Vol 4 Issue 1 ISSN: 2581-3862

ScholarsReport
Open Access Peer Reviewed Journal

Review Article **Subject:** Plant Breeding and Genetics

Effect of salinity stress on cotton growth and role of Marker Assisted breeding and agronomic practices (chemical, biological and physical) for salinity tolerance

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ARTICLE INFO

Article History:
Received: SEP 17, 2018
Accepted: Jan 14, 2019
Available online: April 01, 2019

Keywords: Genetic Engineering; Molecular Breeding; Salinity; Seedling

Abbreviations

(EC) Electrical Conductivity
(BSA) Bulk Segregant Analysis
(NaCl) Sodium Chloride
(QTL) Quantitative trait loci
(G) Gossypium

ABSTRACT

The main factor affecting plant growth in cotton during seedling stage is salinity. During salts stress conditions soluble salts are accumulated in the root zone of plants that causes osmotic and ionic imbalance. Plant growth highly reduces by soil salinity through three different mechanisms like nutrient ion imbalance which reduces K^+, PO_4^-, and NO^- uptake with high concentration of sodium chloride and osmotic stress which inhibits water availability. Plants have specific stress-related genes which make a plant able to adapt different abiotic and biotic stresses. The genetic engineering is helpful for the development of such cotton varieties which will be tolerant against salt stress. Up till now developed cotton varieties against salt tolerance through genetic engineering are unable to achieve the required commercial production level because of minute salt tolerance or not good agronomic practices. The performance of genetically modified plant is much better as compared to other wild types of cotton plants in green house or field condition under salinity stress. This review throws light on new genetic development and molecular breeding against soil salinity tolerance in cotton. The combination of conventional breeding and new molecular techniques will be helpful to develop the salt-tolerant varieties.

Introduction

Among problems in the production of farming crops, salinity is the major one due to its direct effect on seed germination, growth, plant strength and yield.[1] Soil salinity caused by carbonates, bicarbonates and chlorides of calcium, magnesium and sodium among these salts NaCl considered as predominant due to its existence in soil solution as dominant anion Na^+ that inhibits other nutrition process within the soil.[2] When plants are irrigated water which mainly contains anions of calcium, magnesium and sodium,

Citation: Shehzad M, Shafeeq-ur-rahman, Ditta A, Iqbal MS, Malik TH, Hussain SB, Khan MI, Ramzan M (2019) Effect of salinity stress on cotton growth and role of Marker Assisted breeding and agronomic practices (chemical, biological and physical) for salinity tolerance. *ScholReps* 4(1).

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among these anions magnesium and calcium precipitate into carbonates and bicarbonates when water evaporates while sodium anion remains in soil and causes soil salinity.[3, 1] Cotton is important woody perennial crop with family Malvaceae and genus Gossypium. Till now 53 Gossypium species are present in which 46 are diploids (2n=2x=26), seven well established (2n=4x-52) allotetraploid establish this genus. [4, 5, 6] Among these seven well established allotetraploid species two are cultivated such as, G. barbadense and *G. hirsutum* and five are wild species such as, *G. darwinii*, *G. mustelinum*, *G. tomentosum*, *G.ekmanianum* and *G. stephensii* respectively.[4, 5, 7] Cotton is the second major oil seed crop after soybeans which is used for the production of oil in all over the world.[8] Due to high agronomic values its leaves and stalks are ploughed into soil for enhancing soil fertility and structure. Due to high protein concentration some cotton seed is used for bakery products and other food stuff in different countries of the world. In cotton production all over world; china ranked first number second India, third USA and fourth Pakistan. According to suspicious assessment of USDA/ICAC almost 72 present of world total cotton comes from these countries. Moreover, among these four countries China is the world leading cotton producing. On the other hand in cotton consuming countries China, India and Pakistan are at the top by the total consumption of two third of the world which is estimated as 110.3 million 480Ib bales. For the cultivation of cotton wild range of soils can be used but mostly preferable soils are medium and heavy textured, deep, well drained, fertile, clay, alluvial chernozom and laterites soils with good water holding capacity. Acidic and dense sub soils are not good for cotton cultivated due to less root penetration within the soils which prohibit seed germination and ultimately yield of cotton crop. Optimum pH for cotton crop is ranging from 5.5 to 8 with 7 to 8. This is one of the most expansively grown species around the world in tropical and subtropical regions due to its wide range of pests, diseases and agronomic practices with 36 South latitude and 46 North latitude. The production of cotton crop was prohibited by soil salinity in all over the world due to irrigation of salt contaminated water.[9] According to assessment of Ponnamperuma, 1984 every year almost 10 percent of worldwide area is converted into saline soil these millions of hectares are suitable for cultivation but remains under uncultivation due to high economic cost under salinity stress. Soil salinity mostly causes by two factors like primary and secondary. In primary factor salinity causes with accumulation of salt for over long time in soil and underground water by a natural biological process. Secondry factor that causes salinity due to change balance between applied water and plants used waters. Salts have been known to be a problem for so many years decade in those areas where less rainfall and salt move down through plant root.[10].

