthy rice crop. One of these factors is salinity. Rice being a glycophyte has very low tolerance to salt, and salt stress results in a damaged yield. However, rice has its own mechanisms for salt tolerance to a certain level, the knowledge of which scientists have put in use to develop the few improvements in rice varieties providing them with better tolerance. Though these improvements have faced many challenges themselves, science still strives to make further achievements. This study aims to highlight the mechanisms rice use to tolerate salt up to a certain level and how they are affected by salinity at levels above that. It also aims to enumerate the methods biotechnology has developed in the past years to improve the varieties, the advantages they have had, the increase in yield they have shown and the challenges they have had to face. Also, it includes if there are any further developments undergoing and the future scope of salinity tolerance increase in rice, with the help of biotechnology.

I. INTRODUCTION

Rice, (the most commonly used variety being *Oryza sativa*) has fed the world's population since time immemorial. But with the recent trends of population explosion the world is witnessing, naturally produced crops are not enough to feed so many hungry mouths. To add to it, the climate conditions and other factors supporting the crop growth over the past decades have also deteriorated, further decreasing the crop yield.

A. Rice as a Major Crop

As mentioned earlier, rice is a staple plant crop in world and feeds a significant population in the world. It is a unique crop since it shows growth in wet environments and watery land which other crops do not. This quality of rice makes it abundantly grown and harvested in Asia and now it can be grown in all parts of world apart from Antarctica. Rice shows growth in an exceptionally varied environment, from the wettest parts along Myanmar's Arakan Coast that receives an average of 5,100 mm of rainfall to the driest parts at Al Hasa Oasis in Saudi Arabia which naturally receives less than 100 mm of rain. It also shows growth in varied temperatures, from growing in 33 C in Upper Sind, Pakistan to growing in 17 C in Otaru, Japan. When it comes to sunlight required, it can grow under 25% availability in Myanmar and India to 95% availability in parts like Egypt and Sudan [1].

B. World Population and Rice Production

Since the year of 2000, the world has witnessed a stark decrease in the production of rice crop. In contrast, this decline led to the observation that the production since then was less than the worldwide rice consumption. When observed, it was calculated that the world's population was expected to rise from 6.13 billion in 2001 to 8.27 billion in 2030. Whereas the demand for rice was expected to increase from 571.9 M tonnes in 2001 to 771.1 M tonnes by 2030[2]. To secure food availability to the increasing population of coming 30 years, it became necessary to adapt methods that will revert the decline in rice production to enhanced yields.

The International Rice Congress that took place in Singapore held in 2018 hosted speakers from Corteva Agriscience from DowDuPont who brought forth similar facts as mentioned above when discussing with International Rice Research Institute (IRRI). They confirmed the fact that along with the spiking population, the demand of rice globally is supposed to increase by 25% in the span of 20 years from 2010 to 2030 to 550 million tonnes annually [3]. But as it is observed, no matter how accurate the assumptions of the demand are, the production rate is not at par with them. This could be blamed on the deteriorating climatic conditions, rapidly increasing fight for resources and other basic factors that have always been an obstacle in rice production, salinity being one of them. Henceforth, even if rice is a crop that shows growth in versatile light and temperatures, it is highly sensitive to factors like salinity, increase of which in soil and water has been no big feat in recent years.

C. Salinity Affecting Production

Salinity can be described as the presence of more than adequate amount of salts in soil and water, like sodium chloride (NaCl), magnesium and calcium sulphates ($MgSO_4$, $CaSO_4$), and bicarbonates [4]. Salinity although develops naturally, but human interventions and recklessness has led to a rapid increase in the rate with which salinity is increasing in soil and water, the two resource bases that support rice growth. Salinity causes unnecessary fight for water with the salts and also causes intoxication if the nutrient deposition levels exceed in it [5]. Rice being a glycophyte naturally is drastically affected from this unnatural salinity increase and hence even after having defence mechanisms against it, get affected by it. The critical levels of salinity that result in 50% loss of yield of the crop was estimated to be 6.9dS/m for rice [6]. Hence, any levels over it would result in the loss of most of the crop, explaining the reason of inadequate amounts of rice for people. However, to solve this problem, scientists have worked hard to device ways of improving the salinity stress tolerance levels more than usual in rice crops. But before we look into those methods, we must first fully understand how rice defends itself from excessive salt uptake generally.

II. SALINITY TOLERANCE MECHANISM IN RICE

The response of a crop to soil or water salinity comes as a complex phenomenon that depends on many factors. Therefore, the best response lies in the optimization of various, if possible independent, physiological factors. Salinity can be the reason of crop damage by its osmotic effect, specific ion toxicity or by interfering in the essential nutrient uptake of the plant [7].

