Review Article

A REVIEW ON UNDERSTANDING THE EFFECTS AND MECHANISMS OF SALINITY TOLERANCE IN RICE (*Oryza Sativa*L.)

Abstract

Salinity, along with drought, is one of the key abiotic stressors that has posed a danger to the advancement and evolution of cereal crops like rice and wheat. Water shortage and a lack of irrigation water availability are the main causes of salty soil formation. Rice is salt sensitive and glycophyte, wheat is moderately salt tolerant. Wild tolerant cultivars like *Oryzacoarctata* and*Oryzaalta* are more tolerant than traditional cultivars such as Pokkali and Nona Bokra in rice.Salt stress affects crop plants process like ionic imbalance, osmotic and oxidative stress.Na+ should be low in the shoots of plant which is restricted by various transporters in the cell membrane of the roots in soil. High K+ & Na+/K+ homeostasis should be maintained. Many RILs and NILs have been developed which acts as a donor for salinity tolerant genes. FL478 is a recombinant inbred line in which candidate genes are situated in the *Saltol* region of chromosome 1 region which is obtained by cross between Pokkali x IR29.Increase in world's population, rice output must be increased by at least 25% by 2030 and 50% by 2050.Salinity stress is a polygenic character which involves several genes works in harmony. For evolution of salinity tolerant cultivars, we need to access the physiological, biochemical genetic responses of the crop plant which helps in transfer of candidate genes from donor parents to elite high yielding salt sensitive cultivars. Especially in rice salt tolerant mechanisms like,Ion equilibrium regulation, Adjustment of osmotic potential, Reduction of ROS, Nutrient disequilibrium, and Regulation of PGRs.Conventional, MABC, MAS and direct gene transfer by transgenic methods. This review paper's main objective is to understand the mechanisms of the crop plants to salinity effects and development of salt tolerant cultivars by modern approaches which fulfil the food scarcity of staple food crops with increasing population.

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Keywords:*Saltol*QTL,Salinity, anti-transporters, Salt tolerance,PUFs, and Profiling.

1.Introduction

Salinity affects crop plants by various process like mainly development of osmotic stress, ionic toxicity and next to it oxidative stress and indirectly affects photosynthetic process by pigment damaging (Munns&Tester, 2008). Mainly in cereals rice and wheat are the salt sensitive crops which are affected easily because these are the essential & staple food crops for more than 80% of the world's people. To produce high-yielding, salt-tolerant cultivars we need to understand various mechanisms and physiological, biochemical epigeneticresponse of the plants (Hasanuzzaman, M. et al.,2022). Electrical conductivity (Ec)is used to estimate salinity. (Munns and Tester, 2008). In Rice, salinity mainly affects at germination, seedling stage and reproductive stage, in which if Ec is 3 dS/m yield start reducing with a 12% decrease in output per unit as Ec rises (Chinnusamy, et al., 2005; Reddy et al., 2014). Plants have three primary processes. a) ionic removal, b) osmoticresiliance, and c) cellular resilience to salinity stress (Munns and Tester2008). Salinity stress in rice mainlybuild upon Na+ concentration and transporter’s like OsHKT1;5 is a main determinant factor for salinity resiliance. The genetic variability present within crop species of cultivated cereals like rice for saltresiliance is less or narrow, we use donors like land races Pokkali, FL478 and Nona Bokra which also has *Saltol* gene in the respected QTL region mainly in salinity endurance of seedlings. (Bonilla et al.,2002). Progress of varieties which have seedling stage salinity tolerance leads to growth of leaves, stems and rootsthat can increase yield(Hoang, T. M. L. et al.,2016). In India 7mhaland ispretentious by salt(Pradhan, et al.,2013).Due to the frequent use of brackish water from groundwater for irrigation, the inland is salinized(Dolo, J. S. et al.,2012).FL478is a recombinant inbred line which is salt tolerant used as a donor and it is evaluated from cross between IR29x Pokkali (Islam, M. R et al.,2012).Incross Nona Bokra/Koshihikaria transporter called OsHKT1;5 that regulates K+ regulation (Ren, Z. H.et al., 2005). Early maturation, semi-dwarf, and exceptional culinary quality *Saltol* QTL from DP of FL478 was transmitted through Marker Assisted Backcross Breeding (MABB) into recurrent parent Pusa Basmati 1509 (Yadav, A. K. et al., 2020). Until now only two high producing Basmati rice types, Pusa Basmati 1(Singh,V. K. et al., 2018) and Pusa Basmati1121(Babu,et al.,2017b)developed for improved seedling stage salinity tolerance through MABC(Yadav, A. K., et al.,2020).Therice output must be increased by at least 25% by 2030 and 50% by 2050 with the increase in population growth(Li, J. Y. et al.,2014).Salinityexposure for long term in the ionic phase of plants is characterised by

