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# Chapter 13

## Role of Polyamines in Alleviating Salt Stress

Dessislava Todorova, Zornitsa Katerova, Iskren Sergiev, and Vera Alexieva

### 13.1 Introduction

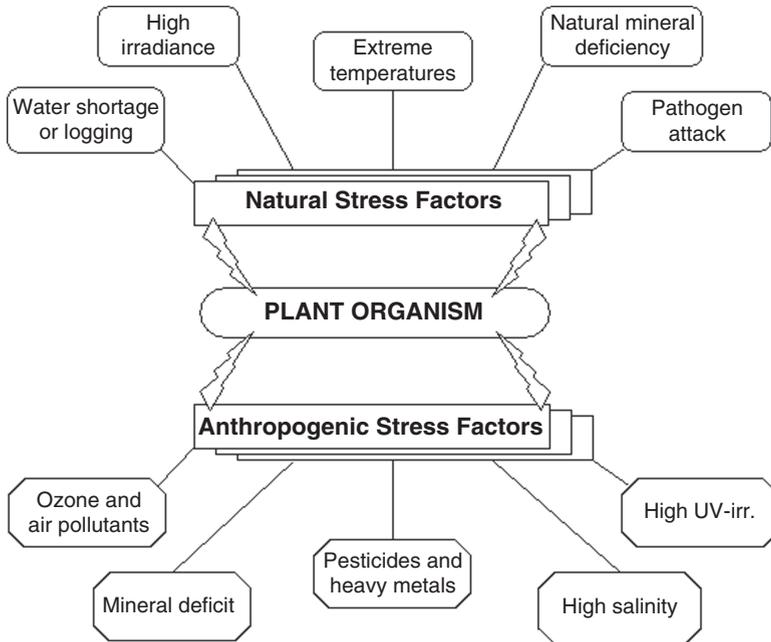
Abiotic and biotic stresses cause alterations in the normal physiological processes of all plants, including the economically important crops. Plant damage and decrease in their productivity take place most often due to naturally occurring unfavorable factors of the environment (natural stress factors). These include extreme temperatures; water deficit or abundance; increased soil salinity; high solar irradiance; early autumn or late spring ground frosts; pathogens, etc. Along with these factors, plants are imposed to a large scale of new stressors related to human activity (anthropogenic stress factors) including, toxic pollutants such as pesticides, noxious gasses ( $\text{SO}_2$ ,  $\text{NO}$ ,  $\text{NO}_2$ ,  $\text{NO}_x$ ,  $\text{O}_3$  and photochemical smog); photooxidants; soil acidification and mineral deficit due to acid rains; overdoses of fertilizers; heavy metals; intensified UV-B irradiation, etc. (Fig. 13.1). All these stresses cause an increased production of reactive oxygen species (ROS) in plants that alter their normal physiological functions, decrease the biosynthetic capacity of plant organisms, and cause damages which may lead to plant death (Mittler 2002; Ahmad et al. 2008; Gill and Tuteja 2010b; Potters et al. 2010).

#### 13.1.1 Reactive Oxygen Species

Independently of the type of stress (natural or anthropogenic), the accumulation of ROS is an undeniably established fact. Currently, overproduction of more than ten

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**Fig. 13.1** Natural and anthropogenic stress factors

**Table 13.1** Reactive oxygen species

Free Radicals		Nonradicals	
Superoxide radical	$O_2^{\cdot-}$	Hydrogen peroxide	$H_2O_2$
Hydroxyl radical	$OH^{\cdot}$	Hypobromous acid	$HOBr$
Hydroperoxyl radical	$HO_2^{\cdot}$	Hypochlorous acid	$HOCl$
Carbonate radical	$CO_3^{\cdot-}$	Ozone	$O_3$
Peroxyl radical	$RO_2^{\cdot}$	Singlet oxygen	$O_2^1\Delta_g$
Alkoxy radical	$RO^{\cdot}$	Organic peroxides	$ROOH$
Carbon dioxide radical	$CO_2^{\cdot-}$	Peroxynitrite	$ONOO^-$
Singlet radical	$O_2^1\Sigma_g^+$	Peroxynitrate	$O_2NOO^-$
		Peroxynitrous acid	$ONOOH$
		Peroxomonocarbonate	$HOOCO_2^-$

oxygen-containing molecules and radicals (Table 13.1) are known to induce oxidative stress. However, most detrimental to all biological systems are  $O_2^{\cdot-}$ ,  $H_2O_2$ ,  $OH^{\cdot}$ ,  $^1O_2$  (Halliwell 2006). In plants, ROS are generated mainly as by-products of various processes requiring high metabolic activity or high rate of electron flow via electron-transport chains. The major targets of deleterious ROS action are cellular macromolecules as phospholipids, proteins, and nucleic acids.

### 13.1.2 Plant Defense Systems

During the phylogenesis, plants have developed a complex of antioxidant protective systems in order to cope with all destructive effects of the unfavourable environmental conditions (Fig. 13.2). In general, the plant antioxidative systems can be divided into, (a) Enzymatic antioxidants including superoxide dismutase (EC 1.15.1.1), catalase (EC 1.11.1.6), guaiacol peroxidase (EC 1.11.1.7) and enzymes belonging to the ascorbate-glutathione cycle – ascorbate peroxidase (EC 1.11.1.11), glutathione peroxidase (EC 1.11.1.9), monodehydroascorbate reductase (EC 1.6.5.4), dehydroascorbate reductase (EC 1.8.5.1), glutathione reductase (EC 1.6.4.2.) and glutathione-S-transferase (EC 2.5.1.18). (b) Non-enzymatic antioxidants including lipid-soluble, membrane associated antioxidants –  $\alpha$ -tocopherol,  $\beta$ -carotene, which directly quench free radicals of lipid peroxidation (triplet chlorophyll and  $^1O_2$ ). Water-soluble antioxidants – glutathione and ascorbate, taking part in the detoxification of  $O_2^{\cdot -}$  and  $H_2O_2$ ; polyphenols (flavonoids, tannins and anthocyanins), proteinaceous thiols, proline, glycinebetaine, and *polyamines*.

