Research Article

The International Journal of Biological Research
(TIJOBR)
ISSN Online: 2618-1444
Vol.5(2);30-June-2022
http://www.rndjournals.com

Biotechnological revolution in plants against salt stress

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Abstract

Salinity limits the production capabilities of agricultural soils in large areas of the world. Salinity/salt stress is the second biggest abiotic factor affecting agricultural productivity worldwide by damaging numerous physiological, biochemical, and molecular processes. In particular, salinity affects plant growth, development, and productivity. Salinity responses include modulation of ion homeostasis, antioxidant defense system induction, and biosynthesis of numerous phytohormones and osmo-protectants to protect plants from osmotic stress by decreasing ion toxicity and augmented reactive oxygen species scavenging. As most crop plants are sensitive to salinity, improving salt tolerance is crucial in sustaining global agricultural productivity. In response to salinity, plants trigger stress-related genes, proteins, and the accumulation of metabolites to cope with the adverse consequence of salinity. Therefore, this review presents an overview of salinity stress in crop plants. We highlight advances in modern biotechnological tools, such as omics (genomics, transcriptomics, proteomics, and metabolomics) approaches and different genome editing tools (ZFN, TALEN, and CRISPR/Cas system) for enhancement of resistance against salt stress.

Key words: Biotechnological tools, Genome editing, Crop Improvement

Introduction

Salinity in soil or water is one of the major abiotic stresses that reduce plant growth and crop productivity worldwide. More than 800 million hectares of land throughout the world are salt-affected (including both saline and sodic soils), equating to more than 6% of the world's total land area. Some of the most serious examples of salinity occur in the arid and semiarid regions. For example, in Iran, Pakistan, Egypt, and Argentina, out of the total land area of 162.2, 77.1, 99.5, and 237.7 million hectares, about 23.8, 10, 8.7, and 33.1 million hectares are salt-affected, respectively (FAO 2008). It is a matter of great concern to fulfill the present and future global food demand, which seems impossible with current agricultural production from already shrinking arable land due to urbanization and land degradation (Hunter et al., 2017). The food for the extra mouths will have to come from the marginal areas; hence, strong efforts and practically effective strategies are needed to enhance crop productivity, especially in the marginal areas in the face of ever-changing climate and various other biotic and abiotic stresses (Raza et al., 2019). Among numerous abiotic stresses, salinity/salt stress is the major abiotic constraint threatening global food security by decreasing agricultural productivity and a major hurdle in accomplishing the "zero hunger" goal proposed by FAO-UN. Millions of people in extreme, rural areas lead stressful lives under hunger and poverty. The number of malnourished people, i.e., facing chronic food poverty, has risen to nearly 821 million in 2017, from around 804 million in 2016. Approximately 1.125 billion hectares of agricultural land and more than 52% (4.03 billion) of the population are affected by salinity. Consequently, poor agricultural land directly leads to food shortage affected by several environmental factors, including salinity stress, which ultimately hinders achieving the "zero hunger" goal (sustainable development goal: SDG2), to "end hunger, attain food security, better nutrition and help sustainable agriculture," by 2030 (Unicef et al., 2019).

Salinity drastically impacts overall plant growth and yield in the long run (Zafar *et al.*, 2020a; Zafar *et al.*, 2021a; Zafar *et al* 2022a; Zafar *et al* 2022c). Salinity negatively affects seed germination by disturbing the physiological activity of seeds, causing an overall reduction in plant: biomass, yield, leaf area, stem, root, and shoot length (Zafar *et al.*, 2020b; Zafar *et al.*, 2021b; Zafar *et al.*, 2021c; Zafar et al., 2022b). In quinoa (Chenopodium quinoa), salinity caused a 49 and 47% decrease in the shoot and root lengths, respectively, in the "A7" genotype. In contrast, in the "Vikinga" genotype, more than 60% of the reduction was observed in shoot and root lengths. In the "A7" genotype, dry weights of root and shoots were reduced to 49%, while in the case of "Vikinga," the reduction percentage was up to 59 and 71%, respectively. The relative water content (RWC) in leaves was also reduced to 33 and 46% in "A7" and "Vikinga," respectively (Parvez et al., 2020). In maize (Zea mays), leaf growth (dry weight) was reduced by 11 and 7%, whereas the reduction in root growth was 30 and 15% at 100 mM NaCl stress level (Hessini et al., 2019). In Libyan hard wheat (Triticum durum Desf.), plant height and dry weight were reduced by 33 and 16%, respectively, while the number of tillers and harvest index were reduced by 27 and 38%, respectively [9]. A significant