Types of salinity and their effects

Salinity is divided into three categories first one underground salinity water, second applied irrigation salinity and third one transient salinity based on soil processes and water availability.[11] Accumulation of salts in dry areas less amount of salt as compared to humid areas and rapid rate of evaporation [12] and water used for agriculture purpose have more amount of salt.[13] Root process of plant is directly affected by saline soil. Usually salt influenced soils have low nitrogen contents, low organic matter and contain high quantity of salt.[14]The chemical properties, physical characteristics and soil structure depend on the salt concentration on soil. There are many types of salts found in underground water and irrigation water such as calcium chloride, sodium chloride and carbonates, but sodium chloride is more affected to cotton crop as compared to others salts.[11]

Types of saline soils

Szabolcs in 1974 classified Salinity soil into two types.[15]

Saline soils

The soils which contain more quantity of salts affect the growth of plant. Sodium chloride and sodium sulfate are found in this type of soil and soil electric conductivity is less than 8.

Sodic soils

In this type of soil contain high amount of sodium salts and other name of this soil are known as alkali soil.

Total salt affected area worldwide

All climatic regions from polar to humid are affected with salinity stress. From below sea level to 5000 meters above to mountains like Tibetan to the Rocky Mountains saline soils can be found.[16] All the continents have salt affected areas but range and distribution of these areas has not be studied in detail. However, the few reports indicate that in North and Central Asia, Australia and South America salinity is most acute (Table 1). FAO/Unesco Soil Map of the World based information on the extent and distribution of salt affected soil is made by F. Massoud.[17] The information about the salt affected areas of European countries was published by Szabolcs.[18] In coming years any attempt to increase crop production will be related to create resistance against salt stress in existing crop cultivars.

Calculation of soil salinity

Electrical Conductivity (EC) is the measuring tool of Soil salinity. The SI unit of EC is 'Siemens' symbol 'S' per meter (S/m). The equivalent non-SI unit is 'mho' and 1mho = 1S. The relationship between other units of conductivity and NaCl concentration (10mM NaCl has an EC close to 1dS/m). For the measurement of Electrical Conductivity saturated pasts extract (ECe) is used. A more convenient and universal method is a '1:5 extract'. In this method first soil is compressed or dried, secondly 1g soil is shacked with 5 ml deionized water, and lastly the probe of hand-hold conductivity meter is placed in the suspension to measure the EC.

Effect of salinity on cotton crop

Till now diminutive research work on salinity has been done on cotton crop at seedling stage. Previous study was stated research work on association mapping for salt stress in cotton crop, but at that time no use of QTLs mapping for salinity study in cotton.[9] Due to salinity stress crop losses increasing year by year.[21] Cotton crop is adversely affected by salinity stress by different ways such as less seedling germination, stunt vegetative growth, which follows to lower crop quantity and quality and reduce total agricultural production. Salinity stress may be affected crop by two ways; first, it reduces water uptake ability of crop which integer the osmotic or water-deficit effect in plant and ultimately leads to reduction of growth.[20] Second, salt-specific or ion-excess effect because of excessive load of salt in the transpiration stream which cause cellular injury in transpiring leaves.[21]