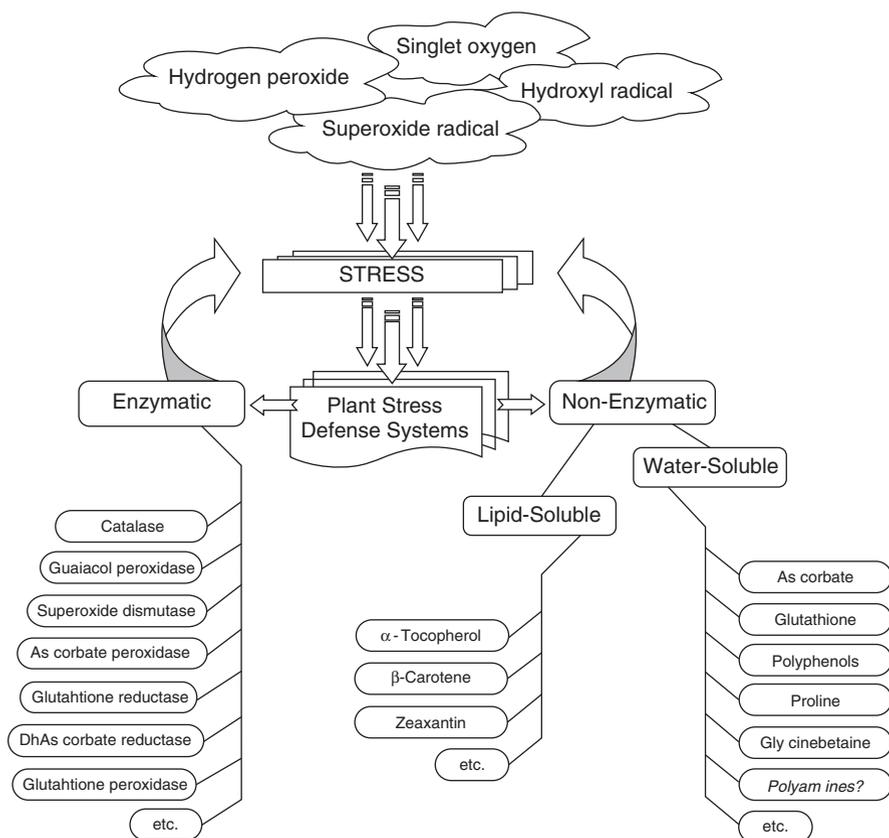


Fig. 13.2 Reactive oxygen species and plant defense systems

By activation of some or all of these systems, the plants are capable of overcoming the oxidative stress. However, in the case of prolonged or acute short stress, the capacity of the defense systems becomes exhausted or overloaded and this leads to considerable damages and even to plant death.

### 13.1.3 Salinity Stress

Salinity of soil as a result from increased quantity of cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ) and anions ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ) which are originated from more water-soluble salts such as  $\text{NaSO}_4$ ,  $\text{NaHCO}_3$ ,  $\text{NaCl}$ , and  $\text{MgCl}_2$  as well as less water-soluble salts such as  $\text{CaSO}_4$ ,  $\text{MgSO}_4$ , and  $\text{CaCO}_3$ , is one of the major abiotic stresses that reduce plant growth and productivity of many crops worldwide. Salinity may originate from natural factors (for example mineral erosion in soil), but also may result from human activities (as irrigation with high mineralized water and/or ineffective draining of the irrigated area, application of fertilizers, etc.). Most often soil salinity is enhanced by the presence of  $\text{NaCl}$  in water and soil, although other salts also can be involved. Detrimental effects of  $\text{NaCl}$  are complex, and include ion toxicity, hyperosmotic stress, and may induce subsequent stresses such as nutritional imbalances and oxidative stress (Zhu 2001).

The metabolic toxicity of  $\text{Na}^+$  is mainly due to its capability to compete with  $\text{K}^+$  for target sites with important cell functions. More than 50 enzymes are activated by  $\text{K}^+$  and elevated levels of  $\text{Na}^+$  or high  $\text{Na}^+/\text{K}^+$  ratio can disrupt a number of enzymatic processes in the cytoplasm. The protein biosynthesis also requires appropriate levels of  $\text{K}^+$  for proper tRNA binding to the ribosomes, and high levels of  $\text{Na}^+$  can negatively influence this process (Tester and Davenport 2003; Bartels and Sunkar 2005).

Increased concentration of  $\text{Na}^+$  can cause a hyperosmotic stress by preventing plant water uptake which results in the so-called “physiological drought” (Turkan and Demiral 2008).

Elevated concentration of  $\text{Na}^+$  can cause a deficit of nutrition elements by inhibiting their intake through the plasmalemma transporters (for instance the selective  $\text{K}^+$  channels) in the root cells. The salinity can lead to a hormonal disbalance and increased ROS production which can result to additional oxidative stress (Azevedo Neto et al. 2008; Turkan and Demiral 2008). Like drought stress salinity can induce accumulation of ABA followed by stomatal closure and respective decrease in the  $\text{CO}_2/\text{O}_2$  ratio in leaves and inhibits the  $\text{CO}_2$  fixation. These conditions are prerequisite for enhanced ROS production.

To unscramble the salinity stress from other plant stresses (as drought and osmotic stress) is difficult because the increased salts alter the ionic chemical balance in plants and affect water availability to plants, and similarly to other stressors may cause oxidative stress via production of ROS. The plant responses to salt stress is documented as multigenic in nature as adaptation to high salt levels involves osmotic adjustment, toxic ions compartmentation and oxidative stress tolerance (Turkan and Demiral 2008).

The investigation on salt stress and modulation of plant salinity tolerance in various cultivars has been extensively carried out using different approaches (Georgiev and Atkins 1993; Tanaka et al. 1999; Apse and Blumwald 2002; Garratt et al. 2002; Rios-Gonzalez et al. 2002; Borsani et al. 2003; Badawi et al. 2004; Flowers 2004; Brankova et al. 2007; Ivanova et al. 2008; Nenova 2008; Ogawa and Mitsuya 2012).

## 13.2 Polyamines: Chemistry and Metabolism

The triamine spermidine (Spd) and tetraamine spermine (Spm), as well as their precursor the diamine putrescine (Put) are the major polyamines (PAs) which are constitutive for all plant species. Polyamines are organic low-weight molecules with straight-chained C<sub>3</sub>-C<sub>15</sub> aliphatic structure with at least two primary amino groups and one or more internal imino groups (Edreva 1996; Groppa and Benavides 2008; Gill and Tuteja 2010a). Besides putrescine, spermine and spermidine, which are common for all plant species, there are also unusual polyamines which occur only in distinct plant species (i.e. diamines cadaverine and 1,3-diaminopropane) or synthesized under certain conditions (i.e. norspermine, norspermidine, thermospermine and caldopentamine) (Table 13.2).