reduction of 32.6% in wheat grain yield was observed due to higher salinity levels. Salinity caused a yield reduction of up to 50% at EC 7.2 dS/m in rice (Oryza sativa) (F Ehtaiwesh et al., 2020). In another study, the yield of "Pokkali" rice varieties was reduced by 20–82% under salinity (Chattopadhyay et al., 2021). In cotton (Gossypium hirsutum), the number of bolls was also reduced due to salinity stress leading to an overall yield reduction (PAKNEJAD et al., 2019). In short, salinity stress impairs plant growth and development, physiological, biochemical, and molecular mechanisms, ultimately reducing overall plant productivity (Zörb et al., 2019).

Salt stress regulation in plant

Soil salinity is major threats for the growth and development of plants across the globe (Khan et al. 2012; Natarajan et al., 2015). Salinity draw damaging effect on plants by affecting physiological, morphological and genetical aspects, such as osmotic balance, ion uptake, photosynthesis, alteration in respiration rates and synthesis of protein, up/down-regulation of gene (Siddiqui et al. 2012). Due to these damaging effects, salinity affected plants show disturbance in the water and minerals (K+ and Ca2+) uptake. The competition between K+ and Na+ helps in the uptake of salt and for their transportation. Similarly, high concentrations of Ca2+ have developed high competence between Na+ and K+, restricted its uptake and transport through Na+ permeable channels. Higher level of Na+ and Cl− salts in the soil reduces the availability of essential nutrients by keeping the ratios of Na+/Ca2+, Na+/K+, Ca2+/Mg2+ and Cl−/NO3 − high (Singh et al. 2014; Liu et al. 2015). It also negatively affects the growth of the plant by impairing metabolic processes by decreasing the ability of a plant to take up water.

EFFECTS OF SALINITY ON GERMINATION AND SEEDLING GROWTH

Environmental stresses affect growth and development of plants significantlyresulting in considerable yield losses. Among the key abiotic environmental factors, salinity is one of the significant threats to the environment as well as crops. Using bad waters like brackish and marine water for irrigation to agricultural areas worldwide hasresulted in further aggravation of the situation (Kaur et al., 2018; Munns et al., 2020). As a global problem salinity induced decline in the crop yields primarily results from the negative effects on the specific growth stage, ranging from germination to seedlingmaturation and yield production (Motos et al., 2017). The early osmotic and subsequent slow ionic phases are seen as two major stresses affecting salinity in plants. In the root zone, osmotic stress has been tested for the excessive salinity that results in delayed water absorption, cell growth and root development, and in prolonged exposure, continued ionic imbalance inhibits the intake of critical nutrients such as K^+, Ca^{2+}, and NO^- therefore contributing to the inhibition of plant growth and hence reducing the crop productivity (Soliman et al., 2018; Sugai et al., 2019).

MORPHOLOGICAL AND ANATOMICAL RESPONSESUNDER SALINITY STRESS

Recent studies have suggested that many ecologically relevant characteristics from morphology, physiology and anatomy to growth and reproductive duration vary. Through these alterations plants maintain ion homeostasis under salinity stress condition (Soliman et al., 2020a). Crop production and performance is significantly influenced by saline water and the effect varies with plant species of the same family,genus and cultivars as well as with the salinity levels of the irrigation water and exposure period (short-term vs. long-term testing) (Acosta-Motos et al., 2017). (Baker et al., 1992) reported that salt accumulation in soil and subsequent inflow into the roots had a considerable damaging effect on the growth and development through water limitation and increased impacts on biochemical functions and cellular activitieslike transpiration, stomatal conductance and the photosynthetic inhibition due to loss of turgidity.

