Chapter

Understanding the Responses, Mechanism and Development of Salinity Stress Tolerant Cultivars in Rice

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Abstract

Rice is the most important staple food crop of much of the world's population. Production and consumption of rice is higher in Asia but adverse environmental conditions critically threaten the rice production. Soil salinity has been a key abiotic constraint affecting the crop production by reducing growth, development and yield of the plant. Rice is highly sensitive to salinity specifically at the early vegetative and late reproductive stages. Therefore, studying the responses of crop at the morphological, physiological, biochemical and molecular level is an effective strategy. Understanding the mechanisms behind the salinity such as osmotic stress and osmolytes, ion exclusion, inclusion and compartmentation, antioxidant response and hormonal regulation. Different screening strategies such as phenotypic and genotypic screening for rice under salinity and select the salt tolerant lines. Using the conventional and molecular breeding approaches is a prerequisite for its effective management and to develop salt tolerant cultivars in rice.

Keywords: rice, salinity, responses, mechanisms, screening, breeding methods

1. Introduction

Rice (*Oryza Sativa)* is a diploid (2n = 12) belongs to the Poaceae family (Umed [1]). It is an important cereal food crop cultivated and consumed across the world population and most of the Asiatic peoples. Rice is a staple food crop and gives 50 to 80% calories to the more than three billion peoples (Umed [1]). The global rice production needs to be increased from 560 million tonnes to 850 million tonnes by 2025 to meet the growing demand of rice [2]. Rice cultivation is continuously threatened by several biotic and abiotic stresses [3]. Salinity is the major soil problem in rice cultivation especially saline prone coastal areas and it is a second most abiotic problem than the drought [4]. Globally, 800 million ha area affected by salt accumulation, which is about 12% of the world land and 20% of the cultivated land [5]. The 1500 million ha of dry land farming and 230 million ha of irrigated farming comes under saline condition and 33% of agricultural land affected with high salinity [6]. In earth surface, the enormous amount of water is available but all the water is not useful for to survival of plants and animals, because 72% of water available as a sea water [7].

Rice is highly is sensitive to salinity at early vegetative and late vegetative stages [8, 9]. Increasing salinity (22 dS m^{-1}) affects the seed germination mainly due to the osmatic stress [10, 11]. It reduces the photosynthetic activity, chlorophyll content, leaf area and stomata where high level of salinity occurs [12]. Yield components such as panicle length, spikelet number per panicle and grain yield were also affected; and panicle emergence and flowering affect the seed set through pollen viability [13].

The present review to show the constrains of the salinity to the rice growth and development and provide the information about the plants responses against the salinity and what are the different approaches are used to develop the salt tolerance rice cultivar.

2. Salinity -abiotic constraint devastating agricultural production

Among abiotic stresses, the salinity which adversely affects the crop growth, development and production [14]. The deposition of salts or salinization is a natural phenomenon with evaporation of saline underground water, sea water infiltration of coastal ground waters, sea water salts in wind and rain and human initiated process such as irrigation with marginal water and poor agronomic practices. Salinity affected almost the 900 M ha of land [15]. In total global cultivated land, 23% contain saline and 37% contain sodic soils and 2.5×10^8 ha of global irrigated lands affected by salinity and water logging. The salinity mostly occurs in the south Asia and Southeast Asia which contributes the 90% of the world's rice production [16, 17]. The abundant accumulation of dissolved salts in soil and water which affect the plant growth that leads to decrease the agricultural production. In ocean water contain 480 mM of Na^+ and 560 mM of Cl^-. If saline water contains the above the optimal level of sodium and chlorine for plant growth and development, they require the techniques to maintain the quality of irrigation water [18]. In some of the plants show the wide range salinity tolerance especially against the Na and Cl salinity. Based on the salinity tolerance and sensitivity, the plants are classified into two types such as glycophytes and halophytes. Glycophytes means which plant grown in low tolerance condition. Halophytes means which plant grown in higher salinity condition. Mostly all plants coming under the glycophytes category.