When growing in salty soil, roots have to deal with two types of stresses, them being osmotic stress and ionic stress [8]. The first causes an inhibition in water uptake and the other causes excessive uptake of Na+ ions which can cause major deficiency of the essential element K+ [9]. When plants encounter such adverse environments, they maintain osmotic and ionic homeostasis via rapid osmotic or ionic signalling respectively. Defence system of the plant to fight the toxicity of salt stress, can be classified into three categories: a) osmotic adjustments to tolerate osmotic stress, b) selective ion uptake and regulated uptake at molecular level of sodium ions, c) tissue tolerance [10].

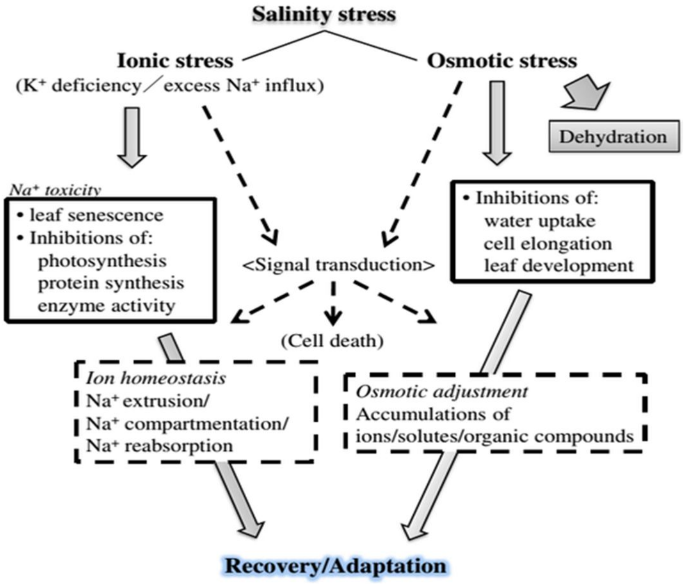

Figure 1 A Schematic Summary of Stresses Plants Suffer under High Salinity Growth Conditions and Corresponding Response of the Plants to survive them [9].