With the increase of salinity stress, most plants become unable to extract water from the soil and face osmotic stress. Soil salinity also disturbs nutrient balance in soil as well as in plants and creates specific ion toxicity in plant. Against salinity stress there is a wide range of salt tolerance plants, but most of the normal crops are not highly salt tolerance and will eventually die out under saline conditions. Salinity stress disturb nutrient balance in plants in two possible ways: first, the uptake and translocation of nutrients highly disrupt by the ionic strength of substrate and second the competition of nutrients with the major salt ion (e.g. Na^+ and Cl^-) in substrate. These interactions leads to Na –induced Ca and/or K deficiencies and Ca –induced Mg deficiencies.[22] Salinity stress adversely effect on ionic balance in plants due to excessive amount of Na^+ and Cl ions and plants growth and survival ultimately depends upon the adaptation of this newly establish homeostasis. Hyper osmotic shock causing loss of cell turgor which cause due to decrease in the chemical activity of water is another effect of salinity stress on plant. Salinity stress inhibits the availability of essential nutrients in plants through the their precipitation with the salt cations such as phosphorus (P) become unavailable to plants in saline conditions due to its precipitation with Ca, Mg and Zn ions in salts. Similarly salt anions, such as Cl^-, they bind together into aggregates and form fine particles in soils and effect soil physical properties and this process is named as flocculation, which is extremely beneficial for soil aeration, root growth and penetration. While, Na^+ has opposite effect on soils: it causes soil dispersion due to its relatively large size, hydration status and single electrical charge.[24] Salt tolerance of plants is defined as the production of percent biomass of plant in saline soil relative to non-saline soil, after growth for a specific period of time. Percent survival is used for the slow-growing, long-lived, or uncultivated species because it is difficult to predict the reduction of their biomass.

Factors influencing tolerance of crops to salinity

Plants tolerance character is varied form species to species or variety to variety and from environmental conditions. This character may also vary with the stage of crop growth of the same species.

Growth stage

Growth stages have very importance when we study tolerance of plant against salt stress. From various studies it has been cleared that many plant species are more sensitive to salt stress during germination or in early seedling stages as compared to maturity stage. In case of rice plant it has been established through many experiments that it is tolerant against high concentration of salt (up to 30 dS/m) at germination, while sensitive to salt stress in early growth stages and then its tolerance increases with age during the tillering phase of growth.[25] In case of cotton crop seedling stage is most sensitive to salinity and the effects of salinity stress can be analyzed by measuring physiological and morphological traits.[27]

If we talk about environmental conditions, they have much effect on the ability of plant to tolerate salt stress. Such as low humidity, high temperature, and high winds trigger the evapo-transpiration through plant leaves which can cause the symptoms similar to water stress and make plants more sensitive to salt stress. Salt–sensitive crop survival increases with high air humidity as compare to salt-tolerant crop.

Environmental factors

Weather conditions have played important role for plant in salt conditions. If weather conditions very hot, humidity in air low, high winds adversely effects on plants and made plant susceptible against salt stress. High humidity in environment beneficial for sensitive plants

Studies by [28] evaporation rate in salinity soil was higher as compared to bulked normal soil. Some people have observed tolerance of plants increased against salt stress in air polluted weather conditions.[29]

Varietal differences in salt tolerance

From past decades the varietal tolerance to biotic and abiotic stresses was existed but it was not identified scientifically but in the latest decades the series of efforts by the scientists have been initiated to point out the genetic potential of salt-tolerant crop varieties through different breeding programmes. The plant salt-tolerant potential may vary with genetic traits. Varieties and rootstocks of specific crops can be quite sensitive to salt stress. Within the same species some varieties are more salt-tolerant than others. In India scientists collect large range of rice germplasm of rice plants from salt affected areas to identify the several genotypes that are highly tolerant against salt stress.[30] Barley crop was exposed in sea water at the University of California to sort out the salt-tolerant its traits in same species.[31]

Plant response to salinity

Plants have showed different mechanisms to evaluate adverse effect of salinity stress. Among these mechanisms there are three possible way to tolerate salinity stress such as:

a) tissue tolerance,

b) Na exclusion from leaf blades,

c) tolerance to osmotic stress.[1]

In this review paper we will discuss these three mechanisms one by one to develop clear view of plant tolerance against salinity stress.