Cereal crops show wide range of response to salinity, for example barley show most tolerance and rice show the most sensitive to salinity [5]. Lutts et al. [19] reported that seedlings of rice are highly sensitive to toxicity of salts. According to Shannon [8] early vegetative and later vegetative stages of rice are highly sensitive to salinity. Khatun and Flowers [20] reported that the salt toxicity affected the panicle length, spikelets per panicle and 1000-grain weight; decreased photosynthesis were observed by Munns and Tester [5] which results in unfilled spikelets in rice. Delay in flowering and ripening, reduced number of tillers and biomass and leaf area also occur due to the salinity [21].

In saline soil contains numerous soluble salts such as Ca^{2+}, Mg^{2+}, Na^+ and anions SO_4^{2-}, Cl^-, HCO_3^-, K^+, CO_3^{2-} and NO_3^- and these soil contain the EC is 4 dS/m or more [22]. Based on the electrical conductivity influence in soil salinity and crop responses are mentioned in **Table 1**.

In arid and semi-arid area where low precipitation and high evaporation leads to accumulation of more salts [24] and accumulation of salts in sea shores is a natural phenomenon of sea water flooding. Worldwide fertility status of rice growing areas were mentioned in **Table 2.**

The quality of irrigation water affected by accumulation of salts. If abundance of salt accumulation in the root zone which alter the water relations in the plant. Due

Electrical conductivity (Ds m^{-1} at 25° C)	**Crop response**
< 2	Salinity effect is practically zero
2 – 4	Reduction in yield of very sensitive crops
4 – 8	Reduction in yield of most crops
8 – 16	Only tolerant crops produce satisfactory yield
>16	Few highly tolerant crops produce satisfactory

Table 1.
Crop response to salinity influenced by electrical conductivity of saturated soil extract [23].

Region	**Percentage of problem soils (%)**	**Percentage of very poor soils (%)**	**Percentage of poor soils (%)**	**Percentage of Good soils (%)**	**Total rice area (ha)**
Western Europe	1.3	46.6	8.4	43.8	25
Southeastern Asia	8.3	43.7	18.6	29.4	49,120
Eastern Asia	1.5	37.6	9.8	51.1	33,425
Western Asia	15.0	17.1	9.4	58.5	156
Asia	5.3	29.6	18.3	46.7	143,429
Southern Europe	1.4	52.8	13.1	32.8	431
Eastern Africa	4.7	62.3	13.1	19.9	3,330
Northern Africa	16.3	5.8	50.4	27.4	558
Southern Asia	4.7	13.9	22.8	58.4	60,526
Central Africa	0.4	76.0	8.6	15.1	736
Africa	3.1	60.5	18.0	18.2	10,466
South America	5.4	61.8	12.7	20.2	5,121
Melanesia	0.9	28.8	13.4	56.9	5
Northern America	7.2	16.9	28.9	47.0	1,259
Central America	1.6	23.5	42.6	32.2	330
Caribbean	7.2	22.7	12.3	57.7	437
Americas	5.6	49.7	16.9	27.8	7,147
Central Asia	42.4	4.8	13.7	39.1	202
Eastern Europe	4.3	10.8	16.9	67.9	248
Western Africa	1.3	62.8	19.0	16.8	5,843
Europe	2.4	38.1	14.1	45.4	704
Australia and New Zealand	24.8	4.6	33.1	37.5	34
Oceania	21.9	7.5	30.7	39.9	39
World Total	5.1	32.5	18.2	44.0	161,784

Table 2.
Worldwide fertility status of Rice-growing areas [25].

to the salt irrigation water induce the some of the effects in plants such as necrosis, chlorosis and interfere the plant physiological activities with depends on the some of the environmental factors such as temperature, humidity, light intensity and soil conditions [26].