Generally, polyamine biosynthesis in plants can be described as a two-phase process – the first stage is the biosynthesis of diamines, and the second stage is spermidine and spermine biosynthesis (Fig. 13.3). The putrescine is synthesized through decarboxylation of L-arginine to agmatine by arginine decarboxylase (ADC – E.C.4.1.1.19), followed by hydrolysis and deamination of agmatine by agmatine iminohydrolase (AIH – E.C. 3.5.3.12) and formation of N-carbamoylputrescine. N-carbamoylputrescine is then subjected to hydrolysis, deamination and decarboxylation by N-carbamoylputrescine amidohydrolase (CPA – E.C. 3.5.1.53) and the final product is putrescine. A parallel pathway for putrescine synthesis is the

**Table 13.2** Naturally occurring polyamines in plants

Name	Structure
1,3-Diaminopropane	$H_2N(CH_2)_3NH_2$
Putrescine	$H_2N(CH_2)_4NH_2$
Cadaverine	$H_2N(CH_2)_5NH_2$
Norspermidine	$H_2N(CH_2)_3NH(CH_2)_3NH_2$
Spermidine	$H_2N(CH_2)_3NH(CH_2)_4NH_2$
Homospermidine	$H_2N(CH_2)_4NH(CH_2)_4NH_2$
Aminopropylcadaverine	$H_2N(CH_2)_3NH(CH_2)_5NH_2$
Norspermine	$H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$
Spermine	$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$
Thermospermine	$H_2N(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$
Homospermine	$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_4NH_2$
Canavalmine	$H_2N(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$

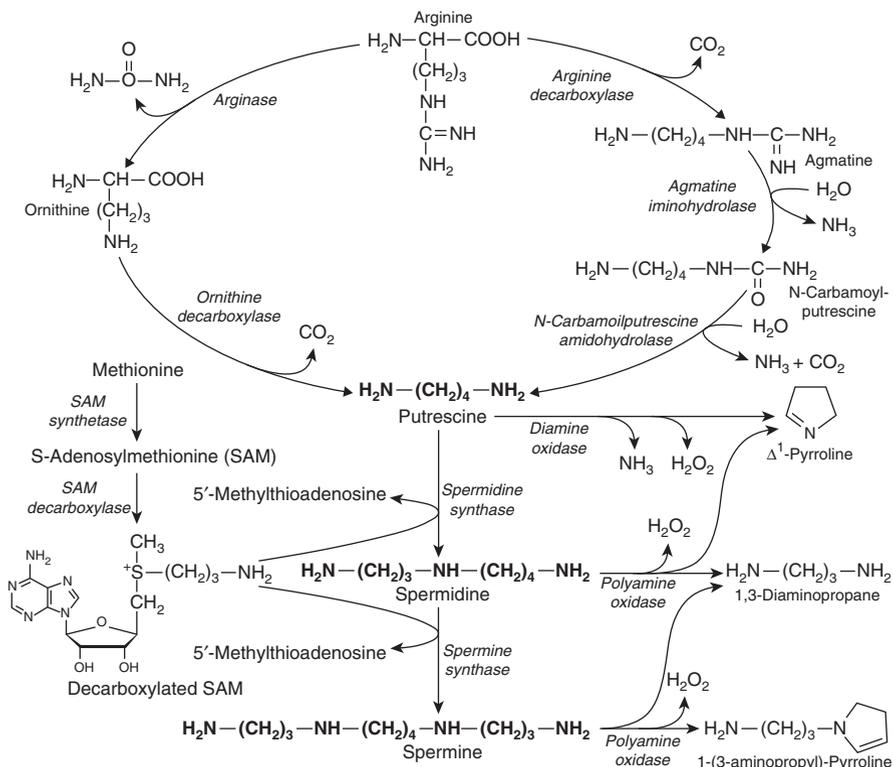


Fig. 13.3 Polyamine metabolic pathways

direct decarboxylation of L-ornithine, catalyzed by ornithine decarboxylase (ODC – E.C.4.1.1.17). The polyamines spermidine and spermine are synthesised by incorporation of an aminopropyl residue from decarboxylated S-adenosylmethionine to putrescine or spermidine – this step is catalyzed by the enzymes spermidine synthase (SPDS – E.C.2.5.1.16) or spermine synthase (SPMS – E.C.2.1.5.22) respectively. The necessary for polyamine biosynthesis decarboxylated S-adenosylmethionine is formed by decarboxylation (S-adenosylmethionine decarboxylase SAMDC – E.C.4.1.1.50) of S-adenosylmethionine (SAM) which is a common precursor of polyamines and ethylene (Slocum 1991).

The polyamine degradation is realized through oxidative deamination catalyzed by aminooxidases, which are copper-containing diamine oxidases (DAO – E.C.1.4.3.6) and flavoprotein-containing polyamine oxidases (PAO – E.C.1.5.3.14). DAO oxidize the primary amino groups of polyamines. The oxidative deamination of putrescine produces  $\Delta^1$ -pyrroline,  $\text{H}_2\text{O}_2$  and  $\text{NH}_3$ . PAO oxidize the secondary amino groups of polyamines and the final products of the process are  $\Delta^1$ -pyrroline (from Spd oxidation) or 1-(3-aminopropyl)-pyrroline (from Spm oxidation), along with 1,3-diaminopropane and  $\text{H}_2\text{O}_2$  (Federico and Angelini 1991).

Under physiological pH conditions polyamines bear positive charge and may conjugate with other negatively charged organic molecules like phenolic acids, proteins, phospholipids or nucleic acids. Thus polyamines in higher plants could be present in free, soluble conjugated and insoluble bound forms. The interaction with macromolecules and cell substructures allow polyamines to participate in a number of important growth and developmental processes in plants (regulation of gene expression; cell division, growth, and differentiation; cell, organ, and tissue senescence; dormancy breaking of tubers and germination of seeds; stimulation, support and development of flower buds; embryo- and organogenesis; fruit set, growth and ripening; plant morphogenesis; etc.). Additionally, PAs act in concert with light and phytohormones and are considered as plant endogenous growth regulators with hormone-like properties. Because of their polycationic nature, polyamines possess free radical scavenging features and antioxidant activity and may confer plant tolerance to different biotic and abiotic stresses (Groppa and Benavides 2008; Gill and Tuteja 2010a).

### **13.3 Polyamines and Salinity Stress Tolerance in Plants**

The participation of polyamines in the scavenging of free radicals, antioxidant activity and modulation of plant stress tolerance to various abiotic stresses has been extensively studied. A number of authors have reported the relationship of PA metabolism with plant responses to nutrient deficiency in the environment (Sarjala and Kaunisto 2002; Camacho-Cristobal et al. 2005), low or high temperature stress (Pillai and Akiyama 2004; Todorova et al. 2007), drought (Yamaguchi et al. 2007; Todorova et al. 2008), UV treatment (Smith et al. 2001; An et al. 2004; Zacchini and de Agazio 2004; Katerova and Todorova 2009), excess of heavy metals (Groppa et al. 2001, 2003; Zhao et al. 2008), and herbicide treatments (Benavides et al. 2000; Durán-Serantes et al. 2002; Szigeti and Lehoczki 2003; Cuevas et al. 2004; Deng 2005). The cited extensive investigations clearly demonstrate that polyamines play a pivotal role in conferring plant stress tolerance under unfavorable environmental conditions.