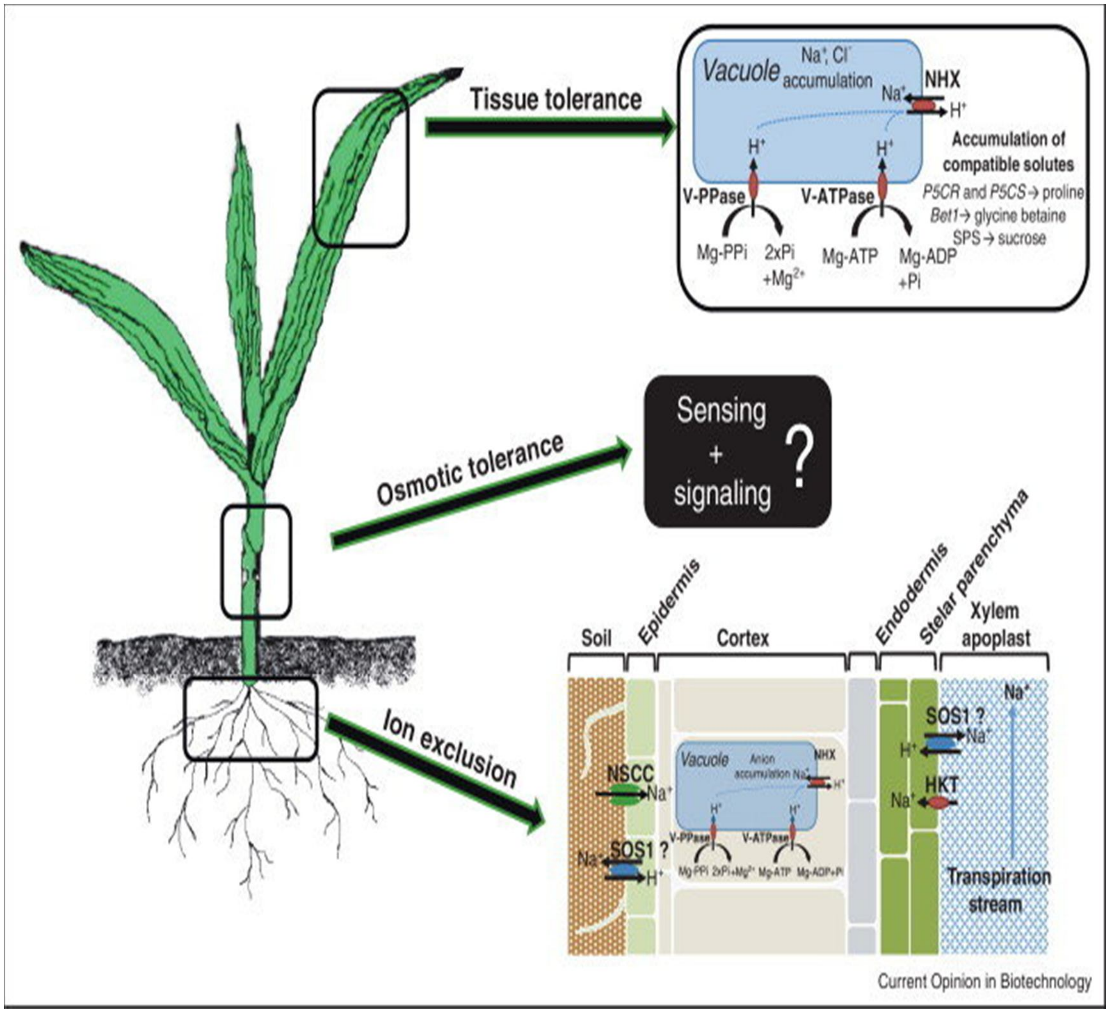

Figure 2 Three main mechanisms of salt tolerance in a plant.
Source: Salt resistant crop plants, Current opinion in Biotechnology. Roy SJ et al. Elsevier.

Due to high salt amounts, osmotic stress leads to rapid increase in Abscisic Acid (ABA) biosynthesis, hence regulating pathways of stress response called ABA dependent. In fact, it is observed that rice cultivars showing salinity tolerance used to vary according to the amount of the ABA the cultivar was synthesising [11, 12].

There are several such genes which are stress inducible and they are ABA- independent. The overexpression of these rice tolerant genes shows tremendous abiotic stress tolerance. Hence this pathway is often named as the ABA dependent pathway [11].

1) *Tolerance to Osmotic Stress:* This immediately results to reduction of cell expansion in the root tip and young leaves, and also leads to stomatal closure. Such a reduced response leads to more leaf growth as well as conductance of stomata, but the resultant extended leaf area will be beneficial to plants with the availability of good amounts of soil water, rice being a fitting example [13].
2) *Tolerance to Ionic Stress:* (Na^+ exclusion from leaf blades) the exclusion of sodium from the roots is a way to make sure that the amounts of sodium do not reach to toxicating levels in the leaves. If there is a failure in doing so, the toxic levels show their effects in a matter of days or weeks (species dependent) and they lead to premature deaths of older leaves [13].
3) *Tissue Tolerance:* Tissue tolerance meaning tolerance by tissues to the accumulated levels of sodium, or in some cases, in some species, to chlorine. This sort of tolerance requires the correct procedures, compartmentalization of sodium and chlorine, that is at the cellular as well as intra cellular high points for avoiding the toxic concentration within the cytoplasm, with special attention to mesophyll cells in the leaves. Toxicity though takes place with time, after the level of sodium increases in the older mature leaves [13].

The Figure 3 shows the various pathways rice crop adopts to control salinity levels along with the numerous necessary genes that make up the working of such an elaborate process.