2.1 Effects of salt stress in rice

The millions hectares of lands continuously salinized and also affect the crop production. It leads to contributing to the future biological catastrophe [27]. Rice is a salt sensitive crop [28]. In rice if increase in salt stress (in terms of 5 to 7.5 dS m–1), the seedling growth and fresh weight decreased [29]. The salt stress decrease the seedling biomass production in rice [10, 11]. The increased salt stress significantly reduce the mean root length, mean root numbers per plant, and shoot length [30]. In rice early stage, leaf mortality increased with increased salt stress [31] and also observed the reduction in growth and development in later stages [32]. Salt stress cause the effects in plant cell metabolism and leaf senescence and old leaves were death which indicate the crucial for the survival of a plant [33]. Due to salt stress panicle sterility observed in pollination and fertilization stage which leads to a decline in grain setting [34]. The lack of transformation of carbohydrate to growth of spikelet which leads to decrease in grain yield due to the salt stress (Sajid [35]). The salt stress contain negative linear raltionship with important factors such as number of tillers per plant, number of spikelets per panicle, and percent of sterile florets [10, 11].

In rice, the salt stress show the physiological effects such as decrease in photosynthetically active radiation (PAR), net photosynthesis (*P*n), stomatal conductance (*G*s), transpiration rate (*T*r), degradation of pigment, and relative water content (RWC) [36]. The chlorophyll and carotenoids contents in rice leaves were decreased after the salt stress [37]. The increased salt stress affect the Na+, Ca2+, K+, and Mg2+ concentration in root and shoot in rice plant [38]. The availability of zinc were decreased and increased cadmium (Cd) toxicity observed where high level of NaCl occur [39].

3. Mechanism and their responses to the salt tolerance in rice

The potential of irrigation water and soil were affected by the accumulation of salts which interfere the uptake of water into plant from soil and presence of higher concentration of Na in soil solution will affected the uptake of some essential nutrients such as K and Ca in root zone [40]. If concentration of Na and Cl reach threshold level, some specific toxicities will alter the water relation [5]. The plants develop the some of the mechanism to grown in salinity condition. The mechanism are such as osmotic stress and osmolytes, ion exclusion, inclusion and compartmentation, antioxidant response and hormonal regulation.

3.1 Mechanism of salt tolerance: osmotic stress and osmolytes

When plants are grown under salinity condition, the plants adjust the losing of water and their potential and it will lead to decrease in osmotic potential, turgor and express the signal that trigger the adaptive responses [41]. The recovery period, osmotic potential and hydraulic conductivity of the membranes is reduced. By the accumulation of the organic and inorganic solutes and the plant turgidity is recovered after the tissue growth occur [5]. In osmotic adjustment, the cell wall elasticity was changed with decrease in RWC (Relative Water Content) and increase water content in the apoplast which decrease the salinity consequences by maintaining turgidity of the tissue. The organic osmolytes used to maintaining the osmotic potential in plants and prevent the salinity effects [42]. When salinity stress occurs, the osmolytes will synthesized and osmolyte biosynthesis and accumulation is important for the salinity tolerance. But the osmolyte biosynthesis may vary based on the plant age and rate of stress occur [43]. The most abundant and compatible osmolytes such as proline and glycine-betaine coming under the organic osmolytes [26]. Proline synthesis is enhanced under salinity [44]. Proline act as a reactive oxygen scavenger, redox buffer and molecular chaperone and stabilizes membranes and proteins under stress conditions [45]. Glycine-betaine involve in protection of enzymes and membrane structures [46].

3.1.1 Ion exclusion, inclusion and compartmentation

Ion exclusion from salt sensitive organs, inclusion in less sensitive locals such as the root and old leaves and organ compartmentation strategies used for decreasing the damaging ion-specific effects of Na and nutrient deficiencies. If the cytoplasm contain excess ions, it will transported across the tonoplast by the Na/H antiporter and compartmentalized in vacuoles which is useful for protect the plant from salinity [47]. Through the exclusion strategies the salts are removed from aerial part by saline vesicle glands in the epidermis which prevent accumulation and transported to roots which is mostly occur in halophytes and glycophytes. Salt sequestering in old leaves which will give salt-induced senescence [48]. When compared to synthesis of organic osmolytes, the ion compartmentation is low cost effective [49].