Osmotic tolerance

Osmotic stress is the ability of any plant to maintain leaf expansion and stomatal conductance through tolerate the drought aspect of salinity stress.[33] Salinity induced osmotic stress reduces the plant ability to salt exclusion and ultimately leads to reduction of plant growth and stomatal conductance.[32] It was established in a study of genetic variation to osmotic stress on 50 international durum varieties and landraces that there is direct proportion between relative growth rate and stomatal conductance in plants under salinity stress and that higher stomatal conductance leads to higher CO_2 assimilation rate.[32] But if the salt accumulation reached at the toxic level than old and young leaves will go to die which ultimately reduces the export of photosynthates and leads to reduction of growth and new leaves production. For this reason, with the increase of osmotic tolerance plant producing new and greater leaves ability will increase and ultimately increase stomatal conductance. The osmotic tolerance mechanism related to water availability, stomatal conductance and the process of photosynthetic capability to sustain carbon skeletons production to meet cell energy demands for growth.

Na^+ exclusion

The plants grown under saline conditions have high concentration of Na^+ before Cl^- that's why most of studies are conducted on Na^+ exclusion and the control of Na^+ transport and concentration within the plant.[1] For that reason, another essential mechanism of plant tolerance against salinity stress is the plant ability to reduce the ionic stress of Na^+ by minimizing the amount of Na^+ that accumulates in the cytocol of cells, particularly in the transpiring leaves. This tissue tolerance process involves up and down regulation of the expression of traffic ion channels and transporters, which control Na^+ transport.[1] The Na^+ exclusion from leaves in cereal crops including durum wheat, bread wheat, barley and rice is concerned with the salt tolerance ability. The Na^+ exclusion from the leaves is correlated with the less uptake of Na^+ by the root cortex cells and the tight control on xylem loading by the parenchyma cells in the stele.[34] The Na^+ exclusion by roots is surety that Na^+ does not accumulate within leaves to toxic concentration. If root cell failed in exclusion of Na^+ then its toxic effect appears after days or weeks depending upon the verieties, species and ultimately it cause the death of older leaves.[1]

Tissue tolerance

The first mechanism, tissue tolerance involved in the survival of old leaves. In this mechanism plants avoid salt ions (Na^+ and Cl^-) toxicity within cytoplasm, especially photosynthetic mesophyll cells in the synthesis and accumulation of compatible solutes in cytoplasm by compartmentalization of Na^+ and Cl^- at cellular and intracellular level.[1] These compatible solutes play a vital role in plant to create tolerance against salt induced osmotic stress by various ways such as: protect denaturation of enzymes, stabilizing membrane or macromolecules, or mediating osmotic adjustment.[35] The role of compatible solute is not only limited to osmotic adjustment; due to their hydrophilic nature they also replace water at the surface of proteins or membranes as acting low molecular weight chaperones.[36] The chemistry of compatible solutes is quite different from other common solutes; they are water soluble, small and uniformly neutral as compared to the perturbation of cellular functions, even when present at high concentrations.[37] In their composition nitrogen compounds participate such as amines, betaines and amino acids, but other organic acids, sugars and polyols.[38]

Cotton molecular breeding for salt tolerance

The use of Advanced Bioinformatics techniques such as Genotype by sequencing(GBS), Single Nucleotide polymorphism (SNP) and Bulked segregant analysis (BSA) third generation sequencing and construction of genetic maps, identification of QTLs and genes that will helpful for marker assisted selection and rapidly development of cotton crop. The promising genomic applications bring a radical change in the plant breeding and genetics.[39] It provides new molecular tools for the identification and tagging of desirable unique/ novel alleles and genes. Present day the use of molecular techniques can augment the breeding efficiency and accuracy. New markers techniques were helpful for understanding of desirable traits like, fiber length and quality [40] through the indirect selection of target traits. DNA based markers don't have any environmental effects or epistatic effects like phenotype markers, and exhibited Mendelian inheritance. DNA markers and/ or gene transformation are particularly beneficial in tagging, cloning, introgression of valuable genes from exotic genetic resources, and quantitative trait loci (QTLs) for specific trait improvement.