Regulation of biotechnology tools under salt stress

Omics

Omics' technologies are primarily aimed at the universal detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological sample. aim to map the genomes and characterize their functional roles, modifications, and biological processes in plants. Multi-omics provide molecular insights to achieve stress-tolerant crop production (Varshney et al., 2020).

a) Genomics

Genomics-assisted breeding involves genomic mapping and subsequent introgression to develop improved cultivars. Mapping quantitative trait loci (QTL) is an important approach for understanding the genetic basis of different complex traits governed by multiple genes. Salinity stress tolerance is controlled by polygenes and hence exhibits quantitative inheritance (Jha et al. 2019; Ganie et al. 2019). The availability of molecular markers, mainly microsatellites or simple sequence repeats (SSRs) and, more recently, single nucleotide polymorphism (SNP), facilitate mapping studies. QTL mapping and other mapping approaches, such as association mapping and Bulk-seq/QTL-seq (using extreme, i.e., low and high performing, bulks), have been used extensively to map salt tolerance associated traits, particularly in rice (Prakash et al. 2020).

Markers	Population	Traits studied	Marker-trait associations (MTAs) identified

Rice			
2 million SNP	204 accessions from Bengal and Assam Aus Panel (BAAP)	Seedling stage hydroponic and soil-based evaluation; 10 traits	97 and 74 MTAs in hydroponic and soil-based evaluation respectively
>33K SNP	155 varieties, Changi Genetic Resources Center, IRRI	8 traits	27 MTAs
Maize			
Sequencing of *ZmHKT1;5* gene	54 diverse maize inbred lines	Survival rate	2 SNP
>580K SNP	399 inbred lines	Seedling stage; 6 traits	57 SNP
Maize SNP50 array; >55K SNP	419 inbred lines	Seedling stage; shot Na+ content	1 candidate gene
Wheat			
90K SNP array;	150 winter and facultative genotypes	13 traits	187 SNP linked to 37 MTAs
90K SNP array; >41K SNPs	100 bread wheat varieties	Na+ exclusion/accumulation	9 linked SNP
660K SNP array	191 accessions from diverse sources	8 phenotyping traits	389 SNP for 11 MTAs

b) Transcriptomics

Advances in high-throughput methods for next-generation sequencing offer a better understanding of salinity stress tolerance in crops by identifying salt-responsive genes using genomics and transcriptomics (Egan et al. 2012; Duarte-Delgado et al. 2020; Kashyap et al. 2020). Transcriptomics can identify stress-associated essential transcripts, the transcriptional structure of genes, their functional pathways, and other post-transcriptional modifications (Wang et al. 2009; Haroon et al., 2022b). RNA-seq and microarray are gene expression approaches (Table 3) with the former being more popular due to its precise transcript measurement ability (Wang et al. 2009).

Genotype	Tissue	Experimental conditions	Technique	Comments
Rice				
Chilbo	Leaves	250 mM NaCl treatment on 14-day-old seedlings for 12 days	WGT	962 upregulated genes identified, mostly belonging to MYB family and ZF family of genes regulating sugar metabolism and amino-acid synthesis

Dongdao -4 Jigeng-88	Leaves	0 mM Na+ (10 mM Na2CO3 and 20 mM NaCl) for 1 day and then 60 mM Na+ (10 mM Na2CO3 and 40 mM NaCl)	RNA-seq	3523 and 4066 DEGs responding to several gene families, involved in functions related to jasmonic acid, organic acid metabolism, iron homeostasis, phenylpropanoid and gibberellic acid synthesis
Wheat				
Qingmai 6 (tolerant)	Shoots and roots (two-weeks old seedlings)	150 mM NaCl, and combination with 100 µM ethylene precursor ACC, and with 150 µM ethylene signaling inhibitor 1-MCP for 3, 6, 12, and 24 h	RNA-seq	Upregulated: *TaCYP450* under stress; six genes played a role in ethylene dependent salt stress
Chinese Spring (*Triticum aestivum*)	Mature leaves, roots, seedlings (30 days)	Half-strength Hoagland solution with 100 mM NaCl from germination to 30 days	RNA-seq,qRT-PCR	Upregulated: LEA, dehydrin and potassium transporter genes in roots, and sodium/cation exchanger and aquaporin genes in shoot
Maize				
G.S. 46	Leaf number 4 of 14-day-old plants	2 mM KCl and1mM CaCl2 for 6 h and 15 mM Ca (NO3) for 2 h. (7 days after salination)	Real-time PCR	ROS scavenging more pronounced in young cells and comparatively reduced in older cells under salt stress. Ascorbate peroxidase and superoxide dismutase significantly higher in NaCl treatment.
B73 maize seedling	Leaf (2 h after NaCl treatment)	200 mM NaCl	RNA-seq,qRT-PCR	Upregulated genes encode oxidoreductase, peroxidase, antioxidant, transcription regulator activities, ERFs, MYBs, b-carotene hydroxylase, and 9-cis- epoxy carotenoid dioxygenase undersalt stress