3.1.2 Antioxidant responses

The oxidative stress occur when plants under the salinity [50]. Under salinity stress, to induce increase the reactive oxygen species such as superoxide radicals and hydrogen peroxide [51]. The antioxidative system is response to the salinity exposure [50]. The chloroplasts and mitochondria are play role in the salt tolerance with the increased antioxidant defenses. The salt sensitive plants express in decreased antioxidant level [52]. Nitric oxide (NO) response to salinity tolerance with the NO donor [53].

3.1.3 Hormonal regulation

The plant hormones such as ABA (Abscisic Acid) and cytokines are increased when plant under the salt stress [54]. The negative effects of salinity such as plant growth, photosynthesis and translocation of assimilates can be reduced by the highly accumulation of hormones. The ABA also involved in compatible solutes and nutritional cations K^+ and Ca^{2+} in vacuoles of roots for salt tolerance [55].

3.2 Response of rice under salt stress

Based on the salinity responses, the plants were classified into halophytes and glycophytes. The halophytes plants tolerate to high concentration of NaCl (400 mM) when compared to glycophytes [56]. In rice, the salinity tolerance is controlled by multiple gene [57] and therefore understanding the plant responses for salinity is very much important for developing tolerant cultivars. Under salt stress condition, the rice plants exposure to different responses such as morphological, biochemical, physiological, molecular response.

3.2.1 Morphological response

Due to salinity stress, the occurrence of morphological changes such as stunted plant growth, leaf burning, chlorosis, low tillering, leaf rolling and poor root growth [58]. Decreased leaf area and changes in leaf anatomy under *invitro* condition was observed by Bahaji et al. [59]. For example, comparable levels of osmolality, the reduction in root and leaf growth were similar for both saline and osmotically-generated stress. Most of the variations in leaf anatomy features caused by the treatments could be ascribed to osmotic stress [59]. The beginning of salt stress, there is no symptoms are observed but shoot and root growth reduction occur. When plants continues exposure to salt condition, leaf senescence occur. After 3-4 days of exposure under salinity, the plants began to develop leaf symptoms such as yellowing and necrotic lesions of old leaf tips. The senescence of older leaves was observed after two weeks of stress. In Nipponbare, root and shoot growth was affected by salinity [60].

3.2.2 Biochemical response

Based on the biochemical response, the effect of salinity in plants which leads into two parts such as initial osmotic effect and later ionic stress (where accumulation salt at toxic level) [5]. The plants express some of the biochemical responses such as oxidative stress, altered metabolism, high Na^+ transport to shoot, lower K^+ uptake and low P and Zn uptake [61]. In initial osmotic effect, water potential is decreased and increased the osmotic potential due to the increased concentration of salts. The salt stressed plants contains the larger amount of proline in higher plants [62] and it act as a osmotic adjustment, shielding the enzymes, membranes and give the energy and nitrogen during salinity [63]. Soluble sugars and starch also responses to salinity as an osmoticum in plants [64]. When rice plants exposure to salinity, sugar content increased in shoot [65] and starch content increased in root, which act as a reservoir for the primary metabolism [66]. Where plants exposure to the salinity, the proteins are synthesized and accumulate as a storage food which is used as a reservoir during salt stress condition and reutilized when absence of stress [67]. The increased protein content is positively correlated to rice seedling tolerance than the sensitive one [68].