QTL (Quantitative trait loci) mapping

QTL mapping is technique used for identification of QTL of different traits in different crops. There are different software's were used in which most commonly used were Win QTLs cartographer 2.5 for identification of QTLs. For this analysis two type of data were needed phenotypic (field data that were measured of different traits) and genotypic (markers data). From this analysis checked genotypic variation in traits. With help of QTLs methods identified different traits QTLs in different crops like salt stress, fiber, drought, heat, yield and quality QTLs that were important for identification of gene and transformation of gene in cotton it's a new strategy for development of cotton.[41] Identified QTLs are helpful for understanding structure, function and nature of agronomical traits.

With help of QTLs understand the nature of traits and role of traits in breeding programmes through marker assisted selection [42] and pyramiding of multiple favorable alleles.[43] The objective of QTLs mapping were identification of exact genomic location, number and interaction of gene. Phenotypic data and genotypic required for QTLs method both segregating among a number of common progenies, derived from two homozygous contrasting parents for the target quantitative trait(s). These are

(a) phenotypic data
(b) genotypic data.

Identified QTL and Genes for Salt Tolerance in different crop species

Identified QTLs and genes related to salt stress traits in different crops such as barley, rice, wheat and Arabidopsis thaliana give in Table 3. Little wok have been done on salt stress and few QTLs identified at that time in cotton.[44]

Improvement measure for saline soil tolerance

Chemical improvement measures

In the earlier a comparative study was carried out to check the effect of newly developed ameliorant and mixture of cow manure and gypsum into saline soil of the north Jiangsu Province. [45] The authors measured the plant (Salicornia europeam L.) and stem root growth. The result of this study presented that the three ameliorants, including cow dung, gypsum, new ameliorant and their combinations developed soil chemical and physical contents and moreover confirmed to improve both plant height and stem diameter.

Biological and ecological measures

Microorganisms with the following characters like salt tolerant with improved genetic variability, synthesizing compatible solutes, producing plant growth promoting hormones, biocontrol potential can successfully interact with the crop plants to improve the saline soil quality.[46] In the microbiological investigations of saline soils, great attention has been paid to halophilic, osmotolerant, and alkali tolerant microorganisms,[47] rhizobacteria is a beneficial organism that increased the growth of wheat plant in under salt conditions.[48] Plants treated with rhizobacteria, tolerance to disease and salt.[49]

Introduction of salt tolerance plants

Cultivation of fodders and bio fuel crops in salt affected area is natural method for controlling of secondary salt and created tolerance in perennial cultivated varieties. However different crop species have different threshold tolerance ECe and yield reduction rate (Table 4).[50]

Conclusion

Even Cotton tolerance against salt stress is very far from the halophytes but it is classified as a salt tolerant crop. For increasing cotton yield and fiber quality under salt stress; effective agronomic and management practices are necessary. Soil salinity results ion toxicity, osmotic stress and nutritional imbalance in cotton crop. Two important strategies often use to mitigate salt toxicity in cotton crop like: stop the accumulation of Na^+ and Cl^- in cotton crop by reducing its concentration in soil solution and genetically improve cotton tolerance against salt stress. By two ways we can enhance salt tolerance of cotton crop like:

1) genetics enrichment
2) chemical, physical and biological treatments.

Salinity stress can be decreased for cotton growth through creating a more suitable soil environment with engineering or agronomic practices and reduction of concentration of salts in salt affected areas. Conventional breeding is time lengthy and time consuming process as compared to molecular breeding, by advance molecular techniques we can save our time and by new functional genomics approaches we can enhance authenticity and accuracy. The information from this study may be helpful in future for development of resistant cotton varieties against salinity and could give economic yield in salt effected areas.

Acknowledgment

We (the Authors) are grateful to all the team and lab mates and friends who helped us during the paper writing.

Source(s) of support in the form of grants, equipment, drugs, or all of these

None declared

Declaration

The author declares that this contribution is original and is not published elsewhere in part or in whole, elsewhere.

