Mugla Journal of Science and Technology, Vol 3, No 1, 2017, Pages 73-85

Mugla Journal of Science and Technology

MOLECULAR AND BIOTECHNOLOGICAL APPROACHES TO INCREASE SALT TOLERANCE IN SOME CROP PLANTS

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Received: 19.01.2017, Accepted: 09.05.2017 doi: 10.22531/muglajsci.286569

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Abstract

Salinity is the most important abiotic stress factor limiting the plant development and production. Soil salinity is a principal constraint in food production. Particularly, it is a problem in the arid and semi- arid areas of the world. Recent biotechnological approaches and molecular markers technology studies have contributed greatly to a better understanding of the genetic and molecular bases of plant salt stress tolerance. The development of molecular studies and in vitro selection technology will provide a new opportunity to improve in salt tolerant crops. The genetic and genomic analysis of the traits of plants can contribute to the breeding of salt tolerant crops through DNA molecular markers associated to salt stress resistance.

In this review, use of in vitro culture methods for increasing salt tolerance in crop plants and also molecular marker and genetic engineering studies were evaluated.

Keywords: Salt tolerance, Plant tissue culture, *in vitro* selection, Molecular markers, QTL, Genetic engineering.

BAZI KÜLTÜR BİTKİLERİNDE ARTAN TUZ TOLERANSINA MOLEKÜLER VE BİYOTEKNOLOJİK YAKLAŞIMLAR

Öz

Tuzluluk, bitki gelişimini ve üretimini sınırlayan en önemli abiyotik stres faktörüdür. Toprak tuzluluğu, gıda üretiminde kısıtlayıcı faktördür. Özellikle, toprak tuzluluğu, dünyanın kurak ve yarı kurak alanlarında önemli bir problemdir. Yeni biyoteknolojik yaklaşımlar ve moleküler markör teknolojisi tuz stres toleransının genetik ve moleküler temelinin daha iyi anlaşılmasına büyük katkı sağlamıştır. Tuza dayanıklı bitkileri geliştirmede moleküler çalışmalar ve in vitro seleksiyon teknolojisinin geliştirilmesi yeni bir fırsat sağlayacaktır. Tuz stresi direnciyle ilişkili DNA moleküler markörlerini tanımlamak için genetik ve genomik analizlerin kullanımı, tuza dayanıklı bitki gelişiminde ıslah stratejilerini kolaylaştırabilir. Bu derlemede, bazı bitkilerde tuz toleransını artırmak için yapılmış in vitro kültür yöntemleri ve aynı zamanda moleküler markör ve genetik mühendisliği çalışmaları değerlendirilmiştir.

Anahtar Kelimeler: Tuz toleransı, Bitki doku kültürü, *in vitro* seleksiyon, Moleküler markörler, QTL, Genetik mühendisliği

1 Introduction

Salt had been the first stress factor faced during the evolution of life. For that reason, organisms had to improve efficient mechanisms to regulate ions and to stabilize protoplasmic units. Salt resistance is very important to use salty soils which are suitable for agriculturing in drought and sub-tropic areas [1]. It has been speculated that around 20% of the irrigated land in the world is affected by salinity. During the year 2050, it is expected that the increase of salinity in agricultural fields will reduce the land available for cultivation by %50 [2]. So plant breeders pay efforts to develop tolerance cultivars to salt. Classical breeding methods are too slow and have had limited contribution in improvement of salt tolerance [3]. *In vitro* cultures are also used as an alternative strategy.

Growing period and development of plants are highly affected from salinity. So growth ratio and biomass production is a reliable measure to determine the salinity degree and salt resistance of plant [1]. However, further research studies should concentrate on combining the growth and measurements of biophysical and biochemical plant characteristics. Thus, such a combined research may promote the discovery of high plant productivity traits in saline environments. Furthermore, crop plant resistance to salinity can be improved by defining the mechanisms of salt tolerance at the germination stage [4].