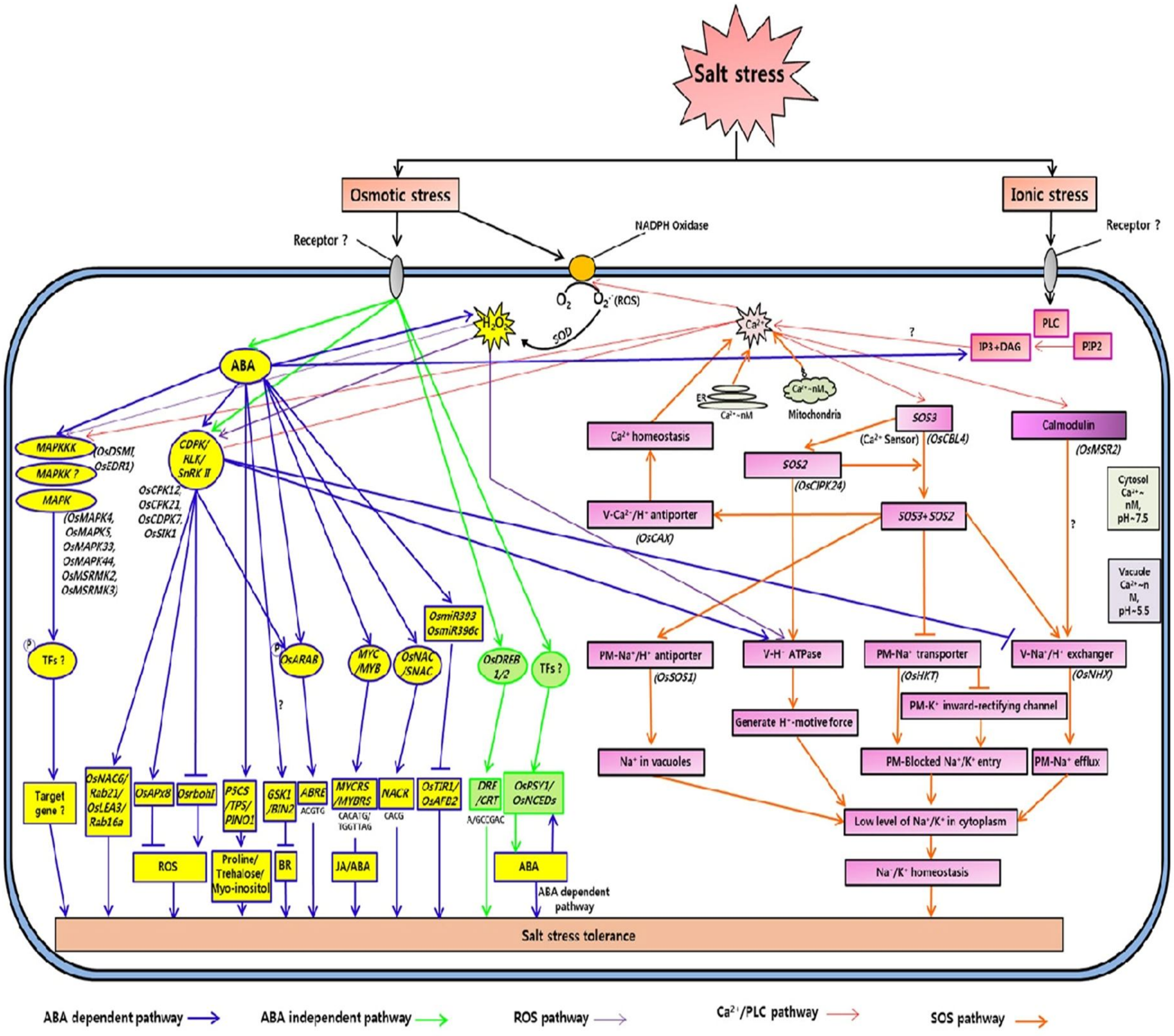

Figure 3 Total signalling pathway during salt stress. Salt stress includes both osmotic and ionic stress. Osmotic stress signalling is transduced using ABA-dependent or ABA-independent pathway. ABA dependent pathway consists mitogen activated protein kinase (MAP Kinase) cascades, calcium-dependent protein kinases (CDPK), receptor-like kinases (RLK), SNF1-related protein kinases (SnRK), transcription factors (OsRAB1, MYC/ MYB and OsNAC/SNAC) and micro RNAs. ABA-independent pathway consists transcription factors (OsDREB1 and OsDREB2) and stress related genes (OsPSY1, OsNCEDs). Ionic stress performs signalling by Ca2+/PLC pathway and salt overly sensitive (SOS) pathway and Calmodulin (CaM) pathway. Ca2+ is sensed by Ca2+ sensor (OsCBL4) and the sensor activates calcineurin B-like protein kinase (OsCIPK24), which in turns activates Na+/H+ antiporter (OsSOS1), H+/Ca+ antiporter (OsCAX1), vacuolar H+/ATPase, vacuolar Na+/H+ exchangers (OsNHX1) and suppress K+/Na+ symporter (OsHKT1) to maintain ionic homeostasis under salt stress. Ca2+ also activates Calmodulin (OsMSR2) which also activates vacuolar Na+/H+ exchanger (OsNHX1). Blue arrow indicates ABA dependent pathway, green arrow shows ABA-independent pathway, violet arrow shows ROS pathway, red arrow shows Ca2+/PLC pathway and orange arrow shows SOS pathway [11].

The study of these pathways makes up the basis of discoveries in the field of biotechnology that leads to the improvement of these very methods. Artificially introducing some such necessary genes or inducing them in a particular cultivar or creating transgenic variety with improved ABA synthesis are a few named achievements whereas there are many that are still underway. Some of these more popular and greatly used biological techniques have been mentioned further below.

III. BIOTECHNOLOGICAL PROCESSES LEADING TO IMPROVED LEVELS OF SALINITY TOLERANCE IN RICE

Biotechnology has shown a huge success in enhancing salt tolerance in a variety of crops along with rice crop. The study of natural salt tolerance processes has led to the discovery of ways where those very processes can be enhanced as well as new processes can be used for improving the rice crop. Two of the most widely used or most successful processes have been the Plant Breeding approach and the Transgenic approach [14]. Over the recent years, genome engineering also has rapidly become an added successful process in improving salt tolerant abilities.

Among them, cell culture and techniques of tissue culture have also been employed to get tolerant plants generally by using two approaches of *in vitro* culture, which includes the given two: selection of the required mutant cell lines from the cultured cells and the *in vitro* of the germplasm of the plant, for the heart of the matter, salt tolerance. Trying to enhance the tolerance or reach appropriate levels of resistance against both the kind of stresses, the hyperosmotic and the ionic stress, can also be attained by molecular breeding in salinity tolerant plants by the use of either molecular marker or genetic engineering [15].