Conflict of Interest

The authors declare that no conflict of interest exists.

Author Contributions

S.F.R, M.S and A.D created the draft of the manuscript and reviewed and studied the manuscript. Ultimately, all authors read and agreed to submit the final manuscript.

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Concepts Guarantor	✓	✓	✓	NA	✓	NA	NA	NA
Design	✓	NA	NA	✓	✓	✓	✓	NA
Definition of intellectual content	✓	✓	NA	✓	NA	NA	NA	NA
Literature search	✓	NA	✓	NA	NA	✓	NA	✓

Clinical studies	NA	NA	NA	NA	NA	NA	NA	NA
Experimental studies	NA	NA	NA	NA	NA	NA	NA	NA
Data acquisition	✓	✓	✓	NA	NA	NA	NA	NA
Data analysis	✓	NA	✓	NA	NA	NA	NA	NA
Statistical analysis	NA	NA	NA	NA	NA	NA	NA	NA
Manuscript preparation	✓	NA	✓	✓	✓	✓	✓	NA
Manuscript editing	✓	✓	✓	NA	✓	✓	NA	NA
Manuscript review	NA	NA	✓	✓	✓	✓	✓	✓
Guarantor	✓	✓	✓	NA	NA	NA	NA	NA

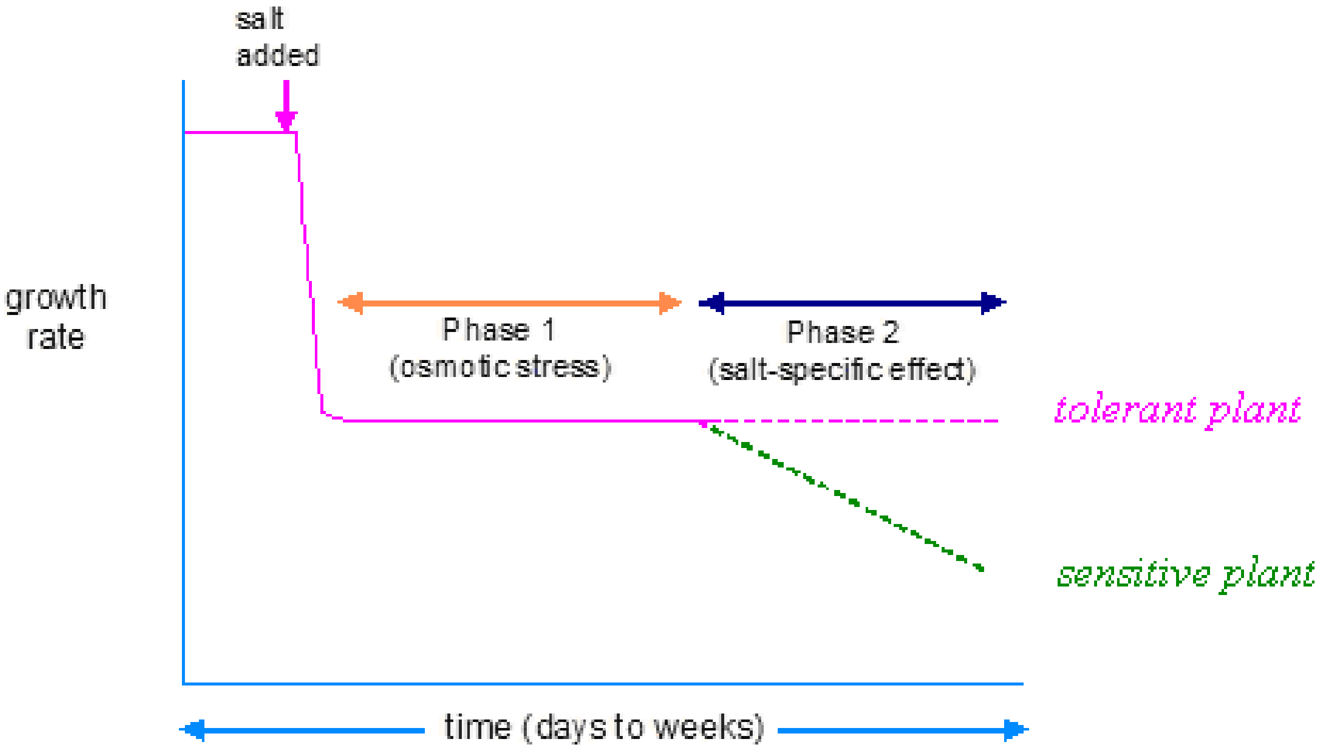

Figure: 1 Effects of salt on the growth of plant (Olias et al.2009) [52]