Selecting the salt tolerant cultivar is one of the important ways to deal salinity [5]. Tissue culture techniques are accepted as important and fast methods [6, 7]. There are many reports about *in vitro* selection related to salt tolerance. It is possible to reach to agriculturally aimed phenotypes with studies based on the production of salt tolerant lines with genetic variability for tolerance to salt in plant tissue cultures, selection of these lines (somaclonal variation and *in vitro* selection and regeneration from these cell lines [3,5,6,7]. Biotechnological studies about salt tolerance in plants have been carrying on as well [8].

Salt stress is typically characteristic with complex quantitative traits. These traits are influenced by a number of environmental and genetic interactions. Salt tolerance traits can be identified through the use of mapping for quantatitative trait loci and genetic approaches. These approaches can facilitate speeding the delivery of crops with improved salt tolerance. So we will continue to use these approaches to identify diverse germplasm in breeding programs.

A significant amount of literature has been reviewed in this study about *in vitro* culture and molecular processes associated with response to salt tolerance.

2 Discussion

In Vitro Cultures and *In Vitro* Selection

Somaclonal variation observed in plant tissue culture, which is useful for breeding, carries a considerable importance. Variation frequency changes with what tissue culture technique is used and somaclonal variation is seen more often in callus cultures and cell suspansion cultures [9]. For that reason, callus cultures were used rather than the other cultures in *in vitro* studies on *in vitro* salt tolerance breeding. Studies on this matter on various plants were given with different culture types in below.

Many strategies are in progress for the devolopment of salt tolerant plants. *In vitro* culture especially *in vitro* selection procedure by genetic transformation through Agrobacterium offers an efficient tool for development of such tolerant lines. A significant amount of papers have been published on developing salt tolerant cell/callus lines using *in vitro* technique (Table 1).

In vitro selection for salt resistance were reported in most plants [2, 5, 10-25]. Using *in vitro* selections widely has a significant effect on getting fast results.

In addition, to control *in vitro* conditions continiously, in comparison to *in vivo* conditions and to have chance to do the selection along the year without some risks as in greenhouses or fields are certain advantages [9]. The development of *in vitro* selection technology together with molecular approaches will provide a new oppurtinity to improve salt stress in plants.

Callus Culture

Successful studies have been carried out, related to obtaining salt tolerant callus/cell lines, selections of those lines and regeneration of tolerant plants using different plant pieces as explants (mature embryo, immature embryo, leaf cotyledon, vb.) in the callus cultures.

Callus cells of 'Shamouti' (*Citrus sinensis* Osb.) which are capable of growing in the presence of NaCl (0.2 M) were selected. Those selected cells for tolerance to NaCl were also tolerant to other sodium salts [26]. Sodium chloride tolerant embriyogenic callus of *Dactylis glomerata* L. were selected and their growth characteristics and some other traits were evaluated. Selected and non- selected lines were compared to understand the mechanisms of tolerance growth. There were significant differences in growth characteristics with increased NaCl but not significant difference due to proline accumulation [27].

Rice is one of the plants, which many studies of salt resistance breeding with tissue cultures have been carried out on. Cell lines which were tolerant to salt were selected with single step selection process in *in vitro* conditions and plants were regenerated from these tolerant lines [28].

Callus culture of mature embryos and regenerated lines from NaCl adapted callus are also salt tolerant [29]. Liua and Baob (1998) released a sufficient protocol for selection of salt tolerant lines on rice (*Oryza sativa* L.)[5].

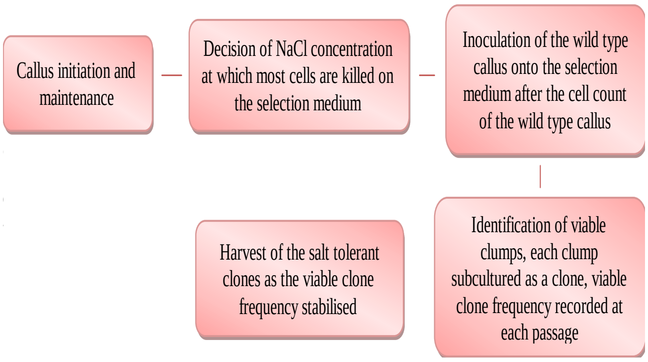

Figure 1. A sufficient protocol for selection of salt tolerant lines on rice [5].