c) Proteomics

Proteomics deals with functional proteins': role, structure, function, localization, connections with other proteins, and their implementation in stress responses or natural conditions. Proteomics allows us to study changes at protein level/post-transcriptional changes in greater detail. Therefore, proteomics becomes an indispensable approach in identifying key stress protein markers that could be useful in generating stress-resilient crops **(**Kosová et al.,2018; Haroon et al., 2022a).

Genotype	**Tissue and developmental stage**	**Experimental conditions**	**Technique**	**Effects**

Rice

OsDRAP1 gene overexpressing line of Nipponbare	Leaves at 3-leaf stage	120 and 150 mM NaCl after 14 days and kept for 7 days	LC-MS/MS analysis	Increased expression of proline, valine, several organic acids (phosphoenolpyruvic acid, glyceric acid, ascorbic acid) and several secondary metabolites
A highly salt-sensitive *Oryza sativa* L. ssp. *japonica* (rice variety 02428)	Leaves at 3-leaf stage	Control, 100 mM NaCl, 10 μM melatonin, 100 mM NaCl + 10 μM melatonin	UPLC and tandem mass spectrometry (MS/MS)	The exogenous application of melatonin in increased salt tolerance. Transcriptomics study indicated that melatonin-mediated pathway contributed salt tolerance specifically AP2/EREBP-HB-WRKY transcriptional cascade and phytohormone (auxin and ABA). Furthermore, 64 metabolites including amino acids, organic acids, and nucleotides were found more in plants treated with salt+melatonin.

Wheat

Wheat cv. Keumgang	Chloroplasts from fully developed leaves; t 12-day-old seedlings	Sandy soil; 150 mM NaCl for 1, 2 and 3 days	Extraction: trichloroacetic acid (TCA)/acetone; linear quadruple trap-Fourier transform ion cyclotron resonance (LTQ-FTICR) hybrid MS	Upregulated cytochrome b6–f (Cyt b6–f), germin-like-protein, c-subunit of ATP synthase, glutamine synthetase, fructose-bisphosphate aldolase, S-adenosyl methionine synthase and carbonic anhydrase. Downregulated (day 1) but upregulated (days 2/3) proteins eIFs 5A-1/2 and 5A-3 subunits, photosystem I reaction center subunits II and IV, germin-like-protein and uroporphyrinogen decarboxylase
Chinese Spring (CS) and amphiploid (tolerant)	Mitochondria of shoots and roots; seedlings	Hydroponic system with 200 mM NaCl gradually on 1,2,3, and 4 days after sowing for 7 weeks.	Extraction: 100% acetone for leaf and TCA/acetone for root; digestion: gel-bound trypsin; quantification: TOF/TOF	Manganese SOD, serine hydroxymethyl transferase, aconitase, malate dehydrogenase, and β-cyanoalanine synthase were expressed higher in amphiploid. Glutamate dehydrogenase and aspartate aminotransferase upregulated in shoots but downregulated in roots.