1) Plant Breeding Approach: Plant breeding is nothing more than the conventional method of crossing two desirable varieties of the crop to produce a fairly better variety. The conventional breeding is meant to occur in two important basic steps. They include: obtaining a breed worthy population that includes high variability in terms of salt tolerance, the other is among those segregating progenies, selecting those ones that can bring together mostly important and useful characteristics of the parents being used and the high levels of salt tolerance [5]. These traits are controlled by a group of various genes that produce a chain reaction of variations, and are known as quantitative trait loci a.k.a. QTLs [16]. To counteract the salt caused effects, a plant reacts in various ways, which also includes the accumulation or exclusion of ions to provide ion homeostasis, or to maintain osmoregulation, the accumulation of organic osmolytes, the change in the pattern of uptake and accumulation of essential mineral nutrients, or to counteract the generation of salt induced reactive oxygen species the production of antioxidants [17]. Over the past years, many researches have been done in various plant species for identification of the QTLs that either direct or indirectly affect the numerous plant responses to the salt levels at different growth stages consisting seed germination, seedling plus reproduction or vegetative growth [18]. The most common rice species *Oryza sativa* shows the following QTLs with respect to their molecular markers and their roles.

Molecular Marker	Locus	Trait Governed
SSRs	qRL-7, qDWRO-9a and qDWRO-9b qBI-1a and qBI-1b	Have significant roles of root length and its dry weight at seed stage under salty conditions
ESTs	qSNC-7 and qSKC-1	Affect the shoot concentration of Na+ and K+, respectively
SSRs	QNa, QNa:K, SKC1/OsHKT8	Regulating homeostasis of K+ / Na+
AFLPs	QNa, QK1, QK2 and QNaK	Improve Na+ , K+ and discriminating uptake of Na+ or K+
SSRs	qDM-3 and qDM-8, qSTR-6	Improve ratio of Na+ /K+ under salty conditions
RFLPs, SSRs, AFLPs and isozymes	qST1 and qST3	Enhancement of shoot salt tolerance
SSRs	qNAK-2 and qNAK-6	Improve ratio of Na+ /K+
SSRs	Saltol	Controls shoot homeostasis of Na+ /K+
SSRs	Saltol and non-Saltol	Controls shoot homeostasis of Na+ /K+
SSRs	QKr1.2	Controls content K+ in root

Table 1. Identification of quantitative trait loci (QTLs) for salt tolerance in *Oryza sativa* [18].

Although it is tough to establish those physiological traits that best define the genotypes of salt tolerance of a plant, the idea of putting physiological criteria to use for salt tolerant screening has been appreciated by many researchers.

2) *Screening [15]:* The problem with breeding is that the traits responsible for the salt tolerance are quantitative in nature and also the problem of establishing a replicable and appropriate environment for testing make it hard to differentiate between salt tolerant lines and sensitive lines. The screening method speed though has a major effect over process of germplasm supposed to be fit into programs of breeding for salt tolerance. The physiological as well as molecular traits of plants provide the base for the efficiency of procedures of germplasm screening through using traditional, molecular breeding, biotechnology. With the in hand knowledge of the basic characteristics of salt tolerance in a specific species, a breeder may plan for more specific targeted crosses to obtain a more salt tolerant crop plant. There is a variety of salt tolerant screening methods that are used for a crop since it is observed that it is not feasible to apply or suggest any one firm method to all varieties or any type of environmental condition. A list of such methods is given below with respect to type or environment:

a) Germination and seedling stage- dry matter, germination rate, seedling vigour
b) Field/greenhouse- the content of chlorophyll
c) In vitro- soma clone variants, induced mutations
d) Doubled haploids- the efficiency of screening increased.

Although an efficient method, the need to use biotechnology proves the lack of resourcefulness in the conventional style of breeding. On the contrary, scientific breeding, as the name suggests, is more science and lesser subjectivity, which means accurate and practical evaluations, and efficient and effective selections [19]. The fast evolving molecular markers and constantly improving molecular assays has led to rise of a new technique in plant breeding: molecular marker-assisted breeding (MAB). Compared to the conventional breeding, it has much significance:

- MAB can be used to select all the desired traits required right at the seedling stage, which saves the time of having to wait for the plant to grow to determine its phenotype. Though, during the plant's later developmental stages, the occurrence of some undesired genotypes is witnessed, which can be easily eliminated by using marker assisted selection (MAS), another widely used biotechnological process in this field [19].
- MAB allows selection at any given time of the year since it is not environment specific, which means selection can occur in any environmental conditions. This proves to be very helpful in case of certain such cases that express only when there is favourable environment. Although, for traits that have less heritability as well as are very much affected from the environment, instead of phenotypic selection, MAS that are reliable markers based which are linked with QTLs of the desired traits tightly are more efficient, effective and hence preferred [19].
- MAB speeds up the process of observing recessive alleles of a plant. Conventional breeding requires self or test crossing to obtain the results of desired recessive alleles whereas MAB uses co- dominant markers like SSR to obtain those alleles in heterozygous status [19].
- Gene masking makes it difficult to distinguish between genes or loci, whereas identification and selection can take place simultaneously using MAB hence making the process easier [19].
- MAB may be considered to have higher effectiveness as well as efficiency in terms of time, resources and the saved efforts since molecular marker based genotypic assays are speedy, less expensive and more accurate compared to the conventional phenotypic assays [19].