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the buildup of Na+ and the early loss of earlier leaves(Singh, D. P., et al.,2014).Despite their lower output and inferior seed quality, landraces have salt tolerantgenes which can’t be used in commercial production (Ravikiran et al.,2018). At the early growth or seedling stage in rice, the *Saltol* QTL maintains Na+/k+equlibrium and exhibits tolerance.(Warraich, A. S.,et al.,2020). The advancement of salt-tolerant cultivars using both traditional breeding methods (Krishnamurthy et al., 2019b)and marker-assisted selection (Singh, R., et al., 2016). MAB avoids linkage drag by limiting the donor parent region through precise transfer of genes in selection of trait (Singh, R., et al 2016). On chromosome 1 of the rice crop, a few molecular markers aid in the identification of salinity resilience genes, and some*Saltol*-linked markers, including RM 3412, RM8094, AP3206, RM493 and RM10793, have been found for MAB & screening (Ismail, A. M., et al., 2007). Pusa44 and Sarjoo52, two high-yielding rice cultivars from India's northwest that are vulnerable to saline conditions at the seedling stage. So,increases in salt tolerance in these varieties through introgession of *Saltol*QTL which is present on chromosome number 1. In most of the plants, roots exclude Na+ and cl- effectively while water is taken from soil. (Munns, R. 2005). Long-term salt exposure in plants causes ionic stress, which accelerates the ageing process and causes grain deformation and yellowing of the leaves (Kumar S, et al.,2017). With the effect of salinitythere is interference in water transport and nutrient homeostasis which leads to osmotic stress and nutrient imbalance. (HaninM, et al., 2016; ZörbC, et al.,2019). Incereals like rice is sensitive, wheat is moderately,and barley is most tolerant to salts(Flowers, T. J., et al.,1977). Crops which are sensitive to saline stress are glycophytes and tolerant ones are called halophytes (Himabindu, Y., et al.,2016).Salinity induces stomatal closure, which rises leaf temperature and limits the development of shoots (Rajendran, K., et al., 2009).

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2.Implicationsofsalt stress oncrop plants

Excess amount of Na+ ion directly cause cellular damage and inhibits K+ uptake due to which hampers photosyntheticpathway (Xiong, L., et al.,2002). Ionic or mineral stress lead the way to Na^+ and Cl^- deposits in plant cells, which causes early dropping of leaves and plant death (Maathuis F. J. et al.,1999). Excess Na+ in plant cell cytosol inhibits enzymatic activity, disrupting different cellular metabolisms such as synthesis of proteins, bio-molecular activities, and chloroplast functions. (Horie, T., et al.,2012).Excess Na+ also

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hampers other macro and micronutrients in cytoplasm(Bulle, M. et al.,2016). Salt induced stress also dismisses cell turgor pressure in plants. (Kerepesi I et al.,2000).Reducing the cytoplasmic Na+ level is critical to improve the saline tolerance mechanism in cereal grains like rice and wheat. (Horie, T., et al.,2012). Osmotic stress also causes decline the CO2assimilation capacity of plants (Munns, R.et al.,2002). Ionic imbalancesharden oxidative stress, which results in the build-up of reactive oxygen species (ROS) which damages lipids, enzymes, DNA & RNA(Lalwani, Z. et al., 2022).