#### ***13.3.1 Endogenous Polyamine Concentrations***

Similarly to other stresses, investigations with different plant species have shown that alteration of polyamine concentrations occur in response to salinity. The effect of saline conditions on endogenous polyamines showed that polyamine concentrations altered in different manner depending on plant species and cultivars, plant organ and developmental stage of tissues, duration and intensity of stress treatment (Liu et al. 2007). Although some authors have found a decrease in endogenous polyamine concentrations under salinity stress (Benavides et al. 1997; Liu et al. 2008; Legocka

and Sobieszczuk-Nowicka 2012), mainly an accumulation of PAs due to salt stress has been reported in plant tissues (Kakkar et al. 2000; Upreti and Murti 2010; Iqbal and Ashraf 2012). Lefèvre et al. (2001) reported that short-term salt stress (50/100 mM NaCl or KCl) markedly increased Put (putrescine) concentrations of roots for the salt-resistant rice cultivar Pokkali compared with the sensitive one (IKP). The authors did not observe a clear relationship between the mean level of salinity resistance and the endogenous amounts of spermidine or spermine. Shoot Put concentration of the salt-resistant cultivar was only slightly increased after exposure to both salt stresses. Therefore, the authors suggested that physiological significance of Put accumulation may depend on the organ considered. It was also found that in salt-stressed heterotrophic maize callus culture total PA content and especially Put amount was higher in the resistant cultivar than in the salt-sensitive one (Willadino et al. 1996).

Most common PAs abundance (and especially of higher polyamine spermidine and spermine) was associated with increased stress tolerance to salinity (Ahmad et al. 2009; Ben et al. 2009; Yamamoto et al., 2011; Alet et al. 2011, 2012). Zapata et al. (2003, 2004, 2008) studied the effect of salinity on polyamine levels in different plant species subjected to 100 mM or 150 mM NaCl. They found that in general Put decreased, while Spd and Spm increased in saline-stressed plants (Zapata et al. 2003, 2004). Additionally the ratio (Spd+Spm)/Put increased with salinity (Zapata et al. 2004), which correlated with the idea of a protective role of higher PAs against salt stress. Similarly, El-Shintinawy (2000) also pointed out that salinity significantly enhanced the augmentation of Spm and Spd accompanied with a reduction in Put amount in wheat cultivars. In their study Tassoni et al. (2008) analyzed polyamine metabolism in *Arabidopsis thaliana* (ecotype Columbia) inflorescences and stalks collected from plants germinated and grown under increasing salt-stress conditions (0–75 mM NaCl). The authors found that free spermidine was the most abundant polyamine and its levels, as well as those of free spermine, increased with salt concentration, supporting the hypothesis for a specific role of higher polyamines in the tolerance to salt stress. In experiment with barley seedlings Zhao et al. (2003) have examined the effect of wide range (0–300 mmol/L) of NaCl on free and bound polyamines and have found that polyamine content raised up in dose-dependent manner in relation to salt concentrations, but amount of bound fractions were reduced by highest concentration. Additionally, they found a positive correlation between the plant growth rate and the ratio of bound PA/free PA levels. The authors suggested that under salt stress, the balance between free PA and bound PA content in roots is important for salt tolerance of barley seedlings. In order to examine the plant responses to salt stress (0, 50, and 100 mM NaCl), Kim et al. (2010) investigated Chinese cabbage seedlings grown as a hydroponic culture. They found that spermidine content decreased as salinity increased, but spermine content increased, which also correlated with proposed idea of polyamine protection under saline environment. Jouve et al. (2004) have shown that *Populus tremula* was able to cope with up to 150 mM NaCl mainly due to accumulation of spermine as well as compatible solutes (sucrose, proline, mannitol and raffinose). Ghosh et al. (2011) found that salt induction had changed the nature of polyamine content in time-dependent manner. Accumulation of polyamines in both rice varieties (Pokkali and Nonabokra)

amplified with increased duration of NaCl treatment. In other experiment with three rice cultivars differing in their salt-sensitivity Roychoudhury et al. (2008) found that salt-tolerant plants (Nonabokra) accumulate less Put than salt-sensitive cultivars (M-1-48) after salinity treatment for 48 h, which was comparable with less significant reduction of growth rate. Correspondingly, Spd and Spm synthesis in the salt-tolerant variety is stimulated to stabilize membrane systems. The effect of salt stress (50, 100 and 150 mM NaCl) on the endogenous levels of free, bound and total polyamines was studied in root tissues of salt tolerant (Coban) and salt sensitive (Sanbro) cultivars of sunflower (*Helianthus annuus* L.) plants for the 5th, 15th and 25th days of the growth periods (Mutlu and Bozcuk 2007). The amounts of free, bound and total Spm increased in root tissues of sunflower plants, while the levels of other polyamine titers were either decreased in general or remained unchanged significantly. The authors suggested that increase in some polyamine concentrations in sunflower root tissues under salinization implies their possible role in diminishing the injurious effect of salinity stress. The salt tolerance in sunflower plants was related to the excessive augmentation of total polyamines in root tissues of salt tolerant cultivar Coban under saline condition (Mutlu and Bozcuk 2007). A marked increase was noted in free Spd and Spm, soluble conjugated and insoluble bound Put, Spd and Spm contents in the roots of cucumber (*Cucumis sativus* L) cultivar Changchun mici (comparatively tolerant to high salinity) than Jinchun No. 2 under short-term salt stress (Duan et al. 2008). Hummel et al. (2004) observed the reduction in polyamines measured in shoots and partially in roots during long term exposure of *Pringlea antiscorbutica* seedlings to severe salinity (300 mM NaCl). Only spermine content was increased in roots after salt stress. The authors concluded that major effect of saline stress was the modification of polyamine distribution between roots and shoots. Higher Spm content in roots was a developmental response to stress and its accumulation in roots facilitated reinitiation of root growth (Hummel et al. 2004).

In general one possible mechanism is assumed which explain the participation of endogenous polyamines (primarily spermine) in plant salt tolerance. In a number of articles where the protective role of endogenous higher polyamines has been shown, it was proposed that under salinity conditions PAs regulated the activity of plasma membrane H<sup>+</sup>-ATPase. Janicka-Russak et al. (2010) suggested that the decrease in PAs could result in the removal of excess cations from the cell. This action of endogenous PAs contributes to ionic homeostasis by modification of the plasma membrane H<sup>+</sup>-ATPase and the vacuolar H<sup>+</sup>-ATPase activities in cucumber roots treated with NaCl. Similarly, Roy et al. (2005), showed that root plasma membranes of rice salt-tolerant cultivars Nonabokra and Pokkali were rich in Spm and Spd, whereas the root plasma membranes of sensitive cultivars (M-1-48 and IR8) were rich in Put only. After treatment of barley seedlings with different concentrations of NaCl (0–300 mM) for 3 days Liu et al. (2006a) have shown that Put content declined, while spermine and spermidine were amplified in roots. The polyamines, especially Spd, enhanced the activities of tonoplast H<sup>+</sup>-ATPase and H<sup>+</sup>-PPase, which are capable to create the proton gradient across the vacuole membrane and to pump in the excessive cytosol Na<sup>+</sup> into the vacuole and to rebuild the relative ion and pH balance. Thus tonoplast H<sup>+</sup>-ATPase and H<sup>+</sup>-PPase play crucial roles in plant salt adaptation and

maintenance of their activities higher is essential for plant growth and survival under salt stress. The authors suggested that the conversion of Put to Spd and Spm and maintenance of higher levels of Spd and Spm were necessary for plant salt tolerance through enhanced tonoplast  $H^+$ -ATPase and  $H^+$ -PPase activities in roots (Liu et al. 2006a).