Maize

Salt-resistant maize hybrid SR12	Root (1 hr after treatment)	25 mM NaCl (1 h)	IEF and 2-DE	10 proteins phosphorylated and six proteins dephosphorylated under salt stress. Enhanced phosphorylated proteins; fructokinase, UDP-glucosyl transferase BX9, and 2-Cys-peroxyredoxine
Salt-tolerant F63 and salt-sensitive F35	Roots (2 days after NaCl treatment)	160 mM NaCl treatment for 2 days	iTRAQ approach	28 proteins (salt-responsive proteins), 22 specifically regulated in F63 (constant in F35) including cysteine proteases, ribosomal protein S8, 60 S ribosomal protein L3-1, and SOS proteins.
CML421, CML448, CML451 and B73	Roots (after 4 weeks of salt treatment)	Pots in green house, NaCl added directly to soil mix (EC = 9.5 dS/m)	Singular enrichment analysis (SEA)	1,747 proteins, of which 209 more abundant in response to salt stress (associated with oxidative stress, dehydration, respiration, and translation) specifically to heat-shock protein (HSP)90-2 (A0A096RTH6) and class III peroxidase (K7U159).
Salt-tolerant Jing724 and salt-sensitive D9H	Seedlings (7 days after 100 mM NaCl treatment)	100 mM NaCl (7 days)	iTRAQ approach	Upregulated DRPs and key DRPs, such as glucose-6-phosphatedehydrogenase, NADPH-producing dehydrogenase, glutamate synthase, and glutamine synthetase, in salt-tolerant line.

d) Metabolomics

Stress can inflict changes in a plant at a: transcript, protein, and biochemical level. Often, the plant responds to stress only at the biochemical level without altering its transcriptional and protein expression (Raza et al., 2020). These biochemical molecules are also called metabolites—the study of metabolites is called metabolomics. Metabolomics allows us to study and explore the in-depth changes in plant cells after sensing stress. They possess different structures and functions, and because of these striking characteristics, metabolites study has become a hot trend in the current scientific research (Razzaq et al., 2019). In another study, tomato (Solanum lycopersicum L.) and cucumber (Cucumis sativus L.) were subjected to varying degrees of salt stress (25, 50, 100, and 200 mM NaCl). Both cucumber and tomato are extremely sensitive to salinity stress and could express some key metabolites useful in breeding programs. For this reason, a metabolomics study was carried out to understand the metabolic response of cucumber and tomato toward salt stress. Flavonoid contents were sharply increased in cucumber and tomato plants under 200 mM NaCl salt stress. The increment percentages of 2 and 30% were recorded in cucumber and tomato compared to their control treatment. The phenolic compounds were accumulated greatly only in tomatoes, whereas no changes were observed in cucumbers. Likewise, saponin content was down-regulated in cucumber under salt stress (200 mM), which inversely increased significantly in tomatoes (Abdel-Farid et al., 2020). It can be suggested that cucumber and tomato plants exhibit different responsive natures to salinity on a metabolic level. Our focus is to skim the metabolomics studies on salt-stressed plants to provide a platform for future beginner researchers.

Genome Editing tools

Rapid, site-directed, sequence-specific, and provide desired modifications at genomic loci to develop multi-stress-resistant plants with improved traits (Adli et al., 2018).

e) TALEN

TALEN is the chimeric protein consisting of repeat variable di-residues (RVDs) mediated DNA binding domain fused with FokI endonucleases (Li, T et al., 2011). The DNA binding domain elements consist of highly conserved 16–20 tandem repeats of about 33–35

amino acids, which are derivatives of transcription activator-like effectors (TALEs), secreted by Xanthomonas spp. through the type III secretion system as a natural host response. The binding specificity of TALEN is strictly determined by two RVDs present at positions 12 and 13 in each repeat which modulate the binding to one of four different types of nucleotides at the targeted DNA sequence (Khan et al., 2019). So far, TALEN has not been vastly utilized in improving tolerance against salinity stress in plants. Therefore, this tool can be used for engineering salinity tolerance in different crop plants shortly.

