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Abiotic Stress Tolerance in Rice (*Oryza sativa* L.): A Genomics Perspective of Salinity Tolerance

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Abstract

Rice (*Oryza sativa* L.) is the main source of staple food for human population. Salinity is the major problem for agricultural production and it affects rice production globally. Different approaches have been developed and exploited to ameliorate the harmful effects of salinity on crop production. Development of salt-tolerant cultivars is the best option which ensures sustainable crop production. Genomics approaches have the potential to accelerate breeding process for the development of salt tolerant crop cultivars. Molecular mapping techniques are the most promising component of genomics. Molecular mapping approaches have greatly helped in the identification of genomic regions involved in salinity tolerance in different crop plants, including rice. Identified genomic regions associated with salinity tolerance accelerated molecular breeding efforts to develop salt-tolerant rice cultivars. Molecular mapping techniques (both linkage and association mapping) are the main components of genomics and these helped in the identification of genomic regions associated with salt-tolerance in rice. In this chapter, a detailed description of molecular mapping techniques, and major findings made by these techniques is presented. Future prospects of these techniques are also discussed.

Keywords: genome-wide association studies, genomics, quantitative trait locus mapping, rice, salinity

1. Introduction

Rice (*Oryza sativa* L.) belongs to family Poaceae and genus *Oryza*. Its genome size is approximately 430 Mb contained in 12 chromosomes. Large part of human population depends on it for staple food. Rice is a salt-susceptible crop. One third of world agricultural land is salt affected [1]. Salinity, both soil and water, has negative effect on rice production [2]. Elevated
Na⁺ levels in agricultural lands are increasingly becoming a serious threat to the world agriculture. Plants suffer osmotic and ionic stress under high salinity due to the salts accumulated at the outside of roots and those accumulated at the inside of the plant cells, respectively.

Projected increase in human population demands a proportional increase in the food supply. This demand of increased food supply can be fulfilled only if we utilize all available land resources to their full potential. An associated phenomenon with the increase in human population is the decrease in world agricultural land area due to its use for human settlements. Due to these constraints, even marginal cultivable lands cannot be neglected. This urges that saline soils should be exploited to their full production potential. For good crop production on saline areas, different practices such as reclamation, agronomic adjustments, and biological amendments are used in combination. Considering sustainable crop production on these areas, the use of salt-tolerant crop cultivars seems to be most suitable option [3–5]. For development of salt-tolerant cultivars, genetic diversity with respect to salt tolerance in crops has to be evaluated. For genetic diversity assessment and identification of genomic regions associated with salt tolerance, molecular mapping approaches have made considerable contribution in different crop plants [6–14]. With the use of molecular mapping approaches, it has become possible to identify the chromosomal regions (quantitative trait loci, QTLs) associated with traits related to salt tolerance in rice. This chapter tries to cover effects of salinity on rice plant’s growth and development, types of molecular mapping approaches, methodology involved in these approaches, and the achievements made through these approaches in salinity tolerance in rice to-date. It also highlights the future prospects of molecular mapping approaches. Thus, it will be a valuable resource for designing future research endeavors to genetically characterize salt tolerance mechanisms and develop salt-tolerant rice cultivars. It will also facilitate molecular breeding efforts for screening rice germplasm for salinity tolerance.

2. Effects of salinity on rice plant growth and development

Salinity affects different morphological, biochemical, and physiological attributes of rice. Salinity has negative effect on percent relative-plant height, total tillers, root dry weight, shoot dry weight, and total dry matter [15]. Biochemical attributes of rice, affected by salinity, include chlorophyll content, proline content, hydrogen peroxide content, peroxidase (POX) activity, anthocyanins, Na⁺ content, K⁺ content, Ca²⁺ content, total cations content [11, 16]. Physiological attributes of rice, which are affected by salinity, include relative growth rate, osmotic potential, transpiration use efficiency, senescence, Na⁺ uptake, K⁺ uptake, Ca²⁺ uptake, total cations uptake, Na⁺/K⁺ uptake, Na⁺ uptake ratio, K⁺ uptake ratio, Ca²⁺ uptake ratio, Na⁺/K⁺ uptake ratio, and total cations uptake ratio [11, 16, 17].