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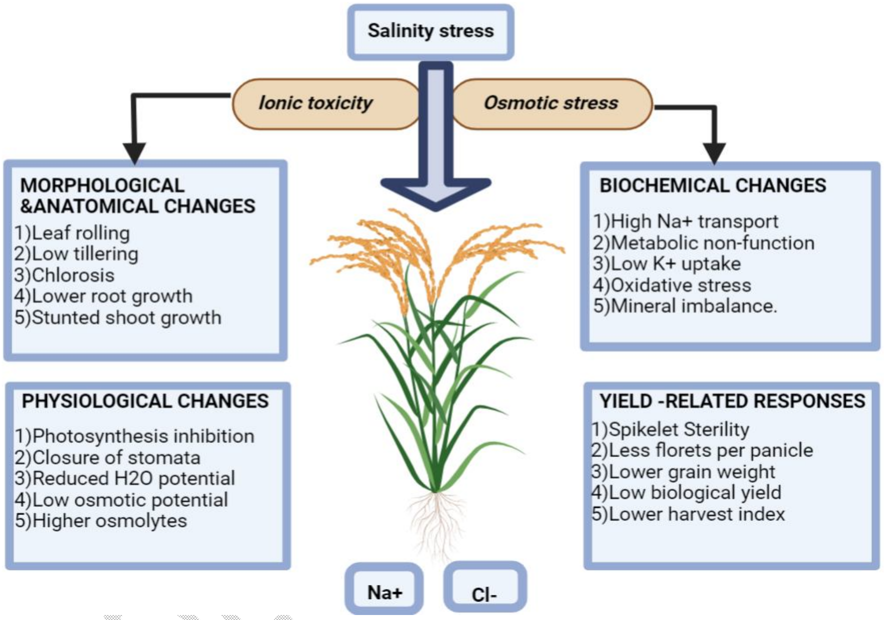

(Hasanuzzamanet al.,2022)

Fig. 1. Schematic representation showing morphological, anatomical, physiological, biological and yield related response of salt sensitive cereals under salinity.

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Two mechanisms exist for plants to be stressed by high salt: Higher concentrations of salinity in soil disrupt the root's ability to absorb water, as well as within the plant can be harmful, inhibits physiological&biochemical processes such as nutrition absorption and acclimatization.(Hasegawa, et al.,2000; Munns, R. 2002;Munns, R. et al.,1995;Munns&Tester2008).Shoot growth is affected by salinity, as illustrated by reduced leafy area and stunted shoot growth (Läuchliet al.,1990).Na+ deposits effects photosynthetic components like enzymes, chlorophyll, and carotenoid pigments in photosynthetic cells(Dawood, M. G et al.,2015).

3. Na^+AND K^+ Content in Shoot and Root of Rice Varieties

Ionic concentration of Na^+ and K^+ in the shoot and root portions of certain rice cultivars show no apparent difference in unstressed conditions but when certain varieties like PB1509 (RP) and FL478 (DP) and NILs are grown under salt stress condition show significant variation in growth of the plant. Here are some of the results in which Na+ concentration in shoots and roots ofamong NIL's substantially less than RP (recurrent parent), but similar to DP (donor parent). Nevertheless, concentration of K^+ in shoots and roots of NIL's is compellingly higher than recurrent parent but similar to donor parent. So, root and shoot Na^+/K+ ratio of NIL's was significantly lower than PB1509 but comparable and similarto FL478(Yadav, A. K et al.,2020).Wild species like *Oryzacoarctata*and*Oryzaalta* are highly tolerant to salinity stress thancheck cultivars which are tolerant such as FL478, Pokkali and NonaBokra.Tolerant native species lines displayed greater levels of Na^+ in the foliage and lower levels of accumulated Na^+ in the root region. Less Na^+ concentration in the shoot is used as a criterion for choice when breeding for salinity resilience varieties in cereals(Prusty, M. R et al.,2018).

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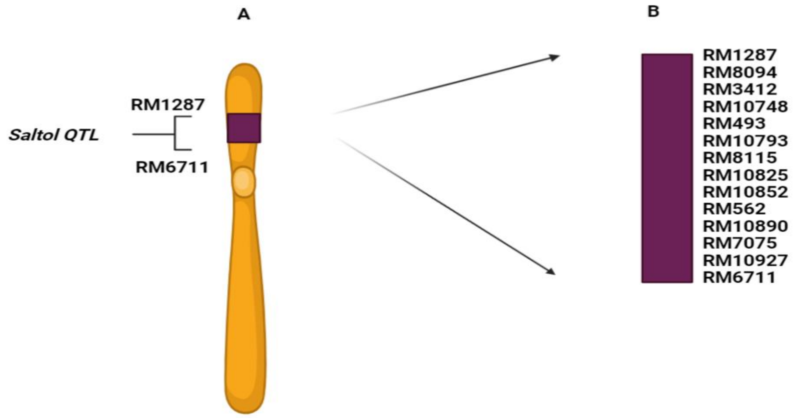
4.*Saltol* QTL Origin and Structure