3.2.3 Physiological response

When plants under salinity, they express some of the physiological responses such as inhibition of photosynthesis, stomatal closure, decreased water content, higher amount of osmolytes and low osmotic potential. The response of rice to salinity, to study the physiological mechanism and it was associated with the plant defense mechanism activated during stress. During salinity, chloroplast and mitochondria are mostly affected compared to other organs [69]. In chloroplast, some of the potential indicators show the effects in the photosynthesis efficiency such as changes in chlorophyll fluorescence and membrane permeability [70]. Salinity affects the mesophyll tissue which leads to affect the vascular bundles. The more accumulation of sodium salts is excited by salt exclusion [71], selective ion uptake [72] and regulation of K+/ Na + ratio [73]. Estimating the different plant parameters such as tiller number, leaf area, panicle length, root length, biomass, dry weight, RGR (Relative Growth Rate) and RWC (Relative Water Content) Zeng et al. [89] from different cultivars, leaf RWC is increased in paddy under salinity and suggested the role of osmo-protectants in preventing cell injury from salt stress-induced dehydration [74].

3.2.4 Molecular response

In molecular response, the main aim is to breed and to develop the salinity tolerance lines. Genetic diversity is a primary work which is used to screen the lines with the various molecular markers such as RFLP, SSLP, RAPD and SSR markers. Salinity is controlled by several genes and inheritance of salinity trait is difficult in rice. These difficulties are overcome by using the positional cloning [75] and insertional mutagenesis [76]. Many genes are identified for the salinity [77]. In rice under stress condition some of the genes were identified such as catalase and several denovo genes. The salinity tolerance controlled by major Quantitative Trait Loci (QTL) is *Saltol* which is mapped in the chromosome 1 of the FL478 Recombinant inbred Line (RIL) line. The *saltol* linked with the flanking markers RM1287 and RM6711 and these QTL region contain 15 SSR markers. The FL478 obtained from crossing between Pokkali and IR29. The *saltol* QTL responsible for the maintain the low Na+, high K+ and Na+/K+ homeostasis in shoots of rice. The *saltol* QTL can transfer into superior cultivar and these transformation confirmed by the candidate gene approaches or Marker Assisted Selection (MAS) [78]. Among the molecular marker analysis, the SSR marker is effective for salt tolerance identification in rice [79]. Several QTLs were identified for sodium uptake, potassium uptake, and sodium: potassium selectivity [80]. The molecular markers are used to identified the QTLs and it gives new platform for salinity study.

4. Screening and development of salt tolerant cultivars

The rice germplasm with diverse and significant varietal differences shows a pool for developing screening techniques. This is important technique for to select varieties for breeding purposes. This techniques are economically feasible, easy and efficient. Rice expresses highly sensitive in early seedling stage and low sensitive in reproductive or late vegetative stage [19]. The continues accumulation of NaCl in older leaves through long exposure [81] which affect the efficiency of photosynthesis and whole plant metabolism [82]. Tolerance in vegetative and reproductive stage of the rice was expressed in seedling stage [83]. So, precise screening method is necessary to identify the salt tolerance line. Seedling stage screening is acceptable and it is rapid screening but it is difficult in vegetative and reproductive stage [4]. From salinity screening, highly tolerant line was identified such as pokkali which is used as a donor for further breeding program of salt tolerance [84].

4.1 Phenotypic and genotypic screening

Salinity tolerance is made as complex in physiologically and genetically [85]. It requires more time and expensive tissue analysis. In early seedling stage of phenotypic screening done in first, second and growing leaves. The phenotypic screening in early seedling to select first, then second and growing leaves. Where salinity occur leaf elongation and new leaf formation is suppressed [86]. In phenotypic screening, for identification of potential rice lines, first step is to screen the already available germplasms of rice. Field level phenotypic screening is difficult because it contain soil heterogeneity, climatic factors and other environmental factors which affect the physiological functions. But, phenotypic screening under laboratory or green house (hydroponics) is better merits than the field screening because it was not affected by the environment and soil factors such as temperature, relative humidity, and solar radiation. In hydroponic phenotypic screening, the seedlings are grown in salinized nutrient solution and where salinity inversely proportional to photosynthesis and chlorophyll content [87]. The vegetative and reproductive stage screening is difficult because decrease in plant height, root length and biomass. In reproductive stage, sterility of florets occurs due to the effect of reduced panicle length, number of primary branches and spikelets per panicle, fertility and panicle weight thus reducing grain yield [88]. It is a rapid, perfect and easy method. Phenotypic screening for salt tolerance is not easy because of environmental effects which hinders the development of accurate and reliable screening technique. Salt tolerance screening at early stage is not correlate with further stages [89]. The germplasm are evaluated by using the high-throughput phenotyping saves time and resources compared to traditional phenotyping methods [90].