f) ZFN

Zinc finger nucleases (ZFNs) bind to a target sequence, thereby dimerizing FokI nuclease. The DSB generated by ZFN cleavage induces DNA repair processes. In the absence of donor template DNA, error-prone non-homologous end joining (NHEJ) can result in 'targeted mutagenesis' (left). The genes against biotic and abiotic stresses reportedly modify loci by ZFN-mediated gene targeting mutations in plants (Townsend JA et al., 2009). ABA-INSENSITIVE4 (ABI4) gene encodes the ERF/AP2 TF family in Arabidopsis thaliana. ZFN-based mutagenesis was carried out, and the mutant plants showed the ABA accumulation and tolerance to salinity along with other various abiotic stresses. Knock-in mutations were identified in maize in which the IPK1 gene was first knocked out, and further biotic and abiotic resistant genes were introduced by ZFN. Similarly, resistance genes were incorporated into endochitinase genes in Nicotiana tabacum via HR-mediated ZFN. However, ZFNs do not have target flexibility due to the inadequacy of recognizing all DNA triplets as compared to TALENs and CRISPR-Cas9 for advancement in genome editing. Limitations caused by ZFN's off-target effects urged researchers to work on other approaches for genome editing with enhanced specificity (Gabriel, et al., 2011). Likewise, more investigations are required in deciphering the ZFN potential in engineering salinity tolerance in crop plants.

g) CRISPR/Cas9

CRISPR/Cas9 (clustered regularly interspaced short palindromic repeat)-Cas9 is a multipurpose technology for genetic engineering that relies on the complementarity of the guideRNA (gRNA) to a specific sequence and the Cas9 endonuclease activity. In rice, NAC TF coding gene OsNAC041 was targeted through CRISPR-Cas9 to determine its function under salt stress. The mutant seedlings showed retarded growth compared to WT seedlings that remained alive under 150 mM L^{-1} NaCl treatment. Mutation in the OsNAC041 gene disrupted the membrane protection system by decreasing activities of sediment oxygen demand (SOD), photochemical oxygen demand (POD), chloramphenicol acetyltransferase (CAT), and a significant increase in ROS accumulation and MDA content, thereby weakening salt tolerance. This study provided evidence that OsNAC041 plays an important role in salinity in rice (Bo et al., 2019). In another study, the function of Auxin Response Factors 4 (ARF4) in tomatoes was determined using CRISPR-Cas9. The down-regulation of the SIARF4 gene resulted in better root development and low stomatal conductance under 150 mM NaCl stress treatment. CRISPR mutant plants (arf4-cr) showed an increased ABA level, coupled with up-regulation of Cu/ZnSOD and mdhar genes resulting in better growth under salinity conditions (Bouzroud et al., 2020).

Conclusion

The recent focus on speed breeding as a robust and time-saving method to boost crop productivity in a controlled environment has opened new avenues for the multifaced integration of technologies. Thus, the amalgamation of omics and genome editing in conjunction with speed breeding can achieve significant results for sustainable agricultural production.

References

1. Raza, A., Tabassum, J., Fakhar, A. Z., Sharif, R., Chen, H., Zhang, C., ... & Varshney, R. K. (2022). Smart reprograming of plants against salinity stress using modern biotechnological tools. *Critical Reviews in Biotechnology*, 1-28.

2. FAO. FAO land and plant nutrition management service. Available online at: http://www.fao.org/ag/agl/agll/spush/. Accessed 25 April 2008; 2008.

3. Hunter, M. C., Smith, R. G., Schipanski, M. E., Atwood, L. W., & Mortensen, D. A. (2017). Agriculture in 2050: recalibrating targets for sustainable intensification. *Bioscience*, *67*(4), 386-391.

4. Raza, A., Razzaq, A., Mehmood, S. S., Zou, X., Zhang, X., Lv, Y., & Xu, J. (2019). Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants*, *8*(2), 34.

5. Zörb, C., Geilfus, C. M., & Dietz, K. J. (2019). Salinity and crop yield. *Plant biology*, *21*, 31-38.

6. Unicef, & WHO, W. (2020). Levels and trends in child malnutrition: key findings of the 2019 edition of the Joint Child Malnutrition Estimates. *Geneva: World Health Organization*.

7. Parvez, S., Abbas, G., Shahid, M., Amjad, M., Hussain, M., Asad, S. A., ... & Naeem, M. A. (2020). Effect of salinity on physiological, biochemical and photostabilizing attributes of two genotypes of quinoa (Chenopodium quinoa Willd.) exposed to arsenic stress. *Ecotoxicology and environmental safety*, *187*, 109814.

8. Hessini, K., Issaoui, K., Ferchichi, S., Saif, T., Abdelly, C., Siddique, K. H., & Cruz, C. (2019). Interactive effects of salinity and nitrogen forms on plant growth, photosynthesis and osmotic adjustment in maize. *Plant Physiology and Biochemistry*, *139*, 171-178.