Rice shows different levels of salt tolerance at leaf and whole plant level [18, 19]. Similarly, behavior of rice plants towards salt stress may be different at vegetative and reproductive phases and this may not correlate with their mean level of relative resistance [20]. It is important to know the specific salt susceptible phase of a rice variety to have a better comparison of performance among varieties under salinity stress.
Vegetative and reproductive growth potential of plant depends upon the process of photosynthesis. Increased sodium concentration in the leaf tissue negatively affects net photosynthesis and essential cellular metabolism [18, 21]. Chlorophyll content is important in photosynthesis. Reports suggest that there is no correlation between the chlorophyll content and photosynthesis under salinity stress. Net photosynthesis was reduced by a sodium concentration which did not affect chlorophyll content [18]. It implicates the disturbance by salinity stress of other cellular processes involved in photosynthesis. Sodium accumulation in the leaf also affects stomatal aperture and carbon dioxide fixation simultaneously [18] and thus it may be one of the reasons for reduced photosynthesis due to sodium accumulation. The most salt-susceptible cultivars had lowest K/Na ratio in the leaves and exhibited strongest yield reductions [22]. Rice plant evolved different mechanisms to cope salinity stress conditions. One of these mechanisms is compartmenting salts within the plant body [23].

3. Molecular mapping approaches: types and methodology

Molecular mapping approaches are of two types, linkage mapping and association mapping, on the basis of mapping population used.

3.1. Linkage mapping

In linkage mapping, bi-parental segregating populations are used. These populations include backcross populations, doubled haploid (DH) lines, F2 populations, introgression lines (ILs), near isogenic lines (NILs) and recombinant inbred lines (RILs). JoinMap [24], MapMaker [25] or QTL IciMapping [26] softwares are used for the construction of genetic linkage maps. WinQTLCartographer [27], QTL IciMapping [26], and QTLNetwork [28] programs are used for the identification of QTLs. Detailed information about input file requirements, statistical parameters thresholds, and the procedure to run the software are provided in the user manuals of these softwares.

3.2. Association mapping

Association mapping uses natural populations for mapping purposes. In this technique, commercial crop cultivars can be employed for the assessment of QTLs. First reported in humans, association mapping is now widely used in plant sciences. Assessment of marker-trait associations is facilitated by controlling underlying population structure in the used plant material for mapping purposes [29]. STRUCTURE software is used for identifying sub-populations in the used plant germplasm [30]. TASSEL software is used for the identification of QTLs in this case [31].

3.3. DNA markers used in molecular mapping approaches

In molecular mapping approaches, different types of DNA markers are used to identify QTLs. Amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP), simple sequence repeats (SSRs), sequence tagged sites (STS), simple sequence length
polymorphism (SSLP), and single nucleotide polymorphism (SNP) [12, 32–35] are different types of DNA markers which are employed for genotyping in molecular mapping studies.

4. Achievements made through molecular mapping approaches with respect to salinity tolerance in rice

By using linkage and association mapping approaches, a number of QTLs linked to salinity tolerance in rice, have been identified. Detail of identified QTLs is given below.

4.1. Linkage mapping

Linkage mapping has been very successful in the identification of QTLs linked to salinity tolerance in rice. A number of significant QTLs associated with salinity tolerance in rice were identified through linkage mapping approach (Table 1). In these studies, the mapping populations used were F₂ population, F₃ population, F₂:4 population, near-isogenic lines, recombinant inbred lines, doubled haploid population, backcross-inbred lines, BC₃F₅ lines, BC₂F₈ advanced backcross introgression lines, and reciprocal introgression lines. Marker systems used in these studies included SSR, RFLP, SSLP, SSR AFLP, and SNPs.

Morphological parameters are supposed to be indicators of salt tolerance. There were various reports in which QTLs related to morphological traits under salt stress were identified [12, 32, 34–43]. In these mapping studies, the plant material was phenotyped at the seedling, tillering, or the maturity stage. Data for different morphological traits were recorded in these studies. These traits included seed germination (%), seedling survival days, seedling vigor, seedling root length, shoot length, fresh shoot weight, dry shoot weight, dry root weight, reduction rate of dry weight, reduction rate of fresh weight, reduction rate of leaf area, reduction rate of seedling height, tiller number, salt tolerance rating, score of salt toxicity of leaves, plant height, and grain yield-related traits. A number of significant QTLs were identified in these studies. These identified QTLs included a QTL for seedling survival days [32]; a QTL for root length flanked by restriction fragment length polymorphism (RFLP) markers RG162-RG653 [36]; QTLs with heritability values up to 53.3% [34]; two significant QTLs, qST1 and qST3, for salt tolerance at seedling stage with 35.5–36.9% phenotypic variance explained values, respectively [38]; same QTLs conferring salt tolerance at both seedling and tillering stages [40], SSR marker RM223 associated with salt tolerance in rice [39], and a major QTL for straw yield, qSY-3 [12]. These studies also suggested that it is possible to combine favorable alleles associated with salt tolerance in a single cultivar through marker-assisted selection (MAS) of main effect QTLs (M-QTLs) [42]. Similarly, pleiotropic effects were found for some QTLs which were found associated with both drought and salt tolerance [43].