Genome wide transcriptome analysis between various rice genotypes is known for spotting of required target genes in *saltol* region inPokkali. (Kumari, S., et al.,2009).*Saltol*QTL (Quantitative trait loci) is locatedon short arm of chromosome number 1 of anPokkali and IR29 were crossed to create the F8 RIL population (Recombinant Inbred Line) at IRRI. The Na^+/K^+ ratio and salt endurance at the sprouting of seed or seedling stage are the two primary effects of the Saltol QTL (Gregorio GB et al.,1997; Bonilla SR et al.,2002)and also observed that under salt stress, rice seedlings have less Na^+ and higher K^+ engrossment,and lower Na^+/K^+ ratio(Gregorio GB et al.,2002). For FL478 a (RIL) Recombinant Inbred Line, pokkali is the source for positive alleles. (Bonilla

SR et al.,2002). Ratherthe*saltol* region in FL478 was donated by IR29 (Walia, H. et al.,2005). Profiling has been done for finding of required target genes in the *Saltol*region whichcodes for signalling related proteins (SRPs) in different genotypes of rice.Salinity Induced factors also known as PUFs (Proteins of unknown function) which codes for genes Which are also helpful in providing salinity tolerance, growth of leaves, roots and stems, viability,fertility, and early blossoming (Soda N et al.,2013).



(Waziri, et al.,2016)

Fig. 2.

A) Chromosome 1 in which*Saltol* QTL (purple colour) is present on short arm, bound by simple sequence repeats (SSR) like RM1287 and RM6711.Central circle portion is Centromere.

B) There are 15 SSR markers are aligned within the QTL region in a position of 10.8 Mb to 16.4 Mb from RM1287 to RM6711 respectively.

In addition to these in the*saltol* region “OSAP1” zinc finger proteins and transcription factors like “HBP1b” gives salinity inducible genes. (Kumari, S et al.,2009; Lakra, N. et al.,2015). MABC and MAS are the major two methods through which salt tolerant cultivars are developed like FL478 and Line IR61920 respectively. In (MABC), simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP) are used in tight linkage with traits for salinity tolerance and biotic stress. (Hasanet al.,2015). MASused in introduction of *Saltol*QTL from IR61920 (DP) to Novator (RP) by microsatellite markers (Usatov AV et al.,2015).

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5. Salt tolerance mechanisms in Rice

a) Regulation of ionic balance

More concentration of Na$^+$ and Cl$^+$ causes salinity stress in the soil(Munns, R. 2002; Ismail, A. et al.,2014). The ions are imported into the crop plants by various transporters at organ and cellular levels. (Deinleinetal.,2014). Na$^+$ and K$^+$ are entranced by same transporters into cell but competes each other for space (Greenway, Het al.,1980). K$^+$ is essential for catalytic enzymes, regulation of osmosis, production of proteins, turgorpressure in cellwalland photosynthesis in leaves therefore its necessary to maintain Na$^+$/K$^+$ homeostasis which helps in grow of plant in salt stress conditions(Lodeyro, A. F. et al.,2015; Fu, H. H.et al.,1998;Ashraf et al.,2004;Freitas etal.,2001).Moreover mechanisms like decrease in cytosolic Na$^+$intake, assortment of Na$^+$ into the vacuole and increment in Na$^+$ outflow, and also salt inducible enzymes, anti-transporter like OsSOS1 helps in carrying away of cytoplasmic Na$^+$ions into the apoplast and outflow of Na$^+$from shoot respectively by increasing salinity tolerance(Yang Y et al.,2018; H. El Mahi et al.,2019).The H$^+$iontranslocatingenzyme (OsVP1)in vacuole transport H$^+$ from cytoplasmic fluid into vacuoles by increase in inducible H$^+$ gradient potential between them and promotes Na$^+$/H$^+$ exchange which enhancesaline tolerance in rice (Liu, S. et al.,2010).

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In rice Na$^+$/H$^+$ anti-transporter (OsSOS1) situated in cell membrane helpful in lowering the Na$^+$/K$^+$ ratio at tissue level whereas the vacuole Na$^+$/H$^+$ anti-transporters (OsNHX1, OsNHX2,OsNHX3, OsNHX4, OsNHX5 and OsARP/OsCTP) has a role of compartmentalization of Na$^+$/K$^+$to determine and development of saline tolerance in rice crop(H. El Mahietal.,2019;Martínez-Atienza, J.et al.,2007;Liu, S. et al.,2010;Fukuda, A. et al.,2011;Uddin, M. I et al.,2008).