9. F Ehtaiwesh, A., & H Rashed, F. (2020). Growth and yield responses of Libyan hard wheat (Triticum durum desf) genotypes to salinity stress.

10. Chattopadhyay, K., Mohanty, S. K., Vijayan, J., Marndi, B. C., Sarkar, A., Molla, K. A., ... & Sarkar, R. K. (2021). Genetic dissection of component traits for salinity tolerance at reproductive stage in rice. *Plant Molecular Biology Reporter*, *39*(2), 386-402.
11. PAKNEJAD, F., RAZAJİ, A., MOAREFİ, M., DAMGHANİ, A. M., & ILKAEE, M. N. (2019). Meta-analysis of the Effects of Salinity Stress on Cotton (Gossypium spp.) Growth and Yield in Iran. *Journal of Agricultural Sciences*, *26*(1), 94-103.
12. Haroon, M., Wang, X., Afzal, R., Zafar, M.M., Idrees, F., Batool, M., Khan, A.S. and Imran, M., 2022a. Novel Plant Breeding Techniques Shake Hands with Cereals to Increase Production. *Plants*, *11*(8), p.1052.
13. Haroon, M., Wang, X., Afzal, R., Zafar, M.M., Idrees, F., Batool, M., Khan, A.S. and Imran, M., 2022b. Novel Plant Breeding Techniques Shake Hands with Cereals to Increase Production. *Plants*, *11*(8), p.1052.

14. Varshney, R. K., Sinha, P., Singh, V. K., Kumar, A., Zhang, Q., & Bennetzen, J. L. (2020). 5Gs for crop genetic improvement. *Current Opinion in Plant Biology*, *56*, 190-196.

15. Kaur G, Nelson K, Motavalli P (2018) Early-Season Soil Waterlogging and N Fertilizer Sources Impacts on Corn N Uptake and Apparent N Recovery Efficiency. *Agronomy* 8:102. https://doi.org/10.3390/agronomy8070102.

16. Munns R, Day DA, Fricke W, et al. (2020) Energy costs of salt tolerance in crop plants. New Phytologist 225:1072–1090. https://doi.org/10.1111/nph.15864.

17. Acosta-Motos J, Ortuño M, Bernal-Vicente A, et al. (2017) Plant responses to salt stress: adaptive mechanisms. *Agronomy* 7:18.

18. Soliman MH, Alayafi AA, El Kelish AA, Abu-Elsaoud AM (2018) Acetylsalicylic acid enhance tolerance of Phaseolus vulgaris L. to chilling stress, improving photosynthesis, antioxidants and expression of cold stress responsive genes. Botanical studies 59:6.

19. Sugai T, Yannan W, Watanabe T, et al. (2019) Salt Stress Reduced the Seedling Growth of Two Larch Species Under Elevated Ozone. Front For Glob Change 2:. https://doi.org/10.3389/ffgc.2019.00053.

20. Soliman M, Elkelish A, Souad T, et al. (2020a) Brassinosteroid seed priming with nitrogen supplementation improves salt tolerance in soybean. Physiology and Molecular Biology of Plants 26:501–511.

21. Acosta-Motos J, Ortuño M, Bernal-Vicente A, et al. (2017) Plant responses to salt stress: adaptive mechanisms. Agronomy 7:18.

22. Kosová, K., Vítámvás, P., Urban, M. O., Prášil, I. T., & Renaut, J. (2018). Plant abiotic stress proteomics: the major factors determining alterations in cellular proteome. *Frontiers in plant science*, *9*, 122.

23. Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nature communications*, *9*(1), 1-13.

24. Raza, A. (2020). Metabolomics: a systems biology approach for enhancing heat stress tolerance in plants. *Plant Cell Reports*, 1-23.

25. Razzaq, A., Sadia, B., Raza, A., Khalid Hameed, M., & Saleem, F. (2019). Metabolomics: A way forward for crop improvement. *Metabolites*, *9*(12), 303.

26. Li, T., Huang, S., Jiang, W. Z., Wright, D., Spalding, M. H., Weeks, D. P., & Yang, B. (2011). TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. *Nucleic acids research*, *39*(1), 359-372.

