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Approaches for enhancing salt tolerance in mulberry (*Morus* L) -A review

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Abstract

Mulberry (*Morus*) is a deciduous woody tree with moderate tolerance to salinity, which has great economic importance as its leaf is used for feeding the monophagous silkworm *Bombyx mori* L. Mulberry leaf is also used as fodder for livestock. The fruits of mulberry are highly nutritious and have many medicinal properties. In this review, effects of salinity on the morphological, physiological, biochemical and genetic characters in mulberry were discussed along with recent developments in salt stress research. Since mulberry is moderately resistant to salinity and a great degree of genotypic variation is found, it is possible to develop varieties with enhanced salt tolerance if appropriate strategies and techniques are adopted. An outline of such strategy was also drawn and discussed. Development of salt tolerant varieties can expand mulberry cultivation into the salt affected marginal lands, thereby increasing the availability of leaf for both sericulture and livestock industries in arid and semiarid regions of India and other Asian countries.

Keywords: Mulberry; sericulture; salinity; silkworm

Introduction

Mulberry (*Morus,* Moraceae) is a fast growing deciduous woody tree with alternate leaves, unisexual to bisexual flowers in the leaf axils, and fleshy fruits (sorosis). It is believed that mulberry first originated in the foothills of Himalayas and later dispersed into Asia, Europe, Africa, and America (Sanchez, 2000a, b). Currently, mulberry is growing in regions between 50^oN Lat. and 10^oS Lat. (Yokoyama 1962); from sea level to altitudes as high as 4000 m (Machii et al., 1999; Tutin et al., 1996). Taxonomically, the genus, *Morus,* is divided into two sections, the *Dolichostylae* (long style) and the *Macromorus* (short style) and each section was further divided into two groups namely *Papillosae* and *Pubescentae* based on the nature of stigmatic hairs. For further classification characters of leaf, inflorescence and sorosis were used (Koidzumi 1917; Engler and Prantl 1924; Bounocore 1941; Ledebour 1951; Hotta 1954, Katsumata 1972). So far, more than 150 species of mulberry have been cited in the Index Kewensis, but majorities of them have been treated either as synonyms or as varieties rather than species, and some have also been transferred to allied genera (Sharma et al. 2000). A few prominent species of *Morus*, which have wide acceptance among mulberry taxonomists and geneticists, are *M. alba, M. indica, M. serrata*, *M. laevigata, M. multicaulis, M. tartarica*, *M. nigra M. australia, M. cathyana*, *M. mierovra M.*

atropurpurea , *M. mizuho, M. rubra, M. insgnis, M. mesozygia,* and *M. macroura*. The species availability in different countries is given Table 1.

Economic importance of mulberry

The economic importance of mulberry is primarily due to its leaf, which is being used for feeding the silk producing insect *Bombyx mori* L. In China, India, Japan, Korea, Pakistan, Bangladesh and in many other Asian countries sericulture (rearing of silkworms for the production of silk fibers) is one of the major rural industries that provide employment to a large number of people. Das and Krshinaswami (1965) estimated that nearly 60% of the production cost of silkworm cocoon is incurred by mulberry leaf production. Thus, mulberry varieties with higher leaf yield are important to sustain profitability in sericulture. Therefore, through traditional breeding methods, a number of high yielding mulberry varieties have been developed in India, China and other countries (Table 2A, B). In addition to the use in sericulture, mulberry leaf is also used as animal fodder because it is highly nutritious, palatable and digestible (70-90 %) to herbivorous animals like cow, sheep goat and buffalo. The protein content in the leaves and young stems varies from 15 to 28 %, depending on the variety. The mineral content in mulberry leaf is also reported to be high and no anti-nutritional factors or toxic compounds have been identified (Sanchez, 2000b). Feeding experiments in countries like Cuba, Tanzania and Guatemala showed that mulberry is excellent forage for livestock, especially during the dry seasons (Benavides et al., 1994; Castro, 1989). Further studies on the economic viability of combining sericulture with dairy production in India produced excellent results (Mehla et al., 1987). Investigations on the possibilities of placing mulberry for direct browsing by cattle in Italy (Talamucci and Pardini, 1993), France (Armand and Meuret, 1995), Tanzania (Shayo, 1997) and Japan (Kitahara, 1999) showed promising results (Sanchez, 2000b). In addition to leaves, mulberry fruits are also valued well (Ercisli, 2004). Some species like *M. laevigata*, *M. rubra*, *M. nigra* and *M. alba* are grown exclusively for their fruits as their leaves are not suitable for feeding the silkworms. Like wise, the black mulberry (*M. nigra*) is extensively cultivated in Turkey (Gökmen 1973) for its fruits. With its Mediterranean climatic conditions, Isparta Province in the Lakes Region is notable for populations of black mulberry (Baytop 1984). The fruits of mulberry has a tonic effect on kidney energy, and thus, it is used as an antiphlogistic, a diuretic and an expectorant (Koyuncu, 2004). Mulberry is also used for landscaping in Asia, Europe and America (Tipton, 1994).

Importance of salt tolerance in plants

It is estimated that nearly 19.5% of the irrigated agricultural lands are considered salt affected (Flowers and Yeo, 1995) and nearly 2 million hectors of agricultural lands are additionally affected by salinity every year (Kalaji and Pietkiewica, 1993). Amelioration and utilization of these saline soils have been the focus of research for the last few decades. Many technical measures, physical, chemical and mechanical, have been developed to reclaim the soil, however, due to economic viability and ecological concerns those technologies became less attractive to the farmers. Under these circumstances, the only viable alternative is to identify or develop suitable salt tolerant crops to cultivate in these saline soils. However, developing salt tolerant crop is not an easy task because salt tolerance is a polygenic trait. Therefore, integration of knowledge on physiological, biochemical and genetic aspects of salt tolerance is essential to make any progress in this regard (Ashraf and Foolad, 2007).