Following studies were in the direction of improvement of callus regeneration in the presence of NaCl as well [30]. This shows that rice improvement through somaculture is promising [31].

In *in vitro* culture of *Cymbopogan martini* (Roxb.) NaCl tolerant lines were produced and obtained by exposing the callus to increasing concentrations of NaCl (0- 350 mM) and thereby the degree of NaCl tolerance of the selected lines were demonstrated [14]. In *Brassica juncea*, NaCl tolerant callus lines were produced with gradual adaptation procedure as well [12,13].

In wheat (*Triticum turgidum* var durum) callus production of 28 cultivars were evaluated with immature embryo culture and *in vitro* salt tolerance. For salt tolerance, the relative fresh weight growth and necrosis percent of callus were used [32]. Plant regeneration from callus initiated at high NaCl levels with immature embryo culture of seven cultivars of durum wheat may be a valid selection method for salt tolerance for future studies. [18]. In durum and bread wheat, studies in order to get optimal callus induction and plant regeneration and improved induction of embryogenic cultures has been described [33].

In potato, salt tolerant regenerants were produced from fast growing cell lines *in vitro* on NaCl (60- 450 mM) supplemented medium following subcultures. It was confirmed that the salt tolerant regenerants differed genotypically from the control with DNA fingerprinting by RAPD's and 70 different primers. That permitted isolation of stable salt tolerant cell lines *in vitro* recurrent selection procedure was reported [34].

In cotton the key factor for succesfully obtaining salt resistant regenerated plants was to select mutants from over one-year-old embryogenic calli [35]. In sunflower, salt-adapted callus were used in the same way [36]. In *Actinidia deliciosa*, NaCl-tolerant somaclons were obtained [37].

Table 1. Selective examples of *in vitro* culture induced by salt tolerance.