Among molecular research tools, quantitative traits loci are useful to study the genotypic of salinity tolerance. These QTL techniques useful in the marker-assisted selection [85]. Saltol is a major QTL which is accounted more than 70% of the variation in salt uptake and these QTLs are incorporated into high yielding varieties by Marker Assisted Backcrossing [91]. While transfer of salt QTL, some of the unwanted traits of linkage drag transferred which is drawback in MABB and genotypic screening [92]. In genotypic screening, the use of molecular markers such as RFLP (Restriction Fragment Length Polymorphism), RAPD (Random Amplified Polymorphic DNA), ISSR (Inter Simple Sequence Repeats), SSR (Simple Sequence Repeats) and AFLP (Amplified Fragment Length Polymorphism) for screening of germplasm is more reliable than the phenotypic screening [93]. Among the above molecular markers, microsatellite markers to be more effective [79].

4.2 Approaches for the development of salt tolerant cultivars

The salt tolerant cultivars are developed by using several ways such as, use interspecific hybridization to raise the tolerance of current crops, use the variation already present in existing crops, generate variation within existing crops by using recurrent selection, mutagenesis or tissue culture and breed for yield rather than tolerance [85]. To improve salinity in rice genotypes by the incorporation of salt tolerance genes into high yielding cultivars.

4.2.1 Conventional breeding

In conventional Plant breeding methods, different approaches are used for development of salt tolerance cultivar such as introduction, selection, hybridization, mutation and shuttle breeding approach. The beginning the salt tolerant rice cultivars (Damodar (CSR 1), Dasal (CSR 2), and Getu (CSR 3)) are developed from pure line selection from the local traditional cultivars. In segregating population, Selection pressure is gradually increased with the generation advancement simultaneously in moderate stress and high stress of sodicity and salinity [94].

In conventional plant breeding, the new and better variety was developed by the combining the different parents genes. In this breeding method, first to generate a breeding population with highly variable and identifying parents for traits of interest. Several conventional breeding programmes are involved in to developing salt tolerance lines such as in vitro selection, pooling physiological traits, interspecific hybridization, using halophytes as alternative crops [85]. Through conventional plant breeding method, some varieties such as CSR10, CSR13, CSR27, Narendra usar 2 and Narendra usar 3 were developed and released as salt tolerant for cultivation [95]. In development of salt tolerance cultivar through conventional breeding is require more time and it depends on the environmental factors. It contains very limited success, due to the complexity of the trait.

4.2.2 Genomics based approaches

In Marker Assisted Selection (MAS), some important strategies are followed such as markers are tightly linked to loci and specific marker alleles are associated with desired alleles at target loci consistently across the different breeding populations [94].

The conventional back cross breeding method drawbacks are overcome by usage of Marker Assisted Backcrossing Method (MABB). Young and Tanksley [96], demonstrated that large amount of DNA from the donor can remain around the target gene even after many generation of backcrossing. In this method, through three important approaches used to reduce the linkage drag such as foreground selection, recombinant selection and background selection.