Saline soils

Generally, salt affected areas are of two categories, the sodic and the saline. The major differences between these two types of salinity are the nature of anions and the pH of the soil. In sodic soils carbonate or bicarbonate are the major ions whereas in saline soil chloride or sulphate dominate. The pH of the sodic soil is mostly above 8.5 while in saline soils it is <8.5. Soil salinity is, generally, measured in units of electrical conductivity (dS $m^{-}1$) of a saturated soil paste extract (ECe). If the ECe is >4 dS m^{-1}, the exchangeable sodium percentage is <15% and pH <8.5 the soil can be considered as saline (Szabolcs, 1994). Although most of the agriculturally important crops cannot grow in saline soils, it is entirely not inimical to growth of all plant species. Some plants grow well in salt affected coastal areas, shores of backwaters lakes and marshy lands. Those plants that can survive and grow well on high concentrations of salt in the rhizosphere are called halophytes. However,

Table 1. The species availability and germplasm conservation of mulberry in different countries

Sl no	Country	Total species available	Endemic species	Total germplasm holdings
1	China	24	17	2600
2	Japan	19	14	1312
3	South Korea	6	1	615
4	India	4	3	1109
5	Indonesia	3	2	NA
6	Taiwan	4	1	NA
7	Thailand	2	2	NA
8	Argentina	1	1	NA
9	Columbia	3	1	NA
10	Mexico	3	2	NA
11	Peru	1	1	NA
12	USA	14	9	NA
13	France	NA	NA	70
14	Italy	NA	NA	50
15	Bulgaria	6	1	140

NA - Not available (Source: Rao 2002)

some other plants cannot even tolerate a salinity caused by 10 % of seawater. Such plants are called glycophytes or non-halophytes. For the sake of convenience, in this review salinity responses of glycophytes will only be discussed giving special reference to mulberry and other woody plants, wherever possible. Halophytes and their response to salinity have been reviewed by Cherian et. al. (1999) and Gorham (1995).

Effect of salinity on mulberry

Salinity affects plant growth and developments in a variety of ways depending on the tolerance level of the plant, growth and developmental stages, physiological condition, severity of the salt stress and influence of many other environmental factors such as relative humidity, temperature, radiation and air pollution (Shannon et al. 1994). The most common effects of salinity on glycophytes are loss of turgor, growth reduction resulting in smaller leaves, shorter stature, early senescence, decreased photosynthesis, respiratory changes, loss of cellular integrity, tissue necrosis, and even death of the plant (Cheeseman, 1988). The major reason for the detrimental effects of low to moderate salt concentrations is the negative osmotic pressure caused by the salts in the root zone (Jacoby, 1994). Depending upon the composition of the saline solution, ion toxicities or nutritional deficiencies may also arise because of the predominance of a specific ion or competitions among cations or anions (Bernstein et al., 1974). The accumulation of high concentrations of Na^+ or Cl^- in the leaves generally results in the formation of burning like lesions (Zhu, 2002). The nutritional deficiency may be manifested similar to those that occur in the absence of salinity. Calcium deficiency symptoms are common when Na/Ca ratio is high in soil water. High salinity can also injure cells in transpiring leaves, which leads to growth inhibition (Tuteja, 2007). The salt that concentrates in the old leaves makes them die early (Munns et al., 2006).

The first visible symptom of salt injury in mulberry is the appearance of yellow patches in young leaves under low to moderate salinity (Vijayan et al., 2008a). The yellowing of leaf may be due to degradation of chlorophyll by the increased activity of chlorophyllase (Singh and Singh, 1999). Under higher salinity burnt like lesions appeared in the leaves (Vijayan et al., 2008a). Early senescence of older leaves and retardation of growth followed under higher salinity as the salt promotes senescence of leaves by increasing the production of abscisic acid (ABA) and ethylene (Kefu et al., 1991; Zhao et al., 1992). Salinity adversely affected the growth and, thus, the leaf yield of mulberry, albeit the severity of which varied depending on the tolerance level of the genotype (Fig 1). An increase in soluble sugar under low salinity followed by a sharp decline under higher salinity was observed in some varieties of mulberry (Vijayan et al., 2008a). The leaf pigments, proline, and Na^+ were also increased under salinity in mulberry (Kumar et al., 2003; Ramanjulu et al., 1993, 1994, 2000; Vijayan et al., 2008a). The antagonistic effects of salinity on proteins by breaking electrostatic bonds and increasing hydrophobic interactions (Melander and Horvath, 1977) was much evident in mulberry as the protein concentrations in the leaves of plants grown under salinity declined significantly (Vijayan et al., 2007, 2008a,b). Salinity also affected plant anatomy as leaves of plants growing in saline soils became thicker and more succulent as compared to that of the plants growing in non-saline soils (Shannon et al., 1994; Vijayan et al., 2008a). The epidermal cell walls and cuticles of leaves in salinized plants also became thicker. This increased leaf thickness may be part of the salt tolerant mechanisms as the leaf succulence may increase the CO_2 absorption per unit of leaf area by increasing the internal surface area per unit of leaf surface (Shannon et al., 1994). In mulberry, the increase in leaf thickness in response to salinity was the result of an increase in number of spongy layers rather than an increase in the size of palisade cells (Vijayan et al., 2008a). However, in *Citrus*, the increase was due to an increase in the size of spongy mesophyll cells (Zekri and Parsons, 1990). This increase in the cell size of leaves of salt affected plants was attributed to a corresponding increase in the cell wall extensibility together with higher turgor pressures (Jennings, 1976). Adverse effect of salinity on the rate of photosynthesis was reported in mulberry as in many other woody plants (Golombek and Lüdders, 1993; Kumar et al., 1999; Lakshmi et al., 1996; Tattini et al., 1995; Ziska et al., 1990). NaCl drastically reduced stomatal conductance (g_s) and photosynthetic rate as well. The extent to which stomatal closure affects photosynthetic capacity can be seen from the magnitude of reduction in g_s. The immediate decline in photosynthetic rate in mulberry and other tree plants like olive trees (Lakshmi et al., 1996; Melgar et al., 2008) under salinity is attributed partly to reduced g_s and partly to a decline in photosynthetic pigment concentrations. However, in long term, the reduction in the photosynthesis is the result of inhibition of leaf formation and expansion as well as early leaf abscission (Kozlowski and Pallardy, 1997). Salinity also injured cell membranes and increased solute leakage (Hautala et al., 1992) and the salinity induced cell membrane damage was greatly influenced by the tolerance level of the genotypes in mulberry (Vijayan et al., 2002). It was also seen that NaCl induced membrane leakage was reduced under higher Ca_2^+ (Leopold and Willing, 1984).