Culture type	Plants	*In vitro* selection	References
Callus	*Hedera helix*	*In vitro* salt tolerant cell lines	50
Callus	*Sonneratia alba*	*In vitro* salt tolerant cell lines	51
Callus	*Spartina patens*	To obtain salt-tolerant lines through tissue culture and plant regeneration	52
Callus	*Oryza sativa*	To obtain salt-tolerant lines through tissue culture and plant regeneration	28
Callus	*Oryza sativa* var.	To obtain salt-tolerant lines through tissue culture and plant regeneration	29
Callus	*Oryza sativa L.*	*In vitro* selection of salt tolerant cells	5
Callus	*Oryza sativa* L.	To obtain salt-tolerant lines through tissue culture and plant regeneration	30
Callus	*Solanum tuberosum*	To produce salt tolerant cell lines	34
Callus	*Citrus sinensis* Osb.	To determine salt-tolerant lines through tissue culture and plant regeneration	26
Callus	*Helianthus annuus* L.	proline, ethylene and polyamine exchange in salt tolerant callus line	36
Callus	*Dactylis glomerata* L.	To determine salt tolerant and non-tolerant lines the differences in growth due to increased salt	27
Callus	*Brassica juncea*	To obtain salt tolerant lines	13
Callus	*Medicago sativa*	To determine the effect of proline and salt in callus lines	53
Callus	*Zea mays*	To determine the most sensitive NaCl lines	54
Callus	*Gossypium sp.*	To obtain plantlets from salt tolerant embryonic callus	35
Callus	*Nicotiana tabacum* L	To determine the effect of salt and osmotic shock in tobacco callus lines	12
Callus	*Triticum turgidum* var. *Triticum aestivum* L	*In vitro* production of salt tolerant plants	32
Callus	*Tritucum turgidum* var. durum L.	To obtain salt-tolerant lines through tissue culture and plant regeneration	33
Callus	*Cymbopogon martini* Roxb	*In vitro* selection	14
Callus	Wheat cultivars	*In vitro* selection of salt tolerant wheat cultivars	18
Callus	Populus euphratica	To investigate effect of salinity on growth	55
Callus	Sugarcane	Selection of callus cultures and response to salt stress	20
Callus	*Chrysanthemum morifolium*	To develop of NaCl tolerant line	22
Callus	*Arabidopsis*	To investigate ethylene and nitric oxide under salt stress	56
Callus	*Sesuvium portulacastrum*	To investigate biochemical, physiological and growth changes in respose to salinity	57
Callus	*Pisum sativum*	To quantify the effect of NaCl treatment of salinity tolerance	58
Callus	*Triticum aestivum*	Plantlets regeneration	59
Callus	*Wheat*	To obtain salt tolerant cell lines	60
Cell suspension culture	*Tomato*	To determine effects of salt adaptation and salt stress	61
Cell suspension culture	*Gossypium herbaceum*	To obtain salinity tolerance and antioxidant plants	42
Cell suspension culture	*Medicago media* Pers.	To regeneration plants from the salt tolerant cultures	39
Cell suspension culture	*Medicago media* cv. Rambler	To regeneration plants from the salt tolerant cultures	40
Embryo	*Persea americana* Mill.	To obtain salt tolerant plants	17
Anther	*Oryza sativa*	To obtain salt tolerant haploid lines	45
Anther	*Oryza sativa*	To obtain salt tolerant double haploid lines	46
Anther	*Wheat*	To investigate the response of wheat genotypes to salinity	62
Anther	*Wheat*	To produce multiple shoots for the tested in different salt concentrations	63
Shoot Apex	*Lycopersicon esculentum*	To obtain salt tolerant in wild tomato species	15
Shoot Apex	*Lycopersicon esculentum*	To evaluate salt tolerance in cultivated tomato	48
Shoot apex	*Eucalyptus camaldulensis*	To determine the effect of salt stess and abscisic acid on proline production, chlorophyl content and growth	64

Cell Suspension Culture

Several scientists also released some methods which were on *in vitro* selections of NaCl tolerant plants with subcultures of fast growing friable callus through callus cultures and cell suspesion cultures obtained from those callus [38-43].

Protoplast Culture

As it was in callus cultures, it has been stated that plants regenerated from protoplasts showed much more variations, compared to those produced from leaf and stem cells. In tomato, salt tolerant lines were obtained from tissue culture and protoplasts [44].

Embryo Culture

Studies were also performed on the mature embryo culture of avocado (*Persea americana* Mill.) and by *in vitro* selection. It has clearly been seen that using embryo culture technique can give supportive results to select rootstocks for their tolerance to NaCl [17].

Anther Culture

In rice, the progress of NaCl tolerance breeding is being fastened by using anther culture technique [45] and the high selection frequency of salt tolerant doubled haploids can be obtained [46].

Shoot Apex Culture

An alternative screening method involving *in vitro* shoot apex culture could be a better system for testing and selecting for salt tolerance [47].

The results suggest that shoot apex culture may be useful for rapid evaluation and screening of tomato segragant populations parameters [15]. Shoot apex cultures were applied to salt tolerance in different tomato species.

Mercado *et al.*, (2000) also investigated two tomato cultivars to screen salt tolerance with *in vitro* tests (0, 43, 86, 129 and 172 mM NaCl) and suggested that this approach may not be a reliable tool to evaluate salt tolerance in cultivated tomato [48]. Mills and Tal (2004) explain that organs or plants grown *in vitro* don't always exhibit the same responses to salinity as the whole plant of same species grown *ex vitro* [49].