### 13.3.2 Biosynthetic Polyamine Enzymes

It is well known that plant ability to control stress, including salinity, is linked to their ability to synthesize PAs (Kasinathan and Wingler 2004; Hamdani et al. 2011). When Kasinathan and Wingler (2004) analyzed polyamine concentration and salt stress tolerance in two *Arabidopsis thaliana* mutants, *spe1-1* and *spe2-1* with reduced activity of ADC, they showed that polyamine accumulation depends on acclimation to salinity and that decreased polyamine formation leads to reduced salt tolerance. Similarly, a marked increase was reported in ADC, ODC, SAMDC and DAO activities in the roots of comparatively tolerant to high salinity cucumber cultivar Changchun mici than in cv. Jinchun No. 2 under short-term salt stress (Duan et al. 2008). It was reported that ADC activity in NaCl-stressed heterotrophic maize callus culture is also significantly incremented, especially for the salt-resistant calluses, which was believed to be related with the rise in putrescine (Willadino et al. 1996). However, long-term (salt treatment 21 days) led to reduced activities of ADC and SAMDC in salt tolerant (var Giza) and salt sensitive (var El Paso) *Oryza sativa* cultivars (Maiale et al. 2004). The activity of SPDS was reduced in the salt tolerant rice cultivar but not in the salt sensitive variety and the authors suggested that SPDS activity has important role for plants subjected to salt stress.

The expression of several genes involved in PA biosynthesis (*ZmODC*, *ZmSPDS2A*, *ZmSPDS2B*, *AtADC2*, *AtSPMS*, *SPMS*, *SAMDC1*, *SAMDC2*) is up-regulated in the presence of salt stress (Li and Chen 2000a; Urano et al. 2003, 2004; Rodríguez-Kessler et al. 2006) in different plants (*Zea mays*, *Arabidopsis thaliana*, *Oryza sativa*). Rodríguez-Kessler et al. (2006) described a spermidine synthase cDNA from *Zea mays* leaves (*ZmSPDS2A*) and discussed the transcriptional regulation of the corresponding gene and other genes related to PA biosynthesis (*ZmODC*, *ADC* and *SAMDC*) under salinity. *ZmSPDS2A* was equally expressed in leaves, stem and roots, however the transcripts of other genes involved in PA biosynthesis (*ZmODC*, *ADC* and *SAMDC*) showed tissue-specific regulation. It was reported that in maize only *ZmODC*, *ZmSPDS2A* and the identified second transcript encoding a spermidine synthase (*ZmSPDS2B*) were up-regulated by salt stress. In *Oryza sativa* L. seedlings, the expression of the *SAMDC1* gene was considerably induced by salinity (Li and Chen 2000a). The transcript levels of *SAMDC1* in two rice varieties differing in salt tolerance was higher in the salt-tolerant japonica rice variety Lansheng, than in the salt-sensitive one (variety 77–170), and occurred more quickly when both varieties were exposed to low salt conditions. The results suggested that the expression of the *SAMDC1* gene in seedlings is positively correlated with the salt tolerance of rice. Hao et al. (2005) studied two SAMDC cDNAs isolated from apple (*MdSAMDC1*

and *MdSAMDC2*) and found that *MdSAMDC2* was positively induced by salt stresses, but *MdSAMDC1* was not activated. In *Arabidopsis* the polyamine biosynthetic gene *ADC1* has been found to be expressed in all tissue tested, while *ADC2* is mainly expressed in siliques and cauline leaves, and is induced upon various stress types, including salinity (Urano et al. 2003, 2004; Bagni et al. 2006). When analyzing the expression profiles of genes responsible for PA biosynthesis in *Arabidopsis thaliana* (two genes for *ADC*, four genes for *SAMDC*, four genes for *SPDS*, and two genes for *SPMS*) under various abiotic stress conditions, Urano et al. (2003), found that *AtADC2* and *AtSPMS* mRNAs (encoding ADC and spermine synthase) increased markedly in response to NaCl. Stress-inducible accumulation of *AtADC2* mRNA correlated with putrescine accumulation under NaCl treatment. Normally ODC activity is involved in Put biosynthesis but in *Arabidopsis* there is no detectable ODC activity (Hanfrey et al. 2001) and ADC is considered as a key enzyme in PA biosynthesis in *Arabidopsis*. The role of stress-inducible *AtADC2* gene in *Arabidopsis* was analyzed through a *Ds* insertion mutant of *AtADC2* gene (*adc2-1*) under salinity conditions (Urano et al. 2004). In the *adc2-1* mutant, which was more sensitive to salinity than the control plants, free Put content was significantly reduced (compared with the control plants) and did not increase under salt stress. The salinity sensitive phenotype of *adc2-1* was recovered by the addition of exogenous Put. The authors concluded that endogenous Put plays an important role in salt tolerance in *Arabidopsis* and *AtADC2* is a key gene for its production under salt stress and normal conditions.

It was reported that *MdADC* expression and ADC activity decreased in apple (*Malus sylvestris* (L.) Mill. var. domestica) in vitro callus experiencing salt recovery, and increased when the callus was subjected to successive salt stress (Liu et al. 2006b). Under the same conditions ODC activity showed a pattern opposite to that of ADC. Moreover, treatment with the ADC inhibitor (D-Arginine) caused serious growth impairment under salt stress. The authors' results clearly showed that the ADC pathway is involved in the salt stress response.

When the localization of *SPMS* gene promoter activity was examined using the *SPMS* promoter- $\beta$ -glucuronidase (*GUS*) gene expressed in transgenic *Arabidopsis* plants the gene was detected in almost all organs during all developmental growth stages (Sagor et al. 2011). However, *ACL5* (encoding thermospermine synthase) promoter activity was predominantly observed in the vascular systems. The authors reported that upon high salt stress *SPMS* promoter activity was strongly detected in all organs except cotyledons, whereas the *ACL5* promoter activity was reduced which is consistent with the decreased levels of *ACL5* transcripts. The results showed that *SPMS* expression differs from that of *ACL5* in respect to tissue specificity and salt stress response, suggesting relevant differences in Spm and thermospermine functions (Sagor et al. 2011).

During the last decade number of authors reported enhanced salinity tolerance in different transgenic plants engineered to overproduce polyamines by overexpression of the genes involved in PAs biosynthesis (Table 13.3).