Mechanism of salt tolerance

The response of plants to salt and other environmental stresses have been extensively investigated for many decades, still we have not been able to understand fully the mechanism which imparts tolerance to some plants and sensitivity to others (Cheeseman, 1988) due to the complexity of the mechanism (Tuteja, 2007). Nevertheless, investigations on physiological, biochemical, genetic and molecular aspects of salinity tolerance in the model plant *Arabidopsis* and many crop plants such as rice, barley, maize, tobacco and alfalfa have identified a number of mechanisms that are actively involved in imparting salt tolerance to plants (Table 3). In general, the mechanisms of salinity tolerance in plants can be categorized into three (1) tolerance to osmotic stress, (2) Na+ exclusion and (3) tissue tolerance (Munns and Tester, 2008). The osmotic stress is caused by high salts which is present in the root zone. Since osmotic stress causes immediate reduction in cell expansion in roots and young leaves, tolerance to osmotic stress results in less reduction in leaf growth and stomatal conductance.The main site of Na^+ toxicity for most plants is the leaf blade rather than the root tips as Na^+ accumulates in the leaf blades due to continuous translocation and deposition due to transpiration. Thus, it is veryimportant that Na^+ doest not reach the leaf blades in excess as the Na^+ relocation from leaves to roots is likely to be only

Table 2a.. High yielding mulberry varieties developed in India

Variety	Region	Developed at	Origin
Kanva-2	South India Irrigated	CSRTI, Mysore,	Selection from natural variability
S-36	South India Irrigated	CSRTI, Mysore,	Developed through EMS treatment of Berhampore Local
S-54	South India Irrigated	CSRTI, Mysore	Developed through EMS treatment of Berhampore Local
Victory-1	South India Irrigated	CSRTI, Mysore	Hybrid from S30 x C776
DD	South India Irrigated	KSSRDI, Thalaghattapura	Clonal selection
S-13	South India Rainfed	CSRTI, Mysore	Selection from polycross (mixed pollen) progeny
S-34	South India Rainfed	CSRTI, Mysore	Selection from polycross (mixed pollen) progeny
MR-2	South India Rainfed	CSRTI, Mysore	Selection from open pollinated hybrids.
S-1	Eastern and NE India Irrigated	CSRTI, Berhampore	Introduction from (Mandalaya, Myanmar)
S-7999	Eastern and NE India Irrigated	CSRTI, Berhampore	Selection from open pollinated hybrids
S-1635	Eastern and NE India Irrigated	CSRTI, Berhampore	Triploid selection
C776	Saline soils	CSRTI, Berhampor	Hybrid from English balck and C. multiculis
S-146	N. India and Hills of J and K Irrigated	CSRTI, Berhampore	Selection from open pollinated hybrids
Tr-10	Hills of Eastern India	CSRTI, Berhampore	Triploid developed from "S1"
BC-259	Hills of Eastern India	CSRTI, Berhampore	Back crossing of hybrid of Mati-gare local x Kosen with Kosen twice
Goshoerami	Temperate	CSRTI, Pampore	Introduction from Japan.
Chak Majra	Subtemperate	RSRS, Jammu	Selection from natural variability
China White	Temperate	CSRTI, Pampore	Clonal selection

Adopted from Datta (2000).

a small portion of what delivered to the leaf. Na^+ enters roots passively, via voltage independent non-selective cation channels and via other Na^+ transporters such as members of the high-affinity K^+ transporter (HKT) family (Laurie et al., 2002). Most of the Na^+ that enters root cells in the outer part of the root is likely to be pumped back out again via plasma membrane Na^+/H^+ antiporters (Tester and Devenport, 2003). Although, identities of the genes that encode the Na^+ efflux proteins are yet to be found out, recent research has confirmed the involvement of a plasma membrane protein *SOS*1 in Na^+/H^+ antiporter activity (Apse et al., 1999; Zhang and Blumwald, 2001). Discoveries of another two genes such as *SOS2* and *SOS3* and their interaction with *SOS*1 in ion homeostasis further confirm the importance of *SOS*1 in sodium ion homeostasis in plants. It is found that under salt stress the *SOS*3, a calcium sensor protein that is constitutively myristoylated and associated with the plasma membrane, activates *SOS*2 kinase in a calcium dependent manner. The *SOS*3-*SOS*2 kinase complex,

in turn, activates *SOS*1 gene expression, this results in a Na^+/H^+ antiporter in the plasma membrane (Chinnuswamy and Zhu, 2003). Since active Na^+ efflux is required in all cells through out the plant, it is likely that other genes encoding Na^+/H^+ antiporters are also exist (Munns and Tester, 2008). The Na^+ that enters into the xylem through the transpirational stream may be retrieved by members of another gene family called *HKT.* In *Arabidopsis* root, *AtHKT*1;1 involved in the retrieval of Na+ from xylem before it reaches the shoot (Davenport et al., 2007). Involvement of *HKT1;5, TmHKT1;4-A2* in maintaining higher K^+/Na^+ ration is becoming more evident in rice *(OsHKT*1:5) and wheat *(TaHKT*1:5) (Blumwald, 1987; Ren et al., 2005). In tree plants like olive trees, the salt tolerance was related to their ability to decrease leaf osmotic potential and Na^+/Cl^- ion exclusion mechanisms in the roots (Tattini et al., 1994). Most of the olive cultivars showed an exclusion capacity of Na^+ such that accumulation of potentially toxic ions in the aerial parts is prevented.