In cerealssome genes like Nax1 and Nax2 used as molecular markers in breeding of salinity resilience, which are situated in 2A and 5A chromosome respectively helpful in control of Na$^+$accumulation (James RA et al.,2006; James RA et al.,2011) and vascular Na$^+$/H$^+$ anti-transporters like TNHX1, TNHX2, and TVP1 are helpful in growth and development of seedlings in wheat through Na sequestration and PH balance(Bulle, M. et al.,2016).

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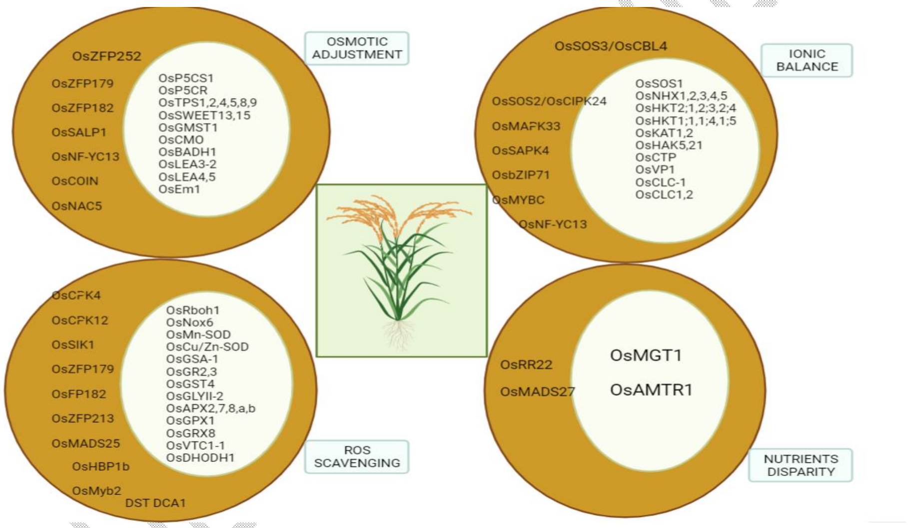

(Liu, C.et al.,2022)

Fig.3. Genes involved in control of salineresilience in rice.

In rice and wheat, many genes are helpful in control of salt tolerance includes a) Ionic balance b) Nutrients disparity c) ROS scavenging d)Osmotic adjustment. The genes in the middle light-yellow colour are functional genes and the outer brown colour has regulatory genes.

The functional genes and its proteins directly protect membranes of the cell, other cell organelles and macromolecules such as DNA, RNA, proteins, carbohydrates,and fats under salinity stress conditions. The regulatory genes and its products such as activators protects crop plants from adverse effects by controlling expression offunctional gene to abiotic stress (Liu, C. et al.,2022).

b) Osmotic potential adjustment

Osmotic stress is caused by salt stress and synthesis of osmolytes which has an impact on osmotic modification it leads to regulation of cell turgor pressure for metabolic activity of the plant and strengthens proteins and cellular frameworks while lowering cell osmotic potential (Yang, Y. et al.,2018). To maintain osmotic equilibrium at the cellular level, osmolyteslikeproline, polyamines, soluble carbs,&proteins such asbetaine and glycine from the late embryogenesis abundant (LEA) family are present(Hare, P. D. et al., 1998).As a physiological indication of salinity tolerance, proline levels are employed.(Liang, W.et al.,2018).Proline synthesis genes like OsP5CS1 and OsP5CR,monosaccharide transporter OsGMST1 andglycinebetaine by OsCMO and OsBADH1 in rice would enhance the salt tolerance by accumulation (Sripinyowanich, S. et al.,2013; Tang,W.et al.,2014).So as to maintain the equilibrium of sugar carbohydrates in rice under dry conditions and salinity circumstances, the outflowing transporters like OsSWEET13 and OsSWEET15 control the transit and dispersal of sugars like sucrose.(Mathan, J. et al.,2020). In wheat betaine helps in inhibit the Na^+&Cl^- from roots and promotes K^+ transport to enhance salinity tolerance (Chen, Z. et al.,2007). Plant specific membrane protein which encodes gene OsSALP1 develops salt stress tolerance by enhancing OsP5CS and prolinesynthesis in amply to salt stress (Yuan, X. et al.,2016).