Some Parameters Measured on *In Vitro* Salt Tolerant Studies

Mainly, properties related to growing on saline medium are evaluated. In addition some physiological properties are evaluated. Some parameters are callus induction, callus yield, cell growth, number of somatic embryos formed per explant, plant regeneration, morphological features, viability, ion compartmentation, cholorophylly fluorescence test, Cl concentrations of roots and shoots, Na+ / K+ ratios, polypeptide patterns, activities of enzymes, proline content polyamines. Patnaik and Debata (1997) stated that the degree of NaCl tolerance of the selected line represented a positive correlation with proline accumulation and a negative correlation with the K+/Na+ ratio [14].

In sunflower, calli adapted or non-adapted to salt stress, a close relationship was observed among proline, polyamines and ethylene biosynthesis. The possibility of control of ethylene production by polyamines as another mechanism involved in salt stress tolerance could not be excluded. There is a direct evidence of the osmoprotectant role of proline in sunflower salt adapted callus [36].

In maize, in a study to select genotypes which are sensitive and tolerant to NaCl; callus colour, proliferative ability, shape, rhisogenesis (morphological particularities), type of callus formed, number of somatic embryos, embryogenic area and callus weight were all evaluated as parameters [54]. There are some studies, with DNA analyses, showing that genotypes selected to be tolerant to NaCl are different from controls [34]. Also Ganggopadhyay et al. (1997b) showed the possibility of using the acid phosphatase isozymic band profile as a spesific marker for osmotic stress and for screening of somaclones [13].

Scientists focused on biochemical changes in superoxide dismutase, glutathione reductase activity, proline content and all basic informations like chromatic effect and root growth at subcultures of plantlets on *in vitro* saline medium.

Paralelly with the increase of NaCl concentration, there was an increase in mitotic inhibition, superoxide dismutase activity and proline content in root but a decrease in root growth.

In Colocosia, as the salinity increases, calcium, potassium, magnesium and sodium contents of tissues decrease [65].

In potato, it was stated that proline accumulation could be used as biochemical marker for salt tolerance and there was a positive correlation of salt tolerance with leaf proline content [51].

In rice, as the endogen proline content of salt tolerant lines are more than the one in control lines [30], there have been some studies that no significant differences could be seen either [28].

Shah *et al.* (2002) observed that as the salinity level increased from medium to higher levels, the Na+ and K+ contents of all lines has also increased and also, the increase in NaCl stress has led to an increase in proline contents of all lines [41].

Molecular studies

Abiotic stress especially salt stress has a major impact on crop yield. The stress tolerance mechanisms a plant needs to employ will be controlled by a variety of factors such as overexpression of specific transcription factors, overproduction of osmoprotectants. For example overexpression of transcprition factor as avaiable way of improving crop salinity tolerance has also been reported numerous times in Arabidopsis, rice, wheat, tomato, alfa alfa (Table 2)[66, 67]. Increasing salt tolerance has been an important breeding aim especially for ecomomically important crops, such as rice, tomato, potato. The molecular marker systems such as RFLP (**R**estriction **F**ragment **L**ength **P**olymorphism), AFLP (**A**mplified **F**ragment **L**ength Polymorphisms), SSR (**S**imple **S**equence **R**epeats), SNP (**S**ingle **N**ücleotide **P**olymorhphisms) led to the construction of high density DNA marker maps of these plants.

Table 2. Transgenic approaches to genetic engineering salt tolerance in some crop plants [66,67].