Once the potentially toxic salt starts accumulating in the leaf tissues, due to enhanced absorption and translocation, it has to be tolerated this accumulation of Na^+. This is achieved by separating and sequestering the toxic ions from the metabolic machinery of the cells because most of the metabolic processes are taking place in cytoplasm and the enzymes involved in such processes are mostly sensitive to high Na^+ toxicity. Hence, salts are sequestered safely in the cytosol through a process called compartmentalization. A salt-inducible Na^+/H^+ antiporter gene is involved in the compartmentalization of Na^+ in the vacuoles (Apse et al., 1999). Overexpression of this vacuolar Na^+/H^+ antiporter gene improved salt tolerance in *Arabidopsis* (Apse et al., 1999), tomato and *Brassica napus* (Zhang et al., 2001), and wheat (Xue et al., 2004). The compartmetalization of the salt in the cytosol creates differences in the osmotic potentials between cytoplasm and cytosol, which in turn nece- ssitates accumulation of non-toxic and compatible solutes such as proline, sucrose, polyols, trehalose, glycine betaine, alanine betaine, proline betaine, choline *O*-sulfate, hydroxyl praline betaine, and pipecolate betaine in the cytoplasm (Pollard and Wyn, 1979; Rhodes and Hanson, 1993; Serraj and Sinclair, 2002). These compatible solutes protect plants from stress through different courses, including contri- bution to cellular osmotic adjustment, detoxification of reactive oxygen species, protection of membrane integrity, and stabilization of enzymes/proteins (Table 4).

Proline is one of the most prominent osmolyte plant accumulates for osmotic adjustment under salt stress in mulberry and other plant species (Ramanjalu and Sudhakar, 2000; Vijayan et al., 2008a). Besides being an osmolyte, it stabilizes sub-cellular structures and scavenges free radicals and maintains the cellular redox potential under stress conditions. It also acts as a protein compatible hydrotrope (Srinivas and Balasubramanian, 1995), alleviates cytoplasmic acidosis, and maintains appropriate $NADP^+/NADPH$ ratios compatible with metabolism (Hare and Cress, 1997). Rapid breakdown of proline upon relief of stress often provides sufficient reducing agents to support mitochondrial oxidative phosphorylation to generate ATP for alleviating stress induced damages (Hare and Cress, 1997; Hare et al., 1998). Proline is also known to induce expression of salt stress responsive genes that possess proline responsive elements in their promoters (Chinnusamy et al., 2005).

Exogenous application of proline has also proved effective in alleviating salt stress by providing osmoprotection and thereby facilitating growth under salinity (Yancey, 1994). In rice, exogenous application of 30 mmol L^{-1} proline on seedlings showed less Na^+ or Cl^- concentrations in the leaves of proline treated plants as compared to the same in control (Krishnamurthy and Bhagwat, 1993). However, excessive application of proline was found harmful to plants as it inhibits growth and affects many cellular metabolisms adversely (Nanjo et al., 2003). Therefore, it is essential to determine optimal concentrations of proline and also the developmental stages at which proline can be applied, which vary depending on species and genotype.

Glycine betaine, another compatible solute, is a quaternary ammonium compound that protects the thylakoid membrane of chloroplast under salt stress (Yang et al., 2003). Glycine betaine is synthesized in chloroplast from serine via ethanolamine, choline, and betaine aldehyde (Rhodes and Hanson, 1993). Choline is converted to betaine aldehyde, by choline monooxygenase (CMO), which is then converted to glycine betaine by betaine aldehyde dehydrogenase (BADH). Although other pathways such as direct *N*-methylation of glycine is also known, the pathway from choline to glycine betaine has been identified in all glycine beatine accumulating plant species (Weretilnyk et al., 1989). Exogenous application of glycine betaine has also been shown to enhance salinity tolerance in plants (Lutts, 2000). A comprehensive study on the ameliorating effects of exogenous application of glycine betaine on salt-induced shoot- and root growth inhibition and ultrastructural damages in rice seedlings revealed that