By using the genomic approaches, the genetic map and genetic diversity in germplasm which is done by usage of the molecular markers [97]. The most efficient markers to screen the salt tolerant genotypes were SSR (Simple Sequence Repeats) markers RM8094, RM336 and RM8046 which were contain higher polymorphic information content coupled with higher marker index value [98]. Two EST markers such as CP3970 and CP6224 and Five SSR markers such as RM1287, RM8094, RM3412, RM493 and RM140 were linked to saltol QTL on chromosome 1 [99]. In salt tolerance rice, transgenic approaches are attempted. Hoshida et al. [100] have reported the transgenic salt tolerance rice by over expresses chloroplast glutamine synthetase gene. These transgenic plants shoots contain more accumulated K^+, Ca^{2+}, Mg^{2+} and less Na^+ compared with those of non-transformed controls [101, 102]. Xujun Chen and Zejian Guo [103] recorded that tobacco OPBP1 increase the salt tolerance and disease resistance in transgenic rice.

5. Conclusion

Salinity is the one of threaten to the rice cultivation, here, the development of salt tolerance cultivar is difficult because it control by many genes and associated with environmental factors. The screening of germplasm used to select the better lines

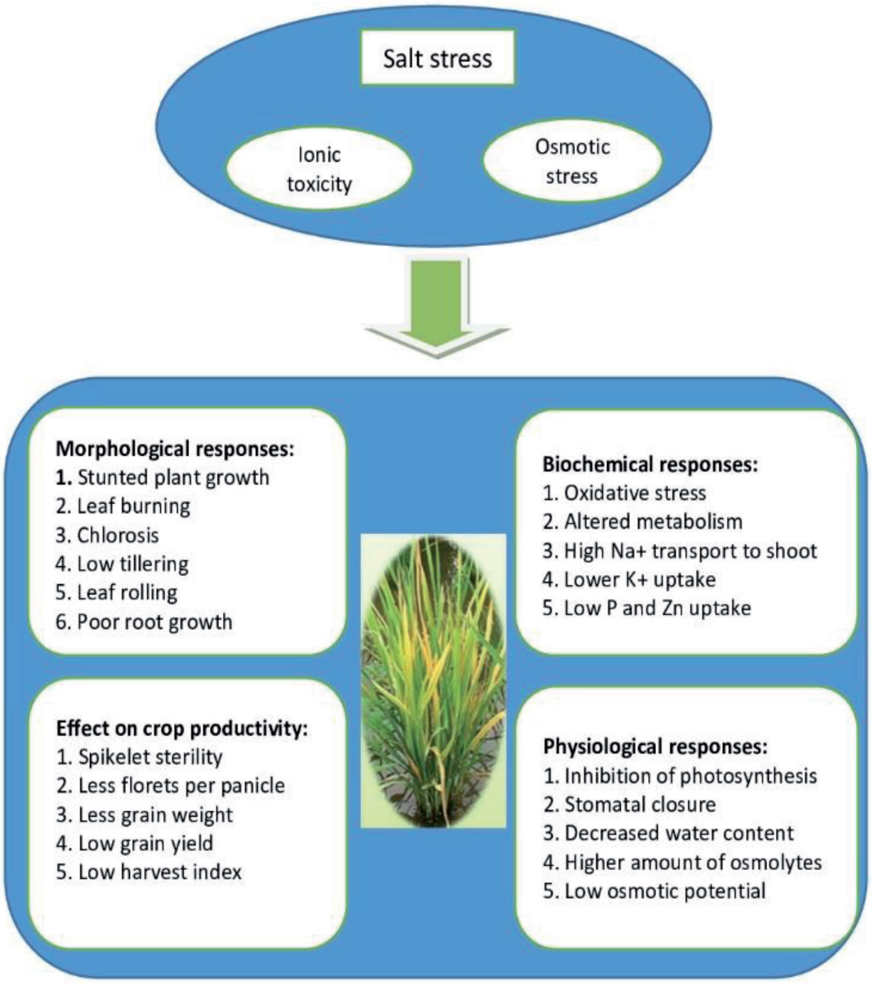

Figure 1.
Plants response to salinity.

which is used for the developing salt tolerance lines. This is done through conventional and genomic approaches. In conventional method it contain limited level of success due to the time consumption and cost. So, the genomic and molecular approaches used to develop the salt tolerant lines with cost effective and in low time period (**Figure 1**).

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