3) *General Procedure for Molecular Assisted Breeding (MAB):* MAB, keeping in consideration the prerequisites are well equipped and available, can be watered down to a step wise process as mentioned in brief below, which also highlights how biotechnological techniques directly comes into use in this process:

a) Planting in the breeding population, the potential segregation for the desired traits, or for the marker being used perform polymorphism [20].
b) Plant tissue sampling, generally at the young age of growth [20].
c) Extraction of DNA from the sample tissue of one individual or the family of population and then preparing that DNA sample for PCR as well marker screening [20].
d) Performing PCR or other elated amplifying operations to obtain the molecular marker associated with the desired trait or QTL [20].
e) Separation and scoring of PCR by using proper detection and separation operations, for example PAGE, AGE, etc. [20].
f) Identifying the family or individual that carries the desired marker allele.
g) Single out the best family or individual that may have both the desired marker allele as well as fairly better other traits, which is done by combined use of marker data and other selection criteria [20].

h) Repeating the above procedure for various generations, depending on marker- trait association, as well as marker allele status, and advancing the selected individuals until the superior line is obtained [20].

4) *Marker Assisted Selection (MAS):* MAS can be considered to work similar to MAB though it relies more on the genotypic aspect of the plant crop contrary to the phenotypic approach of MAB. For plant breeding programs, MAS is an advanced technology more refined than MAB which can help selecting the chromosomal regions that have the appropriate DNA markers tagged to them of the trait desired by humans. Furthermore, MAS works in a combine way, which means, in the shortest means of time, MAS can also collect the desired traits from different donors and then transfer those precise traits or QTLs to a single variety [21].

There are some major steps that need to be followed while using MAS. This is a brief understanding of how the process works:

a) Identifying contrasting parent lines among salt tolerance- This is the primary step in a breeding program, where genetic variability of the desired trait is determined [22].
b) Developing mapping population- The parental lines are cross hybridized. Segregating population is used for genetic matting, which includes early filial or backcross, inbred backcross, recombinant inbred line populations or double haploids. The first step in doing so is to cross breed two contrasting parents in salt tolerance as well as differing markers to produce first progeny [22].
c) The genetic markers showing polymorphism between the parents are identified. A specific enzyme is extracted, different forms are separated based on the electric charge or shape or size via gel electrophoresis. Numerous DNA markers these days are used for QTL mapping or MAS, for example hybridisation based (using RFLP) or PCR based markers [22].
d) Developing Genetic Maps- Before moving on to QTL mapping in a population, it is of utmost importance to first create a genetic map. There are various mapping techniques that help in using the relationship of the markers to the desired phenotypes, which may lead us to the relative positions of the QTLs [22].
e) Screening population for salt tolerance- This is carried out on the basis of certain traits; morphological, physiological or molecular markers [22].
f) QTL analysis and identifying markers associated to salt tolerance- The QTL-marker association identification makes use of various types of statistical analyses [22].
g) In MAS, at least 5 backcrosses are required to pass the desired trait from donor (salt tolerant parent) to the cultivar (salt sensitive parent) (generally selected under controlled environments or greenhouse conditions) [22].

5) *Transgenic Approach:* This approach focuses on using recombinant DNA techniques for obtaining desirable as well as new traits. It has been applied successfully in the production of salinity tolerant rice plants by the introduction of new genes and then testing them against salinity [14]. To generate smart crops, a significant tool is genetic engineering that allows introducing specific traits without the abolishment of native traits, is speedier, is much more effective and can be applied to a large range in species. It has also been proven that if foreign genes are expressed in heterozygous plant systems, they promote higher levels of salt tolerance [23]. Particularly, genetic engineering has been quite successful in attaining crops tolerant to abiotic stresses. However, it will be well to know that the classical approach of genetic engineering that applies transgenic overexpression of genes that control stresses like salinity, comes at a cost [24]. Those are some drawbacks which will be mentioned further in the report. Henceforth, simply put, transgenic approach focuses on genetically modifying crops to the humans’ liking by overexpressing the desired genes in the desired crop until it shows desired results, in the case being tolerance to abiotic stresses like salinity. There are several strategies to acquire transgenics that are suitable for field conditions. Some of the strategies include:

a) Transgene expressions being tightly regulated using stress inducible promoters [24].
b) Engineering protein such as ubiquitination post translational modifications [24].
c) Using wild varieties or halophytes in the crops to express orthologous genes of effectors [24].
d) Regulating the expression of the gene by regulation of the mRNA activity via transgenic expressing of mRNA target sequence [24].
e) Some other ways that have been explored for the generation of the generation of new generations of transgenics may include – gene pyramiding, engineering transcription factors; osmoprotectants; chaperones, late embryogenesis abundant proteins, metabolic pathways, epigenetics [24].