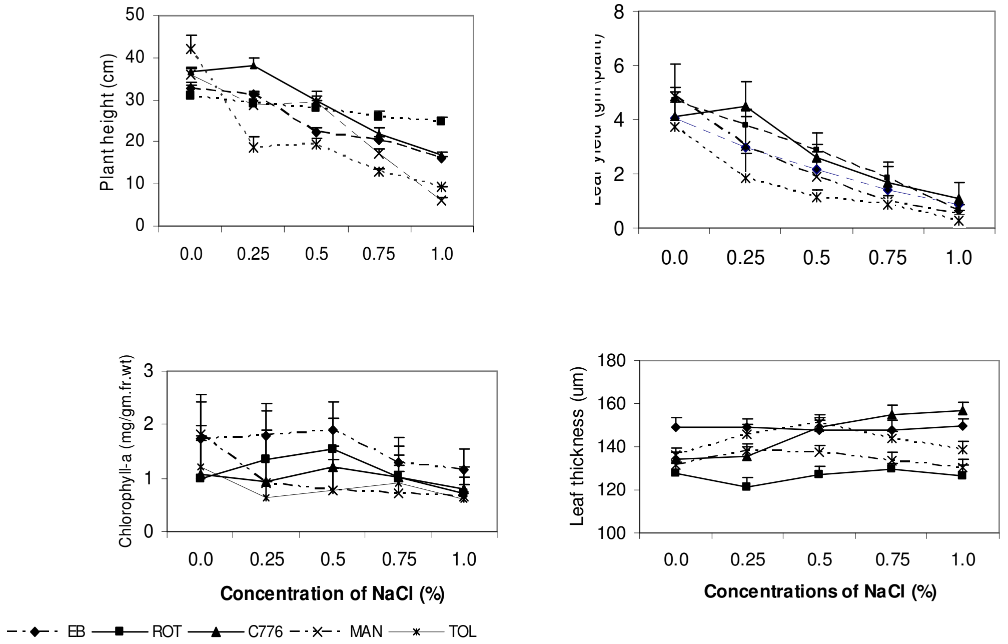

Fig 1. Effect of NaCl on plant height, leaf yield, chlorophyll-a and leaf thickness in four cultivars of mulberry. EB-'English black', ROT-'Rotundiloba',C776-'C776' MAN-'Mandalaya', TOL-'Tollygunj'

glycine betaine treated plants had significantly higher K^+/Na^+ ratio than the controls. Likewise, many of the salt induced ultra-structural damages in the leaf, including swelling of thylakoids, disintegration of grana stacking and intergranal lamellae, and destruction of mitochondria, were less in *Glycine betaine* treated plants as compared with untreated plants (Yang and Lu, 2005).

Another protein that accumulates under high salinity in many plants is the late embryogenesis abundant (LEA) proteins (Rorat, 2006), which protects the plants against salt stress by lowering intracellular water potential, stabilizing membrane structure, binding metal ions and scavenging active oxygen species (Alsheikh et al., 2003). At least six different groups of *LEA* proteins have been reported based on their amino acid sequences, mRNA homology and expression pattern. A stress induced transcription factor *DREB* containing a conserved *AP2*/*EREBP* motif that binds to the promoter region containing the *CRT* (C-repeat)/*DRE* (dehydration responsive element) cis-element, which has the core sequence CCGAC and exists in many *LEA* genes including *ERD*10, *RD*29A, *COR*15a, *COR*6.6 (Yamaguchi-Shinozaki and Shinozaki, 1994). Over expression of *DREB*s induces the expression of these stress-related genes confers the improved tolerance to drought, low temperature and high salinity (Oh et al., 2005). A transgenic mulberry plants overexpressing *HVA1,* a group-3 LEA protein isolated and characterized from barley, showed increased cell membrane stability, higher relative water use efficiency and growth under salt stress in mulberry (Lal et al., 2007).

Salinity stress in plants is thought to enhance the production of many reactive oxygen species (ROS) such as superoxide radicals (O_2^-), Hydrogen peroxise (H_2O_2) and hydroxyl radicals (OH*). ROS are a product of altered chloroplast and mitochondrial metabolism during stress. These ROS cause oxidative damage to different cellular components including membrane lipids, protein and nucleic acids (Vinocur and Altman, 2005). Therefore, plants have developed a number of mechanisms to counteract the damage

Table 2b. High yielding mulberry varieties developed in China.

No.	Variety	Selection and breeding method	Year	Suitable zone
1	Xiansang 305	mutation breeding	2001	The Huanghe River valley
2	Beisangyihao	selected from local seedling mulberry	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
3	Nongsang 8	hybridization breeding	2000	The Changjiang River valley
4	Huangluxuan	selection from local variety	1998	The Huanghe River valley
5	Jihu 4	hybridization breeding	1989	Northeast zone
6	Dazhonghua	polyploidy breeding	1996	The Changjiang River valley
7	Xinyiyuan	mutation breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
8	Nongsang 14	hybridization breeding	2000	The Changjiang River valley
9	Yu 237	hybridization breeding	1989	The Changjiang River valley
10	Xuanqiu 1	selected from local seedling mulberry	1989	Northeast zone
11	7307	selected from local seedling mulberry	1989	The Changjiang River valley
12	Husang 32	selection from local variety		The Changjiang River valley, The middle and lower reaches of the Huanghe River
13	Xiang 7920	hybridization breeding	1995	The Changjiang River valley
14	Canzhuan 4	selected from local seedling mulberry	2001	The Changjiang River valley
15	Huamingsang	selected from local seedling mulberry	1994	chizhou□Xuanzhou□Anqing in Anhui province and Linyi in Shandong province
16	7946	hybridization breeding	1998	The Huanghe River valley
17	Yu 2	hybridization breeding	1989	The Changjiang River valley
18	Shigu 11-6	mutation breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
19	Xuan 792	Selection breeding	1989	The Huanghe River valley
20	Yu 711	hybridization breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
21	Yu 151	hybridization breeding	1989	The Changjiang River valley
22	Hongxin 5	hybridization breeding	1995	The Changjiang River valley, The middle and lower reaches of the Huanghe River
23	Lunjiao 40	selection from local variety	1989	The Zhujiang River valley
24	Wan 7707	selected from local seedling mulberry	1994	chizhou□Xuanzhou□Anqing in Anhui province and Linyi in Shandong province
25	Huangsang 14	selected from local seedling mulberry	1989	The Changjiang River valley
26	Lunjiao 40	selection from local variety	1989	The Zhujiang River valley
27	Shi 11	selection from local variety	1989	The Zhujiang River valley
28	Xinyizhilan	Introduced variety	1995	The Changjiang River valley; The Huanghe River valley
29	Jialing 16	polyploidy breeding	1998	The Changjiang River valley
30	Tang10×Lun 109	hybrid mulberry seed	1989	The Zhujiang River valley
31	Nongsang 12	hybridization breeding	2000	The Changjiang River valley