27. Khan, S. H. (2019). Genome-editing technologies: concept, pros, and cons of various genome-editing techniques and bioethical concerns for clinical application. *Molecular Therapy-Nucleic Acids*, *16*, 326-334.

28. Bo, W., Zhaohui, Z., Huanhuan, Z. H. A. N. G., Xia, W. A. N. G., Binglin, L. I. U., Lijia, Y., ... & Yong, Z. (2019). Targeted mutagenesis of NAC transcription factor gene, OsNAC041, leading to salt sensitivity in rice. *Rice Science*, *26*(2), 98-108.

29. Bouzroud, S., Gasparini, K., Hu, G., Barbosa, M. A. M., Rosa, B. L., Fahr, M., ... & Zouine, M. (2020). Down regulation and loss of auxin response factor 4 function using CRISPR/Cas9 alters plant growth, stomatal function and improves tomato tolerance to salinity and osmotic stress. *Genes*, *11*(3), 272.

30. Abdel-Farid, I. B., Marghany, M. R., Rowezek, M. M., & Sheded, M. G. (2020). Effect of Salinity Stress on Growth and MetabolomicProfiling of Cucumis sativus and Solanum lycopersicum. *Plants*, *9*(11), 1626.

31. Townsend JA, Wright DA, Winfrey RJ, et al. High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature. 2009;459(7245): 442–445.

32. Gabriel, R., Lombardo, A., Arens, A., Miller, J. C., Genovese, P., Kaeppel, C., ... & Von Kalle, C. (2011). An unbiased genome-wide analysis of zinc-finger nuclease specificity. *Nature biotechnology*, *29*(9), 816-823.
33. Zafar MM, A Razzaq, MA Farooq, A Rehman, H Firdous, A Shakeel, H Mo, M Ren, M Ashraf and Y Youlu, 2020b. Genetic Variation Studies of Ionic and within Boll Yield Components in Cotton (Gossypium Hirsutum L.) Under Salt Stress. Journal of Natural Fibers.1-20.
34. Zafar, MM , G Mustafa, F Shoukat, A Idrees, A Ali, F Sharif, A Shakeel et al., 2022b. Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton. Scientific Reports.12(1): 1-11.
35. Zafar, MM, A Manan, A Razzaq, M Zulfqar, A Saeed, M Kashif, A Iqbal Khan et al, 2021b. Exploiting Agronomic and Biochemical Traits to Develop Heat Resilient Cotton Cultivars under Climate Change Scenarios. Agronomy 11(9): 1885.
36. Zafar, MM, A Razzaq, MA Farooq, A Rehman, H Firdous, A Shakeel, H Mo, And M Ren, 2020a. Insect resistance management in Bacillus thuringiensis cotton by MGPS (multiple genes pyramiding and silencing). Journal of Cotton Research 3(1): 1-13.
37. Zafar, MM, A Rehman, A Razzaq, A Parvaiz, G Mustafa, F Sharif, H Mo, Y Youlu, A Shakeel, and M Ren 2022c. Genome-wide characterization and expression analysis of Erf gene family in cotton. BMC plant biology. 22(1): 1-18.
38. Zafar, MM, A Shakeel, M Haroon, A Manan, A Sahar, A Shoukat, H Mo, MA Farooq and M Ren, 2021c. Effects of Salinity Stress on Some Growth, Physiological, and Biochemical Parameters in Cotton (Gossypium hirsutum L.) Germplasm. Journal of Natural Fibers. 1-33.
39. Zafar, MM, X Jia, A Shakeel, Z Sarfraz, A Manan, A Imran, H Mo et al. 2021a.Unraveling Heat Tolerance in Upland Cotton (Gossypium hirsutum L.) Using Univariate and Multivariate Analysis. Frontiers in Plant Science 12: 727835-727835.
40. Zafar, MM, Y Zhang, MA Farooq, A Ali, H Firdous, M Haseeb, S Fiaz, A Shakeel, A Razzaq and M Ren, 2022a.Biochemical and Associated Agronomic Traits in Gossypium hirsutum L. under High Temperature Stress. Agronomy.12(6): 1310.