Although one approach may not be enough to attain crops worth distributing to farmers, but transgenics that are obtained via more than one approach can be crossed [24].

6) *Genome Editing [24]:* Genome editing is not a very popular technique in the field of crop improvement but a very useful one. As the name suggests, it is the target based mutagenesis of genome. The technology in use today, allows us certain changes at particular sites of a genome. Such technology makes use of customised reagents of DNA cleavage plus repair pathway of cellular DNA. Usually, the reagents used are engineered nucleases that cleave the target DNA at the sequence specified by the user further using non homologous end joining (NHEJ) or homologous recombination (HR) to repair the double strand breaks. These DNA repairs or erraneous repairs are manipulated to obtain customized modifications in DNA like insertions, or donor cassette insertions, or deletion. There are four known engineered nucleases that are utilized in genome engineering. They are: Zinc finger nucleases (ZFN), transcription activator- like effector nucleases (TALEN), meganucleases and CRISPR/Cas RNA-guided nucleases.

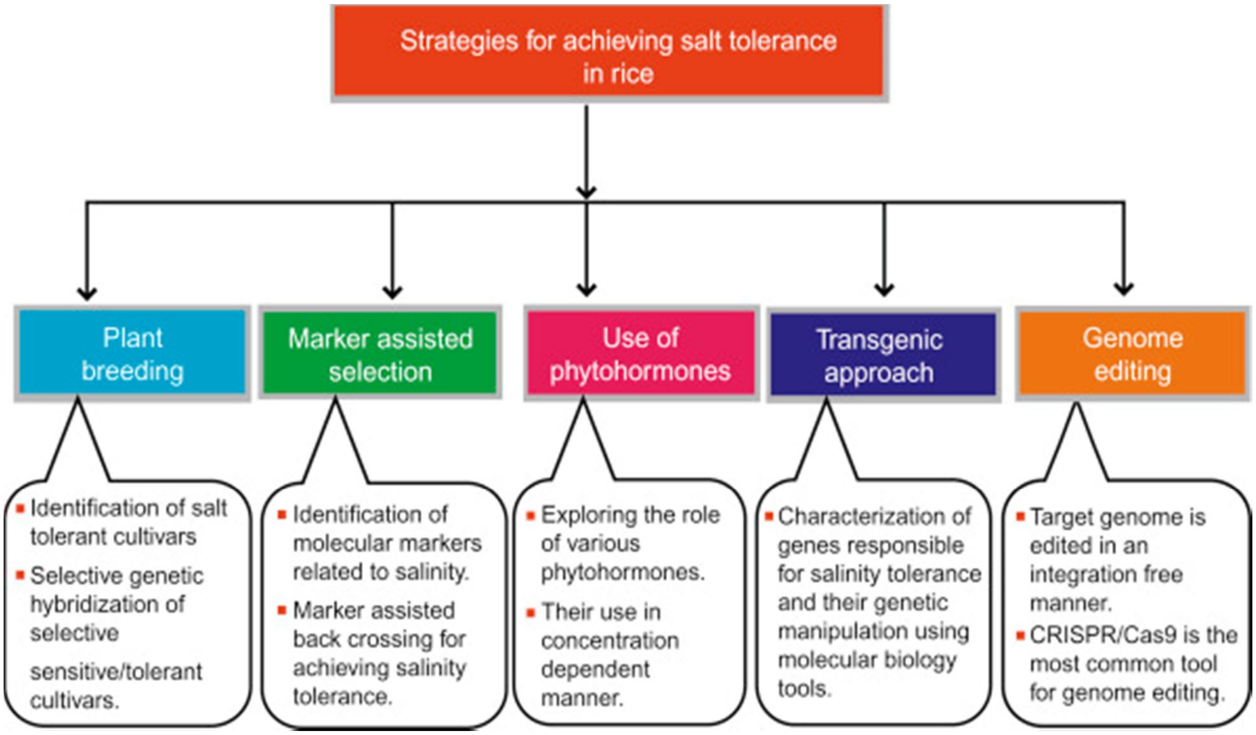

Figure 4 Strategies used to develop salt tolerant variety of rice.

Source: Deciphering Strategies for Salt Stress Tolerance in Rice in the Context of Climate Change, Advances in Rice Research for Abiotic Stress Tolerance. Kaur N, et al. Woodhead Publishing.