caused by ROS. Synthesis of low molecular mass antioxidants such as ascorbic acid and reduced glutathione and a diverse array of enzymes such as superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX), glutathione S-transferases (GST) and glutathione peroxidases (GPX) to scavenge ROS are a few among them (Zhu, 2002; Tuteja, 2007; Chinnusamy et al., 2004). The improved seed germination and seedling growth of a transgenic tomato overexpressing S-transferase (GST) and glutathione peroxidase (GPX) indicated that the increased glutathione-dependent peroxidase scavenging activity and associated changes in glutathione and ascorbate metabolism lead to reduced oxidative damage in plants, which in turn enhanced the plant tolerance to salinity stress (Roxas et al., 1997). In mulberry, Harinasut et al. (2003) investigated the salt induced changes in antioxidant enzymes using a salt tolerant cultivar 'Pei'. With increasing salinity, the amount of hydrogen peroxide and the activity of guaiacol-specific peroxides, superoxide dismutase, ascorbate peroxidase and glutathione reductase were enhanced up to a salinity imposed by 150mM NaCl.

Abscisic acid (ABA) plays a central role in root-to-shoot and cellular signaling in salt stress and in the regulation of growth and stomatal conductance. Under salinity ABA level increases due to the activation of genes responsible for ABA biosynthesis. Studies on the tissue distribution and regulation of *AtNHX*1 expression by salt stress and abscisic acid (ABA) showed that the steady state level of *AtNHX*1 transcript is up-regulated by treatment with NaCl, KCl, or ABA. The substantial increase in the promoter activity of *AtNHX*1 under the influence of NaCl, KCl, or ABA demonstrate that salt and ABA regulate *AtNHX*1 expression at the transcriptional level (Shi and Zhu, 2002). Similarly, accumulating evidence now shows that members of a class of negative regulators of growth, the *DELLA* proteins, mediate the growth promoting effects of gibberellins in a number of species and integrate signals from a range of hormones and abiotic stress conditions, including salinity (Munns and Tester, 2008)

Genetics of salt tolerance

The adaptive capacity of plants to perceive and respond to salt stress has been described as a quantitative genetic trait (QTL), thus, it is difficult to manipulate salinity tolerance through conventional breeding and phenotypic selection. Hence, it is necessary to identify QTLs that contribute to natural variation in response to salt stress for understanding the complexity of the genetic control of salt tolerance in tree plants. Although no such work has been carried out in mulberry, recent attempts in developing molecular based linkage map construction (Venkateswaralu et al., 2006) and other genetic assessment of mulberry under salinity (Vijayan et al., 2008b) are pointing to positive developments in this direction. Vijayan et al. (2008b) for the first time investigated the effect of different genetic components and their interaction on the development of various agronomic traits under saline conditions. Under salt stress conditions, a shift from non-additive gene effect to additive gene effect was observed for most of the agronomically important traits and absorption and translocation of Na+.

Effect of microbes in salinity tolerance of plants

Beneficial effects of application of Vesicular arbuscular mycorrhizae (VAM) on plants under salinity have been reported from many plants (Declerck et al., 1995). In most of the cases, it was found that application of VAM is more effective during plant acclimatization (Yano-Melo et al., 1999). Although their sporulation and colonization is inhibited by salinity in some areas, VAM can promote plant growth through improvement of plant nutrition and production of osmoregulators. Foliar application of *Azatobacter chroococum* in mulberry under salinity was found effective in increasing leaf yield and mitigating salinity effects on biochemical constituents in the leaf (Vijayan et al., 2007)

Approaches to develop salt tolerant varieties in mulberry

Improving salinity tolerance of mulberry by genetic means has been an important but largely unfulfilled object of sericultural research in India and many other Asian countries. At the genetic level, salinity tolerance in plants has been considered as a quantitative trait, and, thus, has been resistant to improvement by conventional plant breeding methods. However, the recent advancements in understanding the biochemical mechanisms of salinity tolerance, molecular cloning of genes involved in various pathways that are related with salt tolerance, even from unrelated plants like halophytes, offer new approaches to resolve these perennial problems.

Exploitation of natural genetic variations in mulberry as in other woody trees has long been recognized as a potential means of developing salt tolerant varieties. Accordingly, mulberry germplasm resources from different regions, especially from areas with saline influence like coastal areas, have been collected and are being assessed for their salt responses in

Table 3. Processes, genes and mechanisms of salinity tolerance in plants (reproduced from Munns and Tester, 2008)

Processes	Genes involved	Osmotic stress	Na^+ exclusion	Tissue tolerance
Sensing and signaling in roots	*SOS3, SnRKs*	Modification of long-distance signaling	Control of net ion transport to shoot	Control of vacuolar loading of ions
Shoot growth	--	Decreased inhibition of cell expansion and lateral bud development	---	Delay in premature senescence of old leaves
Photosynthesis	*ERA1, PP2C, AAPK, PKS3*	Deceased stomatal closure	Avoidance of ion toxicity in chloroplasts	Delay in ion toxicity in chloroplasts
Accumulation of Na+ in shoots	*HKY, SOS1*	Increased osmotic adjustment	Reduced long distance transport of Na^+	Reduced energy spent on Na^+ exclusion
Accumulation of Na+ in vacuoles	*NHX, AVP*	Increased Osmotic adjustment	Increased sequenstration of Na+ into root vacuoles	Increased sequenstration of Na^+ into leaf vacuoles
Accumulation of organic solutes	*P5CS, OTS, MT1D, M6PR, S6PDH, IMT1*	Increased Osmotic adjustment	Alteration of transport process to reduced Na^+ accumulation	Accumulation of high concentrations of compatible solutes in cytoplasm