In *Oryza sativa*, the overproduction of polyamines was achieved by overexpression of *ADC* from *Avena sativa* and *SAMDC* under the control of ABA inducible

**Table 13.3** Salt stress tolerance in transgenic plants engineered to overproduce polyamines

Gene	Gene source	Transgenic plant	PA overproduction	Reference
<i>ADC</i>	Oat	Rice	Put	Roy and Wu (2001)
<i>ADC</i>	Oat	Eggplant	Put, Spd (particularly conjugated forms) and free Spm fraction	Prabhavathi and Rajam (2007)
<i>ODC</i>	Mouse	Tobacco	Put	Kumria and Rajam (2002)
<i>SAMDC</i>	Tritordeum	Rice	Spm and Spd	Roy and Wu (2002)
<i>SAMDC</i>	Human	Tobacco	Spd, Put, especially conjugated fraction	Waie and Rajam (2003)
<i>SAMDC</i>	Carnation	Tobacco	Put, Spd and Spm	Wi et al. (2006)
<i>SAMS</i> ( <i>SsSAMS2</i> )	Suadea salsa	Tobacco	Free Spm, Spd and Put	Qi et al. (2010)
<i>SPDS</i> ( <i>MdSPDS1</i> )	Apple	European pear	Spd	He et al. (2008)
<i>MdSPDS1</i>	Apple	European pear	Spd	Wen et al. (2008)
<i>MdSPDS1</i>	Apple	Tomato	Spd and Spm	Neily et al. (2011)
<i>SPDS</i>	Fig leaf gourd	Arabidopsis	Spd, especially conjugated fraction	Kasukabe et al. (2004)
<i>SPDS (FSPD1)</i>	Fig leaf gourd	Sweet potato	Spd	Kasukabe et al. (2006)

promoter and it was shown that transgenic plants had higher salinity tolerance as compared to non-transformed plants (Roy and Wu 2001, 2002). The transgenic *Nicotiana tabacum* plants, overexpressing the putrescine synthesis gene *ODC* from mouse possessed enhanced salt tolerance (Kumria and Rajam 2002). The introduction of *SPDS* gene into *Arabidopsis* and *Ipomoea batatas* cv. Kokei 14 led to the enhanced tolerance against multiple abiotic stresses including salinity (Kasukabe et al. 2004, 2006). Transgenic eggplants overexpressing *ADC* gene from *Avena sativa* accumulated polyamines and exhibited an increased tolerance to multiple abiotic stresses including salinity (Prabhavathi and Rajam 2007). The transgenic tobacco overexpressing human *SAMDC* gene has showed increased PA levels (especially conjugated Put and Spd fractions) and tolerance to salinity (Waie and Rajam 2003). Salt-induced damage was attenuated in the transgenic *Nicotiana tabacum* L. plants overexpressing *SAMDC* from *Dianthus caryophyllus* L. flower (Wi et al. 2006). Recently, it was reported that salt stress induced damage in tobacco transgenic plants overexpressing the suadea salsa full-length S-adenosylmethionine synthetase (*SsSAMS2*) gene under the control of cauliflower mosaic virus 35S promoter, was attenuated (Qi et al. 2010). The authors showed that *SsSAMS2* overexpression in transgenic tobacco plants leads to increase in free Spm, Spd and Put content, and as a result promotes salt tolerance. Transgenic European pear (*Pyrus communis* L. 'Ballad') and tomato overexpressing

apple *SPDS* (*MdSPDS1*) performed attenuated susceptibility to NaCl stress in relation to the wild plant (Wen et al. 2008; Neily et al. 2011). Additionally, He et al. (2008) documented that the same transformation led to enhanced enzymatic (SOD, APX, MDHAR, GR) and non-enzymatic antioxidant capacity in response to salinity. The overexpression of fig leaf gourd (*Cucurbita ficifolia*) *SPDS* in *A. thaliana* enhanced the tolerance of transgenic plants to various types of environmental stresses, including salinity, suggesting the key role for spermidine as regulator in stress signaling pathways (Kasukabe et al. 2004). The authors suggested that Spd could be involved in stress tolerance phenomenon as a direct stress-protecting compound and as a stress-signaling regulator.

### 13.3.3 Aminooxidase(s)

Under normal and stress conditions PA content in plant cells depends not only on their biosynthesis and transport but also on their catabolism where aminooxidases (copper-containing DAO and flavin-containing PAO) are involved. PAOs are grouped into families, which are participating either in terminal catabolism or back-conversion of polyamines. DAOs exhibit high affinity for diamines, while PAO oxidize secondary amine groups from Spd and Spm (Alcazar et al. 2006). However, it was noted that in soybean plants CuAO preferred Cad over Put and the catabolic enzyme was active even under salinity (Campestre et al. 2011). DAO and PAO are localized in the cytoplasm and cell walls where they provide  $H_2O_2$  required for suberization, lignification and formation of cross-bridges between the components of the cell wall that confer cell wall-stiffening (Cona et al. 2003; Kuznetsov and Shevyakova 2007). In contrast to DAO, which are known to occur in high amounts in dicotyledonous, PAO occur in high levels in monocotyledonous plants (Šebela et al. 2001; Cona et al. 2006). However, Maiale et al. (2004) did not detect PAO activity in crude extracts of salt tolerant (var Giza) and salt sensitive (var El Paso) *Oryza sativa* cultivars subjected to long-term salt stress.

The activities of CuAO and PAO lead to a reduction in the PA levels. Additionally, along with the other products, CuAO and/or DAO generate  $H_2O_2$ , which is known to act as a signal molecule in low concentrations, and might coordinate adaptation processes in plants (Kuznetsov and Shevyakova 2007; Alcazar et al. 2010; Campestre et al. 2011). Therefore the PA catabolism and the associated  $H_2O_2$  production are important in the induction of abiotic stress (including salinity) tolerance in plants. Moschou et al. (2008) revealed the importance of the  $H_2O_2$  derived from PA catabolism in the induction of salinity-induced tolerance in tobacco (*Nicotiana tabacum* cv Xanthi) using transgenic plants overexpressing or downregulating apoplasmic PAO. In addition, Rodríguez et al. (2009) reported that PA oxidation by the activity of PAO could be the major source contributing to ROS production involved in maize leaf elongation under salinity. The authors showed that maximal activity of PAO was not affected by salinity, and suggested that the enzyme in *Zea mays* is tolerant to salt stress. As expected, the total activity of PAO was found to be up to 20-fold higher than that of the CuAO.

Campestre et al. (2011) examined the possible relationship between polyamine catabolism mediated by CuAO and the elongation of *Glycine max* L. hypocotyls from plants exposed to NaCl. Salinity caused a rise in CuAO activity in segments of the hypocotyl elongation zone in in vitro and in vivo experiments. The expression of *GmCuAO1* gene (found by Delis et al. 2006 predominantly in tissues characterized by rapid extension growth, as the apical segments of etiolated hypocotyls) was detected in the elongation zone but, surprisingly, there was no change in its expression level under salinity (Campestre et al. 2011). The authors conclude that the higher CuAO activity found under salt stress could not be attributed to a higher expression of *GmCuAO1* gene.