Gene /protein	Source	Transgenic crop	Defined transgenic plants performance	References
Ascorbate peroxidase	*Arabidopsis*	Tobacco	Maintenance of photosynthetic efficiency	68
Calcineurin- B like interacting protein kinases (CIPK)	*Arabidopsis*	Barley	Altered Na $^+$,K$^+$, Cl $^-$accumulation	69
Na+/H+ antiporter (SOS1)	*Arabidopsis*	Tobacco	Altered shoot and root accumulation of Na $^+$,K$^+$	70
AtRabG3e/vesicle	*Arabidopsis*	*Arabidopsis*	Induction of salt tolerance	71
ProDH/proline dehydrogenase	*Arabidopsis*	*Arabidopsis*	Antisense transgenics more tolerant to freezing and high salinity	72
AtNHx1/ Na+/H+ antiporter	*Arabidopsis*	Tomato	Transformants with sustained growth in high salt (200 mM) without Na + accumulation in fruits	73, 74
CodA/choline oxidasea	*Arthrobacter globiformis*	*Arabidopsis*	Transformants tolerant to salt	75
Gly1/glyoxylase-1	*Brassica juncea*	Tobacco	Overexpressors with tolerance to methyloxal and high salt	76
Mitogen activated protein kinase (MAPK)	*Chickpea*	Tobacco	Improved biomass production	77
GST/ GPX- Glutathione-s-transferase/glutathione peroxidase	*E. coli*	Tobacco	Overexpressors of GST/GPX are stilumated	78
BetB/betaine aldehyde dehydrogenase	*E. coli*	Tobacco	Transformed plants with better growth in osmatic stress	79
Mt1D/ mannitol-1 phosphate dehydrogenase	*E. coli*	Tobacco	Transformants with better growth under salt stess	80
mtD1/ mannitol-1 phosphate dehydrogenase	*E. coli*	Tobacco	Transformants more tolerant to salt	81
Mannitol-1-phosphate Dehydrogenase (mt1D)	*E.coli*	Rice	Inreased growth	82
ectA, ectB, ectC/ L-2,4- Diamino-butyric acid acetyltransferase, L-2,4-diamino butyric acid transaminase, L- ectoine synthase	*Halomonas elongata*	Tobacco	Transformants with increased tolerance to hyperosmatic stress	83
Myo- inositol O-methytransferase	*Masembryanthemum crystallinum*	Wheat	Maintenance of photosynthetic efficiency	84
Imt1/ Myoinositol –o-methytransferase	*M.crystallinum*	Tobacco	Transformants better adapted to salt stress	85
Gpd Nicotinamiddinükleotid dependent glyceraldehydes 3-phosphate dehydrogenase	*Pleurotus sajor-caju*	Potato	Transformants with salt stress tolerance	86
L-myo-inositol 1-phospase synthase (MIP)	*Porteresia coarctata*	Tobacco	Reduced wilting	87
OSISAP1/ Zinc finger family	Rice	Tobacco	Improved growth under several stress conditions	88
Oscdpk7/Ca dependent protein kinase	Rice	Rice	Induction of stress- responsive genes in response to salinity	89
Na+/H+ antiporter	Rice	Tomato		90
Trehalose-6-phosphate Phoshatase(TPP)	Rice	Tomato	Improved plant survival	91
Na+/H+ antiporter (SOD2)	*Salicornia brachiata*	Rice	Provided a potential tool for improving salt tolerance	92
Tsi1 /tobacco stressinduced gene	*Tobacco*	Tobacco	Tolerance to salinity	93
Hal1/ protein involved in regulation of K+ transport and Na+ extrusion	Tomato	Tomato	Transfotmants with higher level salt tolerance	94
Hal1/ protein involved in regulation of K+ transport and Na+ extrusion	Tomato	Tomato	Transgenics able to retain more K+ than control under salt stress	95
Glutathione S-transferase (GST)	Tomato	Rice	Maintenence of growth	66
Cnb1/calcineurin	Yeast	Tobacco	Strong tolerance to salinity	96
Na +transporter (HKT subfamily 1) **Na+/K+ transporter (HKT subfamily 2)**	Yeast	Barley	To increased salt tolerance by reinforcing the salt inclucing	97

Several crops have already been screened for salt tolerance. A lot of traits (such as; germination rate, shoot growth, photosynthesis, shoot Na$^+$ accumulation, shoot K$^+$ accumulation, ion transport and grain filling etc.) for salinity tolerance for which QTL (**Q**uantitative **T**rait **L**oci) have been identified (Table 3)[98]. Additionally a lot of strategies have been suggested about genes/proteins related to salt stress tolerances in plants. This strategies involve many molecular processes such as mutation breeding (designing new phenotypes), transgenic modification, map-or genome sequance based QTL analysis, genomics, signaling molecules, transcriptional and post transcriptional mechanism, translational mechanism [68, 99].