comparison with salt sensitive ruling varieties. Since, efficient screening of large number of mulberry accessions is essential for identification of salt tolerant and susceptible accessions, attempts were made to develop easy, reliable and economically viable screening methods. Screening for salt tolerance in the field is not a suitable method as the levels of salinity in field varies depending on season and soil depth Thus, screening should be done in soil-less culture with nutrient solutions of known salt concentrations. Hossain et al. (1991) and Vijayan et al., (2003) developed an efficient screening method for mulberry, wherein axillary buds were cultured *in vitro* saline conditions and based on the growth and development of shoots and roots under different salt concentrations salinity tolerance level of accessions were determined. Seed germination in a saline gel on Petri-plates was also used for identification of salt tolerant maternal parents (Vijayan et al., 2004). In general, mulberry and other woody plants are relatively sensitive during the emergence and young seedling stages but become progressively more tolerant with increasing age through the reproductive stage (Shannon et al., 1994). Significant amount of genotypic variation was observed in mulberry and other woody plants (Hossain et al., 1991; Vijayan et al. 2003; Tal 1986). Often salt tolerance of closely related species varies widely as found in the case of mulberry (Hossain et al., 1991; Vijayan et al., 2003, 2004), *Acacia* (Craig et al., 1990), *Casuarina* (Clemens et al., 1983;), *Melaleuca* (Van der Moezel and Bell, 1987), and *Eucalyptus* (Dunn et al., 1994). Once the salt tolerant genotypes are identified, the next step is transfer of the salt tolerance to the desired variety through crossbreeding. However, the success of traditional breeding strategies is limited by the complexity of stress conditions and lack of efficient selection techniques. The quantitative trait loci (QTL) that are linked to tolerance at one stage of development can differ from those linked to tolerance at other stages of development. Nonetheless, marker-assisted selection can help breeders to speed up selection procedure at different stages of development. In mulberry, although a recent study with RAPD, ISSR and SSR markers resulted in a genetic linkage map (Venkateswaralu et al., 2006), no molecular marker has been identified that is associated with salt tolerance or even leaf yield. Therefore, urgent attention is required to be given on this aspect. Another strategy being used widely for developing salt tolerance is induction of somaclonal variants through cell cultures (Winicov, 1996). However, attempts to regenerate plants with increased salt tolerance in a number of laboratories in a variety of plants including mulberry have not met with much success due to the complexities like formation of albino plants dwarf plants with limited fertility or no fertility.

The advent of molecular cloning and sequencing along with gene transfer has opened new avenues for developing salt tolerant plants. Present day genetic engineering tries to transfer one or several genes that encode either biochemical pathways or end points of signaling pathways that are controlled by a constitutively active promoters. Engineered over expression of biosynthetic enzymes for osmoprotectants, scavengers of reactive oxygen species (ROS) and stress induced proteins are some of the important strategies being attempted in a number of crop plants including some of the woody plants. For instance, increased expression of the *A. thaliana* tonoplast membrane Na^+/H^+ antiporter, *AtNHX1*, under a strong constitutive promoter, was found increasing salt tolerance in tomatoes (Zhang and Blumwald, 2001). The transgenic tomato plants grown in the presence of 200 mM NaCl were able to flower and set fruit. While the leaves accumulated high concentrations of sodium, the tomato fruits continued to contain only low concentrations of sodium. The *NHX1* system seems to be highly conserved between many different plant species and manipulation of this system in crop species is likely to result in improved salt tolerance. Similarly, betaine aldehyde dehydrogenase (BADH) gene from a salt tolerant mangrove species, *Avicennia marina,* was transferred to tobacco and *Brassica* successfully. These transgenic tobacco and *Brassica*, which over-expressed the BADH from *Avicennia*, conferred salinity tolerance up to 250 mM NaCl (Swaminathan, 2001). Overexpression of enzymes leading to increased mannitol, ononitol, fructan, trehalose and proline were also reported in tobacco (Kishor et al., 1995). Overexpression of dehydration-responsive element binding protein *DREB*/ C-repeat 9CRT) binding factors (CBF) in and wheat has resulted enhanced stress responsiveness in the transgenic plants. In order to develop a transgenic plant, efficient plant regeneration systems coupled with good gene transfer techniques are essential. In mulberry, attempts have been made to develop protocols suitable for plant regenerations from leaf discs (Bhatnager et al., 2000), cotyledons (Bhatnagar et al., 2000), callus (Narayanan et al., 1989). Gene transfer using particle bombardment (Bhatnagar et al., 2002) and via *Agrobacterium tumefaciense* (Bhatnagar et al., 2003) were also achieved. Taking advantages of these technological advancements, Lal et al. recently (2008) developed a transgenic plant overexpressing the late embryogenesis abundant (LEA) protein gene (*HAVI*) from barley (*Hordeum vulgare*) aleurone. This transgenic plant was shown to have better membrane integrity, improved photosysnthetic capacity higher plant water status, better water use efficiency under salt stress as compared to the control plant. In a similar way, a pear (*Pyrus communis* L. 'Ballad') plant overexpressing an apple spermidine synthase (SPDS) gene (*MdSPDS1*) was developed and it was shown that the transgenic plant has higher tolerance to salinity (Wen et al., 2008). These studies clearly revealed the potentials and possibilities of developing transgenic plants with enhanced salt tolerance in mulberry and other woody crop plants. Since the salt adaptive capacity of species may be related to constitutive expression of genes that encode salt-tolerance determinants, emphasis should also be given to the identification of native genes that respond to various stress stimuli. Analysis of transcripts from EST data is a rapid and powerful approach for such gene discovery, if a large number of ESTs can be isolated from cDNA libraries developed from plants grown under normal and stress conditions. Generally the salt inducible genes fall in to two categories depending on their functions such as the osmoprotectants, molecular chaperons, proteins involved in ion transport and signal transduction, and the transcriptional factors. Transcription factors are critical regulators of the changes in gene expression that drive developmental processes and environmental stress responses. Over 1,600 transcription factors, representing approximately 6% of the total number of genes, have been identified in the Arabidopsis (*Arabidopsis thaliana*) genome (Gong et-

Table 4. Response to salt-stress-accumulating products and their function(s) in conferring tolerance (reproduced from Pari and Das, 2005).