There are also a number of reports of QTLs identified for different physio-biochemical traits through linkage mapping [11, 33, 44–52]. Traits which were studied in these reports were shoot Na⁺ concentration; shoot K⁺ concentration; leaf Na⁺ concentration; leaf K⁺ concentration; Na⁺ uptake; K⁺ uptake; Na⁺ absorption; K⁺ adsorption; Na⁺/K⁺ absorption ratio; K⁺/Na⁺ ratio;
<table>
<thead>
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<th>Marker system used</th>
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<td>RFLP</td>
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<td>RFLP</td>
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<td>Plant height; panicle length; tillers per hill; spikelets per panicle; grain yield</td>
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<td>Seedling height; dry shoot weight; dry root weight; Na/K ratios in roots</td>
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<td>Plant height; root length; shoot dry weight; shoot fresh weight</td>
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<td>RILs</td>
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<td>Salt tolerance traits</td>
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<td>Salt tolerance traits</td>
<td>F(_2) and F(_3) populations</td>
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<tr>
<td>–</td>
<td>140 RILs</td>
<td>SSR</td>
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<td>Leaf Na(^+) concentration; K(^+)/Na(^+) ratio; K(^+) concentrations; ratio of leaf Na(^+) to sheath Na(^+) concentrations</td>
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<td>RILs, F(_2)</td>
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<td>Physiological traits</td>
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<td>Pollen fertility; Na(^+) concentration and Na/K ratio in the flag leaf</td>
<td>F(_2) population</td>
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<tr>
<td>Sodium (Na(^+)), potassium (K(^+)), and calcium (Ca(^{2+})) accumulation traits</td>
<td>F(_2) population</td>
<td>SSR</td>
<td>[11]</td>
</tr>
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</table>

Table 1. QTLs identified through linkage mapping studies.
ratio of leaf Na\(^+\) to sheath Na\(^+\) concentrations; sodium (Na\(^+\)) and potassium (K\(^+\)) in roots; Na\(^+\) concentration and Na/K ratio in the flag leaf; and sodium (Na\(^+\)), potassium (K\(^+\)), and calcium (Ca\(^{++}\)) accumulation traits. Major discoveries in these studies included a major QTL (QKr1.2) identified for K\(^+\) content in the root on chromosome 1 explaining 30% of the total variation [48]; pollen fertility, Na\(^+\) concentration and Na/K ratio in the flag leaf were found as the most important attributes for salt tolerance at the reproductive stage in rice [52], QTLs for sodium and potassium uptake were identified on different linkage groups (chromosomes) [33] suggesting that different pathways are involved in Na\(^+\) and K\(^+\) uptake; and a major locus controlling Na\(^+\) uptake (QTLsur-7) was identified on chromosome 7, with \(R^2\) value of 72.57% [11].

### 4.2. Association mapping

In recent years, association mapping is widely used to identify QTLs in plants. Association mapping approach is relatively new arrival in plant genetics. There are some reports of association mapping for salt tolerance in rice [13, 53–58]. Main findings of these association studies are presented in Table 2. In these studies, rice mapping populations used consisted of European Rice Core collection (ERCC) containing 180 japonica accessions [53], 96 rice germplasm accessions including Nona Bokra [55], 220 rice accessions [56], 341 japonica rice accessions [57], 94 rice genotypes [58], and 24 indica rice genotypes [13]. Traits for which data were recorded in these studies included Na\(^+\)/K\(^+\) ratio, survival days of seedlings, shoot K\(^+\)/Na\(^+\) ratio, Na\(^+\) uptake, Ca\(^{++}\) uptake, total cations uptake, Ca\(^{++}\) uptake ratio, K\(^+\) uptake ratio, Na\(^+\)/K\(^+\) uptake and salinity tolerance scoring. Major findings made in these studies included an observation that distribution of favorable alleles associated with salt tolerance was random in ERCC [53]; 40 new allelic variants found in coding sequences of five salt-related genes [54]; STS marker, RM22418, for SKC1, on Chr. 8 was found associated with salinity tolerance [55]; region containing Saltol was found associated with Na\(^+\)/K\(^+\) ratio [56]; marker RM3412 was found associated to salinity tolerance at seedling stage due to its close linkage to SKC gene [58]; and the report that other QTLs, in addition to Saltol, might be involved in salinity tolerance [58]. These reports highlighted that in rice germplasm there might be other genomic regions involved in salt tolerance. These genomic regions need to be characterized in future to add a wealth