Table 3. A selection of QTL and genes along with salinity tolerance in some crop plants-motified aftter Roy et al 2011-[98].

Trait measured	Species	Markers	Chromosome	References
Chlorophyll content	Barley	RFLP	QFv2H, QCh7Ha,Qch2Ha and QWSC2H	100
	Bread wheat	Not mentioned in the article	3D,7A	101
	Bread wheat	SSR	5B	102
	Rice	AFLP, SSR	2, 3, 4	103
Dry matter production	Barley	AFLP, SSR	2H, 4H, 5H	104
	Barley	AFLP	7H	105
	Barley	Not mentioned in the article	1H,2H,5H,6H	106
	Barley	DArT, SSR	2H	107
	Bread wheat	Not mentioned in the article	1A, 3B	101
	Rice	Not mentioned in the article	5, 6, 10	108
	Rice	AFLP	6	109
Germination rate	*Arabidopsis*	AFLP	RAS1	110, 111
	Arabidopsis	Not mentioned in the article	QTL1 QTL2, QTL3, QTL5	106
	Barley	Not mentioned in the article	4H, 5H	106
	Bread wheat	Not mentioned in the article	6H,7H, 3A, 4D, 5A	105
	Barley	DArT, SSR	6H	107
	Rice	RFLP	6,7	108
Grain Yield	Bread wheat	AFLP RFLP, SSR	1B,2B,3D,4A,4B	112
	Rice	Not mentioned in the article	7	113
Seedling survival	Barley	SSR	1H,2H,5H,6H and 7H	114
	Rice	ESTs	1, 6, 7	115
Seedling vigor	Rice	AFLP, SSLP	1,3	116
	Rice	RFLP	7	117
	Rice	RFLP	6	107
	Rice	SSR	qRL-7,qDWRO-9a and qDWRO-9b, qBI-1a and qBI-1b	118
Shoot K^+ concentration	Bread wheat	EST	5A	119
	Bread wheat	SSR	5A,5B,5D	120
	Rice	AFLP	1,4	109
	Rice	ESTs	1	115
	Rice	AFLP, SSR	1	103
	Rice	EST	qSNC-7 qSKC-1	121
Shoot Na^+ concentration	Barley	RFLP	1H	122
	Barley	DArT, SSR	2H,	107
	Barley	CAPS, DArT, SSR	7H	123
	Bread wheat	DArT, SSR	2A,2B,7A	120
	Durum wheat	SSR	2A	124
	Rice	AFLP	4,6	109
	Rice	ESTs	7	115
	Rice	AFLP, SSR	1	103
	Rice	RFLP	1, 4, 12	125
	Rice	ESTs	qSNC-7, qSKC-1	115
	Rice	AFLP, SSR, RFLP, isosyme	qST1, qST3	116
	Wheat	ESTs	TmHKT7-A2	126
Na^+:K^+ ratio	Barley	DArT, SSR	6H	107
	Bread wheat	AFLP, SSR, RFLP	5A	127
	Bread wheat	AFLP, SSR, RFLP; RFLP	4D	124, 127
	Rice	AFLP	1,4	109
	Rice	RFLP, SSLP	1	128
	Rice	AFLP, SSR	1,9	103
	Rice	RFLP	1, 4, 12	125
	Rice	SSR	QNa, QNa: K, SKC1/OsHKT8	129
	Rice	SSR	qDM-3 and qDM-8, qSTR-6	130
	Rice	SSR	Saltol	103
	Rice	SSR	Saltol and non Saltol	131
	Rice	SSR	qNAK-2 and qNAK-6	132
Tiller number	Barley	AFLP, SSR	7H	104
	Barley	DArT, SSR	4H	101
	Barley	SSR	SSR	133
	Bread wheat	SSR	5A	120
	Rice	Not mentioned in the article	6	113

The use of advanced molecular biology techniques may faciliate devolopment of plants with improved salt tolerance as described in Table 2 and Table 3.