Classical approaches, using inhibitors of enzymes involved in polyamine metabolism, pointed to their possible role in plant adaptation to NaCl stress. Using a DAO specific inhibitor aminoguanidine and exogenously applied Put, Su and Bai (2008) reported that a 15–20 % of proline accumulated in *Glycine max* leaves under salt stress (50–150 mM NaCl) could result from PA (Put) degradation products. Additionally, the activity of DAO was also enhanced. Shevyakova et al. (2006) suggested that salinity (400 mM NaCl) induced peroxide in the leaves of the halophyte *Mesembryanthemum crystallinum* L. was produced via DAO activity. This assumption was supported in experiments with addition of the inhibitor aminoguanidine to the root medium in the presence of NaCl. In addition, their study indicated that activation of DAO combined with H<sub>2</sub>O<sub>2</sub>-peroxides reaction in leaves and roots is implicated in the regulation of free and conjugated PA concentrations under salinity. In a study conducted with similar model system Kuznetsov et al. (2007) also noted enhanced DAO activity, which correlated with the increased H<sub>2</sub>O<sub>2</sub> production in the presence of NaCl. Salinity (50, 100, 150 mM NaCl) strongly increased the activity of DAO in *Glycine max* (cv. Suxie-1) roots (Xing et al. 2007). Accumulation of  $\gamma$ -aminobutyric acid (GABA) levels also increased with the increasing NaCl concentrations. Expectedly, PA degradation under NaCl treatment led to reduced amounts of free Put, Cad and Spd. The authors observed a close correlation between the alterations in DAO activity and GABA accumulation also after treatment with the aminoguanidine inhibitor. It was suggested that higher GABA accumulation induced by treatment with NaCl might result from PA degradation, concluding that PAs could execute their functions through formation of GABA under salinity. Soybean plants treated with N,N<sup>1</sup>-diaminoguanidine (an inhibitor of CuAO) showed a decrease in CuAO and significant reduction of reactive oxygen species in the elongation zone, even under salinity (Campestre et al. 2011). N,N<sup>1</sup>-diaminoguanidine ceased the increased hypocotyl length in control and salinity exposed plants. The authors suggested that the activity of CuAO may be partly contributing to the hypocotyl growth under NaCl stress, via generation of H<sub>2</sub>O<sub>2</sub> by polyamine catabolism – thus reinforcing the importance of polyamine catabolism and H<sub>2</sub>O<sub>2</sub> production in the induction of salt tolerance in plants. Supporting the idea of Moschou et al. (2008), Rodríguez et al. (2009) and Campestre et al. (2011) for the important role of H<sub>2</sub>O<sub>2</sub> produced by PAO and/or DAO, Shores et al. (2011) noted that under salinity PAO activity was highly increased in the leaf-growing zone in *Zea mays*, possibly providing the ROS required for elongation. The authors suggested that the

enhanced PAO activity was an adaptive mechanism directed to restore the normal levels of ROS at the expansion zone where NADPH oxidase could no longer provide the required ROS for growth.

It was reported that under 0–200 mM NaCl PAO activity was increased in the roots of barley seedlings (Zhao et al. 2003). Interestingly DAO activity was also increased in transgenic eggplants (overexpressing oat *ADC* gene) that exhibited an increased tolerance levels to multiple abiotic stresses including salinity (Prabhavathi and Rajam 2007). Duan et al. (2008) also reported that under short-term salt stress DAO activity increased along with the biosynthetic PA enzymes (ADC, ODC, SAMDC) in the roots of comparatively tolerant to high salinity cucumber cultivar Changchun mici than in cv. Jinchun No. 2.

The polyamine metabolic pathway is interconnected with other metabolic routes engaged in the formation of different signaling molecules (as H<sub>2</sub>O<sub>2</sub> and NO) and metabolites (ethylene, SAM, proline, GABA) which are important in plant stress (including salinity) responses (Kuznetsov et al. 2007; Xing et al. 2007; Su and Bai 2008; Alcazar et al. 2010).

### 13.4 Application of Polyamines for Enhanced Salinity Stress Tolerance

Large number of evidence suggested that exogenous application of PAs could be used as promising tool to enhance plant tolerance under salt stress conditions. Ali (2000) showed that exogenous Put reduced the net accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in different organs of *Atropa belladonna* subjected to salinity stress. Put alleviated the negative effect of NaCl during germination and early seedling growth and increased endogenous Put of *A. belladonna*. Verma and Mishra (2005) also reported that Put counteracted the salinity induced decrease in seedling growth and biomass accumulation, and increased the activity of antioxidant enzymes and carotenoids in leaf tissues of salt stressed *Brassica juncea* seedlings. Similar results were reported by Tang and Newton (2005), who showed that polyamines (mainly Put) lessened salt-induced oxidative damage by increasing the activities of antioxidant enzymes and decreasing lipid peroxidation in callus and seedlings of Virginia pine. Putrescine (0.5 mM) was completely able to amend the toxic effects of salt stress (100 mM NaCl) on electrolyte leakage and lipid peroxidation and partially on relative water content in chickpea plants (Sheokand et al. 2008). Additionally, Put had a positive effect on antioxidant enzyme activities under salt stress. In other study (Shi et al. 2008), exogenous putrescine (100 μM) was added to nutrient solution 3 days before cucumber (*Cucumis sativus* L. cv. “Jinyan No.4”) seedlings were exposed to 100 mM NaCl treatment. Putrescine considerably diminished the negative effects of NaCl on root growth through decreasing of Na<sup>+</sup> uptake and increasing of potassium accumulation in roots. Demetriou et al. (2007) reported that enhanced salinity provoked negative changes in the photosynthetic apparatus of green alga *Scenedesmus obliquus* by affecting both its structure and function: 10‰ NaCl increased the effective

antenna size and decreased the active reaction center population, accompanied by a significant inhibition of the photosynthetic rate. Exogenously added Put altered these negative changes, by inducing the reciprocal reorganization of the photosynthetic apparatus (Demetriou et al. 2007). Increased salinity (150 mM NaCl) provoked a negative modification in plasma membrane phospholipids and polyamine treatment (particularly putrescine and spermidine) caused a favorable effect in maintaining of plasma membrane stability and functions of wheat roots under surplus salinity (Mansour et al. 2002).