Table 1: Total salt effected area all over the world

Total area mentioned in hectare			
Continent	Saline affected area	Affected area from sodium	Total
North America	6191	9564	15755
Mexico and Central America	1965	-	1965
South America	69410	59753	129163
Africa	46831	25659	80438
South Asia	83312	1798	85108
North and central Asia	91383	437	211448
Southeast Asia	19983	-	19983
Australia	17597	339971	357568
Europe	292.8	919	1895.7

Table 2: Identified QTLs related to salinity traits in different cultivated crops

Crop plants	Molecular markers	Number of f QTLs	function	References
Cotton (*G. hirsutum* L.)	SSR	qRL-Chr16-1, qRL-Chr22-1, qSh-Chr15-1, qSh-Chr24-1, qChl-Chr24-1, qSfw-Chr15-1, qSdw-Chr9-1, qSdw-Chr15-1, qSdw-Chr26-1, qRfw-Chr15-1, qRfw-Chr23-1	Salt tolerant	[19]
Wheat (*Triticum aestivum* L.)	EST	One QTL TmHKT7-A2	Reduction of sodium chloride in leaf of plants	[52]
Arabidopsis thaliana L.	AFLP	One QTL RAS1	Increase the growth of plant at early seedling stage by inhibition ABA activity.	[54]
Rice (*Oryza sativa* L.)	SSR	Four QTLs 1-qRL-7, 2-qDWRO-9a and 3-qDWRO-9b qBI-1a and 4-qBI-1b	Increase root weight and root length under salt conditions	[56]
Barley (*Hordeum vulgare*)	SSR	1-bPb-1278 and 2- bPb-8437	Increase number of tillers per plant height and number of spikelet's per spike	[60]
Sunflower (*Helianthus annuus* L.)	EST and SNP	1-ORS674, 2-ORS784, 3-ORS235, 4-ORS681	Increase uptake of $Ca2^{+}$ and rapidly removal f Na^{+}	[64]
Tomato (*Solanum lycopersicum* L.)	RFLP	Seven QTLs on chrom1, chrom 2, chrom3, chrom 7, chrom 8, chrom 9 and chrom 12	Increase germination under salt stress condition of parents and offspring's	[65]

Table 3: identified genes used for development of cotton crop against salt stress.

Name	Identified from	Gene function	Production	Reference
AtNHXl	Arabidopsis thaliana	antiporter of Na+/H+	Salt tolerance increased biomass	[74, 75]
TsVP	Thellungiellahalophila	This responsible for accumulation salt in vacuoles	Increase tolerance against salt stress Increased biomass	[76]
AhCMO	Atriplexhortensis	synthesis of glycine betaine	Improved salt tolerance and increased plant biomass	[77, 78]
AnnBj1	Mustard	Ca2+ phospholipid protein and cytoskeleton protein	Increase dry weight and amount of water against salt stress,	[79]

Table4: Name susceptible crops against salt stress (Bauder et al. 2004; Chinnusamy et al. 2005)

Sr. No	Name of crops with affects of salts on reduction of crops yields
1.	Corn (yield reduced twelve Slop % per dSm-1 percent against concentration of salt one point seven dSm-1
2.	Sugarcane (yield reduced five point nine Slop % per dSm-1 percent against concentration of salt one point seven dSm-1
3.	Rice, (yield reduced twelve Slop % per dSm-1 percent against concentration of salt three dSm-1
4.	Wheat (yield reduced seven point one Slop % per dSm-1 percent against concentration of salt six percent dSm-1
5.	Cotton (yield reduced seven point seven Slop % per dSm-1 percent against concentration of salt five point five dSm-1

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