IV. ADVANTAGES

The basic advantage of using biotechnology in agriculture is obviously for obtaining improved crops. In this case too, biotechnology makes it possible to obtain and grow rice varieties that are more salt tolerable than the conventional crop which makes it possible to produce an impressive yield even in areas of fairly high salinity in water and soil, hence proving to be a successful step towards matching produce to world population demand. There are, however, certain process specific advantages too.

How MAB is advantageous over conventional breeding in the world of agriculture has already been discussed above. MAS, too, shows similar advantages in the plant breeding approach. Though how genetic engineering has proved to be advantageous can be seen in the points mentioned below:

A. Increased crop productivity [25].
B. Enhanced crop protection to other abiotic stresses along with salinity [25].
C. Improved nutritional value [25].
D. Fresher produce [25].
E. Environmental benefits being a very significant advantage [25].

V. DISADVANTAGES

Although biotechnology doesn't show specific disadvantages when used for salt tolerance improvement in rice, it may show some general disadvantages to the world of agriculture. They may be the fact that if improved variety of crops are available to farmers, they may switch completely to it which in future may lead to the extinction to certain varieties [26].

Also, growing genetically modified may lead to, as scientists suggest, to the creation of new allergens [26].

Furthermore, these processes being expensive introduce unfair competition between farmers [26].

It also affects, indirectly, the insects that feed on the natural variety of the crop [26].

VI. CHALLENGES

Even though biotechnology has made great improvements in the past few years and has reached great heights, there are still some challenges the methods face when applied. Some of them may include:

A. All markers may not be breeder friendly. Although scientists have tried to find a way around it where they convert such markers to other markers that are breeder friendly and it may be useful [19].
B. All of the markers can't be applied across population due to the lack of reliable marker trait association or marker polymorphism [19].
C. The recombination between markers and QTLs may lead to false selections. Although reliable selections may be made using flanking markers [19].
D. The process may be less efficient or slower because of inaccurate estimations of the location and effects of QTLs [19].
E. More accurate estimates may be obtained using fine mappings with high density marker for great populations and well defined phenotyping in multiple environments [19].
F. MAB may not be as successful a technique for more practical and large scale breeding programs [19].
G. High labour costs and start-up expenses [19].
H. Transgenic plants developed too, on field level are a matter of concern since they only overexpress one gene and not the rest which may render the one improved irrelevant [24].
I. Also, since the overexpressed gene consumes resources required for normal cell growth, it may led to growth and development defects [24].

VII. FUTURE SCOPE

Biotechnology although today is sufficient in itself and has shown tremendous results in improving the salinity tolerance of rice, but there always is scope of improvement. Various genes pertaining to salinity tolerance have been identified and tested, however, only a handful of them have been validated functionally in the transgenic rice plants for salt stress tolerance. Since under field conditions crops are exposed to multiple stresses, it is a challenge for scientists to look for genes that can improve multiple tolerances at once. This can be done by either combining multiple genes that take the same protective pathway or by the combination of primary regulatory genes of diverse protective pathways [27]. Also, genome editing is one process that scientists only have touched upon to this date. The method has immense potential and further studies on it are imminent in the near future. The challenges mentioned above are also being closely studied and possible counter ideas are being developed. There is a need for a well- focused approach that will combine physical, molecular and metabolic aspects of the tolerance of abiotic stresses to bridge the gap between the knowledge of short- term and prolonged gene effect and also their products as well as between molecular and cellular expression of genes and entire plant phenotype under stress. Ongoing fine tuning of molecular marker technologies assure the production of marker-assisted selection for significant QTLs or participant genes cheaper and much more effective in coming times [15]. Transgenic technology also will definitely go on to help the search of those cellular mechanisms that underlie tolerance, though complexity of trait is probably to mean that the road to applying such tolerance among sensitive species will be long [15].

VIII. CONCLUSION

It is imminent to point out that although biotechnology has been tremendously useful to the field of agriculture, it still has miles to go when it comes to improving abiotic stress tolerance in plant crop. We have discussed above the methods that have been a breakthrough in the study of plant biology and its enhancement using biotechnological tools, but we have also observed how they still face so many challenges, so many speculations and have so many drawbacks. It can be safely said that when it comes to improving salinity stress tolerance in rice crop, however the use of above mentioned processes is underway, since salinity in rice is controlled by multiple genes, science is to still see the breakthrough where these processes come down from theory to field.

They still require work to be able to be used on large commercial scales in the field, to be available to the farmers worldwide, to have as less drawbacks as possible. It is still to see if technology in true sense can be connected to the biology of rice plants and be developed accordingly. It is still being observed if the speculative wild rice variety going to be the much coveted hope that scientists need to rely on for a brighter future. Simply said, it cannot be denied that, with the increase in demand of rice and simultaneously the increase in the salinity of soil and water, biotechnology has evolved and helped in the increased production and salinity tolerance too, but with the constantly increasing population, that is not enough and hence constant progress is a need to be able to develop a sustainable produce in coming times.

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