The effects of higher polyamines (Spd and Spm) on physiological and biochemical changes in 12-day-old rice seedlings (salt-tolerant Pokkali and salt-sensitive M-1-48) were investigated during salinity stress (Chattopadhyay et al. 2002). Both polyamines considerably prevented the electrolyte leakage, amino acid and chlorophyll loss, and inhibition of photochemical reactions of photosynthesis, as well as the downregulation of chloroplast-encoded genes like *psbA*, *psbB*, *psbE* and *rbcL* induced by the salinity stress. In similar experiments with roots of salt-tolerant (Nonabokra) and salt-sensitive (M-1-48) rice cultivars treated with 150 mM NaCl and 1 mM Spd, Gupta et al. (2012b) for a first time showed that polyamines participate in the salt stress signaling due to spermidine-mediated phosphorylation and activation of 42 kDa  $\text{Ca}^{2+}$ -independent non-MAPK protein kinase. This fact allowed the authors to presume that this protein kinase plays a key role in the activation of different stress regulatory biomolecules, indicating its importance in salinity mediated signal transduction (Gupta et al. 2012a, b). In other study with rice seedlings (salt-tolerant Pokkali and salt-sensitive KDML 105), the application of 1 mM spermidine helps the plant to withstand the negative effect of NaCl (Salethong et al. 2011). The authors concluded that exogenous spermidine enhanced the salinity tolerance of rice by stabilizing membrane, scavenging free radicals and maintaining  $\text{K}^+/\text{Na}^+$  balance.

Salt stress reduced all evaluated growth parameters and yield components, content of leaf pigments, carbohydrates, protein, spermidine and spermine as well as amylase activity of *Vigna sinensis* plants (Alsocari 2011). Exogenous application of spermine mitigated the deleterious effects of salinity stress on growth and yield of the stressed plants. The protective effect of spermine on *V. sinensis* plants appeared to be mainly due to the increased chlorophyll and protein content and endogenous polyamines (Alsocari 2011). Similar results were reported by Sakr and El-Metwally (2009) who showed that exogenous spermine partially alleviated the harmful effect of soil salinization on wheat growth and yield. Spermidine application in salinized nutrient solution during short-time stress resulted in alleviation of the salinity-induced membrane damage in the roots and plant growth and photosynthesis inhibition, together with an increase in polyamine and proline contents and antioxidant enzyme activities in the roots of salt-sensitive cucumber cultivar Jinchun No. 2 (Duan et al. 2008). Recently, Roychoudhury et al. (2011) provided an additional information concerning protective effect of exogenous application of spermidine and spermine on NaCl-treated rice seedlings. The authors showed that mitigation of inhibitory effect of salinity stress was conferred by preventing growth inhibition, averting different forms of cellular injuries, maintaining  $\text{K}^+/\text{Na}^+$  balance or increasing the level of compatible osmolytes and activity of antioxidant enzymes.

Similarly to endogenous polyamines, the beneficial effect of the exogenous polyamines is related to the improvement of the ion balance in salt-treated cells due to their polycationic nature. As was illustrated in series of experiments, the application of 1 mM Put decreased the  $\text{Na}^+$  accumulation of rice calli and rice plants exposed to salt stress (Ndayiragije and Lutts 2006, 2007). The protective effect of exogenous polyamines under high salinity is associated mainly with improving of the  $\text{K}^+/\text{Na}^+$  homeostasis through restricting the  $\text{Na}^+$  influx into roots and thus preventing the loss of  $\text{K}^+$  from cells (Zhao et al. 2007). Zhao and Qin (2004) also proposed that one of the possible mechanisms involved in the alleviating of salt injury in barley seedlings by PAs application was to maintain tonoplast integrity and function under saline conditions. Furthermore, Zhu et al. (2006) suggested that exogenous Spd inhibits  $\text{Na}^+$  transport from roots to shoots under conditions of high salinity by reinforcing the barrier effects of Casparian bands, which are beneficial for attenuating the salt injuries in barley seedlings. Salinity stress severely inhibited the  $\text{H}^+$ -ATPase activity in rice plants, but spermidine treatment significantly recovered its activity (Roy et al. 2005). Shabala et al. (2007) showed that application of polyamines in micromolar concentrations was efficient in preventing NaCl-induced  $\text{K}^+$  efflux from the pea mesophyll. The authors suggested that polyamines may directly block the  $\text{K}^+$  efflux through non-selective cation channels and activate the plasma membrane  $\text{H}^+$ -ATPase, so restoring the membrane potential (Shabala et al. 2007). Moreover, maintaining of cytosolic  $\text{K}^+/\text{Na}^+$  balance seems to be the major beneficial effect of polyamine regulation of membrane transport activity (Shabala and Cuin 2008). However, preliminary application of 1 mM Spm, Spd or Put prevented NaCl-induced  $\text{K}^+$  leak only in the mature root zone of hydroponically grown maize and Arabidopsis. In contrast, in the distal elongation root zone, PA pre-treatment resulted in an even larger NaCl-induced  $\text{K}^+$  efflux, so PAs affect the cell membrane transporters in a highly specific way (Pandolfi et al. 2010). The authors concluded that the ameliorative affect of PAs is a result of combination of several issues which probably incorporate PA transport, accumulation and metabolism in cell, and the functional expression of specific target proteins or signaling components (Pandolfi et al. 2010).

### 13.5 Conclusion and Future Perspective

The importance, actuality and inevitability of the salinity stress, as well as its negative impact on the physiological processes and plant productivity have drawn the attention of a number of investigators to study the problems of salt stress. The research in this area is routed mainly into five directions:

- Studies on the physiological response of the plant organism subjected to salinity stress
- Comparison of the effects of salt stress on several plant organisms, differing in their species, variety or genetically determined resistance to the stress factor

- Searching for possibilities to decrease the unfavorable consequences caused by a salinity by induction of adaptation or by means of application of xenobiotics, including polyamines
- Selection using cell cultures and conventional plant breeding
- Genetic engineering for improved stress tolerance

One popular procedure for increasing plant salt tolerance is the using of exogenous application of different chemicals. For now the polyamine large-scale agricultural benefit application is rather limited.

Alterations in polyamine content, expression of genes and/or activity of polyamine biosynthetic enzymes due to salt stress have been explored in a large number of plant species, for example rice (Krishnamurthy and Bhagwat 1989; Chattopadhyay et al. 1997; Quinet et al. 2010), tomato (Santa-Cruz et al. 1997a, b, 1998; Botella et al. 2000), wheat (Li and Chen 2000b), sunflower (Alvarez et al. 2003), *Fraxinus angustifolia* (Tonon et al. 2004), Arabidopsis (Urano et al. 2004), *Lotus glaber* (Sanchez et al. 2005) and *Lupinus luteus* (Legocka and Kluk 2005). Most of the studies demonstrate that polyamine abundance is associated with enhanced plant salinity tolerance. Hence, perspective and modern strategy for improvement of plant tolerance to cope with salinity is the using of transgenic and molecular genetic approaches to increase cellular PAs levels. Current studies with gain- or loss-of-function mutants have also suggested a close relationship between plant stress tolerance and the level of endogenous PAs in plants (Alcazar et al. 2006). Therefore, screening of varieties that maintain high level of endogenous polyamines is promising approach to enhance salinity tolerance.

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