Product group	Specific compounds	Suggested function (s)	References
Ions	Sodium, Chloride	Osmotic adjustment Potassium exclusion/Export	Blumwald et al. (2000); Hasegawa et al. (2000) ; Koyro (2000)
Proteins	Osmotin SOD/Catalase	Pathogenesis-related proteins Osmoprotection Radical détoxification	Singh et al. (1987) ; King et al. (1988) Bohnert and Jensen (1996); Allen et al., (1997); Hernandez et al. (2000)
Amino acids	Proline Ectoine	Osmotic adjacent Osmoprotection	Khatkar and Kuhad (2000); Singh et al. (2000); Lippert and Galinski (1992)
Sugars	Glucose, fructose, sucrose, Fructan	Osmotic adjustment Osmoprotection, carbon storage	Kerepesi and Galiba (2000); Bohnert and jensen (1996) ; Pilon-Smits et al. (1995)
Polyols	Acyclic (e.g manitol) Cyclic (e.g. pinitol)	Carbon storage, osmotic adjustment Osmoprotection, osmotic adjustment Retention of photochemical efficiency of PSII Radical scavenging	Popp et al. (1985); Bohner et al. (1995) Ford (1984); Bohnert et al.(1995); Sun et al (1999); Smirnoff and Cumbes (1989) ; Orthen et al. (1994)
Polyamines	Spermine, spermidine	Ion balance, Chromatic protection	Tiburico et al. (1993); Santa Cruz et al. (1998)
Quaternary amines	Glycine betaine, β-Alanine betaine, Dimethyl-sulfonio propionate, Choline-o-sulfate	Osmoprotection Preservation of memebrane integrity Osmoprotection Osmoprotection Osmoprotection	Khan et al. (2000) Wang and Nil (2000) Rhodes and hanson (1993) Rhodes and Hanson (1993) Hanson (1998); Trossat et al. (1998) Nuccio et al. (2001)
Pigments	Carotenoids, Anthocyanins, Betalaines	Protection against photo inhibition	Adams et al. (1992); Foyer et al. (1994); Kennedy and De Fillippis (1999)

al., 2004). These transcription factors can be classified into several families based on the structure of their DNA-binding domains. Members of the *MYB*, *ERF*, *bZIP*, and *WRKY* transcription factor families have been implicated in the regulation of stress responses (Singh et al., 2002). Over expression of many of these transcription factors like *AtMYB44* has resulted in enhanced abiotic stress tolerance in *Arabidopsis thaliana* (Jung et al., 2007). Furthermore, application of native plant promoters in transgenesis need to be expanded as this can avoid transgene silencing, which is often associated with the presence of promoters of non-plant origin in the plant genome (Yevtushenkova et al., 2004).

Effect of salinity on herbivores

Since, the primary purpose of mulberry cultivation in most of the Asian countries is to feed the silkworm with its leaf, the impact of the salt that accumulated in leaf of mulberry on silkworm's growth and development need to be investigated in detail. Unfortunately, no detailed investigation in this aspect was reported till date. Nevertheless, a few studies have been conducted on the effects of salinity on herbivorous insects, which fed on salt affected glycophytes. The result suggested that salinity adversely affect the growth and development of glycophytic herbivores (Moon and Stiling, 2002a). Studies with leaf miner performance under salinity revealed that salinity reduced performance of dipteran leaf miners and gall formers (Moon and Stiling, 2002b) attacking goldenrod host plants. It is presumed that the osmotic stress imposed by salinity also impedes the ability of the herbivores to absorb essential sugars from host tissues. This could be more severing on early instar insects as they are particularly vulnerable to reduced plant nutrition (Mopper et al., 2000). However, detailed investigation on the effect of leaves from salt affected mulberry on survival, growth and development of silkworms as well as the effect of excessive salt in the leaf on cattle need to be conducted before under taking large scale cultivation of mulberry in salt affected areas.

Conclusion

Thus in short, research during the last few decades, on various aspects of salinity tolerance in crop plants and other glycophytes, have revealed many mechanisms responsible for salinity tolerance in plants. Modification of different pathways by overexpressing the concerned genes has greater effect on salinity tolerance in plants. Further, knowledge on genetic basis of salinity tolerance in mulberry and other woody plants through identification of QTLs will facilitate utilization of molecular markers for identification desirable parents for cross hybridizations and screening of hybrids at early developmental stages. Emphasis should also be given to explore the natural genetic variations in salt tolerance among crop plants and their wild relatives. Regarding mulberry, recent developments in genetics, tissue culture, transgenesis, and linkage mapping show that research in the coming years will definitely change the salt tolerant capacity of mulberry. However, unlike many other crops, in mulberry leaf is the primary product with which silkworm is fed, the effect of increased salt concentration in the leaf on the growth and development of silkworm also needs further investigation. Similarly, emphasis has to be given for developing mechanisms by which mulberry plant can regulate excessive ion uptake and translocation to leaves.

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