c) Reactive oxygen species scavenging

Oxygen is essential in cellular metabolism which is converted to ROS during plant metabolic process. Excess of ROS is produced during the under-salt stress conditions eg: O_2, $O_2^{\,-}$,H_2O_2 and OH^-(Qin, H. et al.,2020; Miller et al.,2010). Salt stress reactions can be triggered by low ROS production,and excessive build-up results in destruction of plasma membranes, phytotoxic reactions such as DNA mutation, peroxidation of carbs, lipids, and proteins, permanent metabolic failure, and ultimately death of cell. (Ahanger et al.,2017; Miller et al.,2010). Crop plants uses antioxidants to mollify ROS stress which is of 2 types mainly a) enzymatic b) Non-enzymatic (You, J. et al.,2015).Some of the enzymes that act

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as antioxidants are nicotinamide adenine dinucleotide phosphate oxidases (NOXs, also called as respiratory burst oxidase homologs [Rbohs]), ascorbate peroxidase (APX), catalase (CAT),glutathione peroxidase (GPXs) (Yang, Y. et al.,2018;Postiglione, A. E. et al.,2020;Mittler et al.,2017;Torres, M. A. et al.,2005).Non-enzymatic antioxidants includes glutathione (GSH), ascorbic acid (ASH),phenolic compounds, tocopherol, carotenoids, glutathione (GSH), flavonoids and alkaloids.(Gill, S. S.et al.,2010;Borghesi, E. et al.,2011).CAT and APX enzymes helps in removing of H_2O_2 for crop tolerance (Ashraf M et al.,2004). Due to salt stress, rise in ROS causes lipid peroxidation in cell membranes, which makes MDA a critical product for reducing oxidative stress and boosting rice salinity tolerance (Latef et al.,2021). From the above these studies states that removing of ROS would effectively increase the rice salt tolerance.

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d)Nutrients disparity

Due to salinity stress nutritional deficiencies occur and reduces transportation of the nutrients within the plant body (Razzaqetal.,2020). Magnesium ion transport in root zone increases OsHKT1;5that limits Na^+cumulation in shoots which leads to enhance in saline tolerance (Chen, Z.C. et al.,2017).In rice aminotransferase and cytokinin type -B response regulator control stress associated proteins and Zn-transporter genes respectively which maintains salinity tolerance (Gao, S. et al.,2019).

e) Plantgrowth regulators adjustment

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In addition to above mechanisms the PGRs also helps in salt tolerance. Phytohormonesvaries during untimely salt stress and also salinity induced signalling cascade which leads to adaptive responses. PGRscontrol plant growth and development in tough and difficult environmental conditions. (Van Zelm.E.et al.,2020). To manage and adjust to salt stress, multiple PGRs must be integrated and coordinated like (IAA) Indole acetic acid, (GA) Gibberellic acid, abscisic acid (ABA), cytokinin (CK), ethylene (ETH), brassinosteroids (BR), jasmonic acid (JA), triazoles (TR), salicylic acid (SA),which controlstandard growth and moderate counter to salt stress (Sahet al.,2016).

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6) Conclusion

Salinity stress increases in salt sensitive cereals like rice and wheat which leads to decrease in growth, development, and excess of it causes death of crop plant. So, to demonstrate salt stress there is a need to development of various tolerant varieties through MABC and MAS which stands in adverse climatic conditions. Some of the nutrients like Na+ and K+ plays important role in control of abiotic or salt stress. As Na+ increases in shoot of the crop plant which causes imbalance in ions, oxygen levels and PGRs.In rice various transporters and genes are able to cope with salt stress by developing mechanism like ionic homeostasis, ROS scavenging, osmotic adjustment. Genes like OsHKT1;5 located in *Saltol* QTL region is incorporated from salt tolerant fewer yielding landraces like pokkali, Nona Bokra to cultivated salt sensitive high yielding varieties by wide transcriptome analysis. Even though the mechanisms within the plant and the various conventional breeding techniques as well as modern Marker Assisted Backcross and Marker Assisted selection techniques are developing new varieties and direct transfer of targeted genes into selected plant through genome editing like CRISPR/CAS9 and transgenic methods, as they do not accomplish the required need of growing populations and increase in salinity soils. Therefore, in staple food crops like rice and wheat, further research must be carried to elucidate various biochemical, molecular mechanism and physiological mechanism of salinity tolerance by modern approaches to develop climate resilient crops. There is a further need to study how to enhance and develop salinity tolerant crops.

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