Genomics-based strategies such as gen dicovery, QTL/physical mapping, MAS, genetic engineering lead to the process of crop improvement against abiotic stress. Several screning and selection schemes have been proposed for abiotic stress tolerance. For example, Datta (2002) has designed multidisciplinary approach for abiotic stress tolerance in rice (Figure 2) [134].

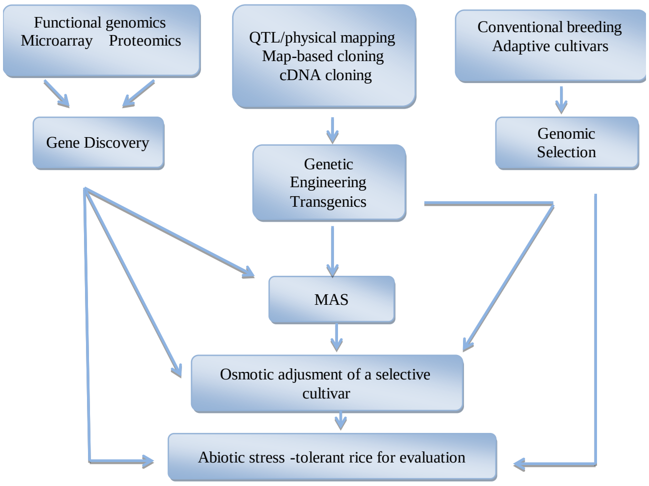

Figure 2. After, Datta (2002) A schematic illustration of multidisciplinary approach for abiotic stress tolerance in rice [134].

Transgenic plants with halophyte genes have shown positive results with their tolerance to salt and other abiotic stress [135, 136]. Research *Arabidopsis* and rice have been essential for plants to cope with salt stress in different crops [99]. A number of genes (**Ion transporter**: *Arabidopsis*; 136-141, Tobacco; 142, 143, rice; 144-146, **osmolyte biosynthesis:** *Arabidopsis*; 135, Tobacco and rice; 147, **genes antioxidative enzymes:** *Arabidopsis*: 148, 149, Rice: 150, Tobacco: 151) isolated from haloypytes have been transferred to glycophyte plants trough different genetic transformation techniques [152].

Higher accumulation of NA^+ was related to salt sensitivty of plants by the studies of some researchers [153]. Na^+/H^+antiporter would be a promising target for genetic engineering for improving salt tolerance [154].

Overexpression of the *Arabidopsis* tonoplast membrane bound Na+/H+antiporter AtNHX1 gene, under a strong constitutive promotor resulted in salt tolerant *Arabidopsis* as reported for tomato [73], wheat [155], tobacco [156].

3 Conclusion

Salinity is one of the most serious threats to agriculture in many parts of the world. To obtain salt tolerant plants, two essential subjects for plant breeding are the presence of genetic variation and an efficient selection method. Somaclonal variation in tissue cultures is used as fast variation resource. On the other hand *in vitro* selection of mutants have some advantages compared to *in vivo*. For that reason, successful studies show that somaclonal variations and *in vitro* selections are important potentials for plant breeding.

The development of molecular studies and *in vitro* selection technology will provide a new opportunity to improve in salt tolerant crops. The genetic and genomic analysis of the traits of plants can contribute to the breeding of salt tolerant crops through DNA molecular markers associated to salt stress resistance. Molecular biology techniques and molecular markers can be used to map QTL for salt tolerance and to dissect the genetic basis of salt tolerance in plants. Therefore, it is estimated that future studies on molecular marker technology and genetic transformation may contribute to the development of salt-tolerant crop plants. Similarly, salt tolerance in plants may also be developed by the use of marker assisted selection.

Among the avaliable molecular and biotechnological tools for genetic engineering crop breeding based on introgression of genes have proved to be the most effective approaches for developing salt tolerant plants.

4 References

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