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<tbody>
<tr>
<td>Salinity tolerance</td>
<td>180 japonica accessions</td>
<td>SNPs, SSR</td>
<td>[53]</td>
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<tr>
<td>Na(^+)/K(^+) ratio equilibrium; signaling cascade; stress protection</td>
<td>392 rice accessions</td>
<td>SNPs</td>
<td>[54]</td>
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<tr>
<td>Salinity tolerance</td>
<td>96 germplasm accessions</td>
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<tr>
<td>Survival days of seedlings and shoot K(^+)/Na(^+) ratio</td>
<td>341 japonica rice accessions</td>
<td>SSR</td>
<td>[57]</td>
</tr>
<tr>
<td>Seedling stage salt tolerance</td>
<td>94 rice genotypes</td>
<td>SSR</td>
<td>[58]</td>
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Table 2. QTLs identified through association mapping studies.
of information in the present rice genetics knowledge pool. Random distribution in the rice germplasm of favorable alleles associated with salt tolerance is a worthwhile finding which should be considered while exploring and selecting crossing parents in breeding programmes.

5. Future prospects and conclusions

Climate change has affected world agriculture a lot. The most pronounced effects of climate change are the heat stress and periodic drought conditions in major rice producing countries of the world. Due to periodic drought conditions, the already existing problem of high amounts of salts in the upper surface soil has intensified. So, there is a dire need to opt for a coordinated approach to address the problem of salinity stress for rice production. Genomics has great potential to assist in this coordinated programme. With the help of molecular mapping approaches, a number of major and minor QTLs associated with salinity tolerance in rice have been identified in recent years and there are further accelerated research efforts underway in this direction. The identified QTLs are valuable resources for marker-assisted selection (MAS) to develop elite salt tolerant rice cultivars. Great task is needed to be done in this regard so that marker-assisted breeding (MAB) approach can be implemented successfully in routine breeding programmes. In future, efforts should be directed to develop climate-smart rice cultivars which can perform stably under diverse environmental conditions. Identified QTLs and rice germplasm found tolerant to salinity stress can be exploited in three major ways: (a) to understand the molecular genetics of salt tolerance in rice; (b) salinity stress tolerant rice germplasm might be incorporated into salt-tolerant rice cultivars development molecular breeding programmes; (c) identified QTLs incorporated into MAS for screening rice germplasm against salinity stress. New genes involved in salt tolerance will be identified by this approach. Genome sequence of rice, both indica and japonica subspecies, is available now. In the next phase of annotation of the rice genome, molecular mapping results can be of help in combination with the comparative genomics approach.

Lot of work related to molecular mapping for salinity tolerance in rice is to be performed yet. The main cautious point is the plant phenotyping for salt stress tolerance. Accuracy in the phenotyping work is the key in the authentic identification of QTLs related to salt tolerance. Hydroponics should be tried for this purpose. Under salinity stress conditions, phenotyping at germination, seedling, tillering, and reproductive phases require different strategies and care. In case of quantitative traits, such as salinity stress tolerance, there is pronounced effect of environment. Efforts should be made to design a judicious phenotyping plan which can minimize effect of environment. In case of plant genotyping work, robust marker systems with high resolution power such as SNPs should be preferred over other marker systems. Genotyping-by-sequencing (GBS) is another option.

Previous research efforts have pointed out that the distribution of favorable alleles, associated with salt-tolerance, is random among the rice germplasm [53]. So, it is possible to pyramid favorable alleles of salt-tolerance in an elite rice genotype through well-planned crossing programme. This elite rice cultivar will have great potential with regard to salt tolerance. In view of inland intrusion of the seas, we have to concentrate on the coastal areas to fully exploit
their agricultural production potential. This is also imperative in view of alarming increase in human population and to feed this population we have to exploit every available land for agricultural production. It is hoped that genomics approaches will play a greater part in this exploitation of land by providing salt tolerant crop cultivars.

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