

Genetic Basis of Salt Tolerance In Micobres and Plants - A Brief Review

Sonia ¹ and Gaurav Sharma ²

1. Department of HealthCare Sciences, School of Pharmaceutical and Healthcare sciences, CT University, Ludhiana, India

2. Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara, India

Abstract

Salinity stress is the worldwide loss adversely affecting the growth and productivity of living organisms. For understand the mechanism of stress tolerance, there is a need for salinity stress tolerant species to mitigate the affect of salinity stress that helps in production of compatible solute which resumes the normal hemostatis under salt stress conditions. Identification of genes is the primary diagnosis for molecular studies involved in the production of compatible solutes. This review emphasize on different studies of salt stress tolerant mechanism from different halophilic / halotolerant microbes and plants.

Introduction

Deposition of salt in agricultural land leads to damage the soil fertility. According to Yeo, 1999, 20% of the irrigated land is presently affected by salinity. Use of novel genes for the identification of beneficial traits has developed for sustaining the food production. With the aid of biotechnology technique, It becomes accessible to deploy genes in target species. For instance crystal protein gene from the soil bacterium *Bacillus thuringiensis* in cotton (Jenkins et al. 1991). Therefore, it is imperative to explore the elite and meticulous type of organism including microbes which are having important genes for enhancing the agricultural production.

Osmoregulation

Osmoregulation is process of maintenance hemostasis under either high salt concentration or low salt concentration in organisational fluid (Solomon and Martin, 2002). With time, the bacteria has evolved osmoconformation to the changing osmotic balance between cellular fluid and external environment (Wood, 2011). During the course of time microbes has continue to develop a complex stress tolerant machinery to survive the change in external environment. These extremophilic microorganism has now been considered as good source for biotechnology and commercial

significance. Greater metabolic diversity are seen in halophilic or halo-tolerant eubacteria (Margesin and Schinner, 2001).

Microorganism is been classified in to slight halophiles that can tolerate 3% w/v NaCl in sea water,

moderate halophiles can tolerate 3-15 % NaCl, extreme halophiles at 25% w/v NaCl and borderline extreme halophiles at 12 % w/v salts (Kushner, 1988).

Several characterization has been carried out for salt tolerance in microbes (Das et al. 2015) identified and characterize bacteria *Staphylococcus epidermidis* to vary salt concentration where as *Bacillus cereus* can survive up to 20 % salt concentration (Behera et al. 2013a, 2013b). (Behera et al. 2015a, 2015b) reported isolates from east indian sea coast which are resistant to high salt concentration generally belongs to firmicutes and proteobacteria group.

Compatible solutes and salinity tolerance

Compatible solutes are low molecular weight, highly water soluble sugar, other alcohols, amino acids or their derivatives known to be osmotically active substances make bacteria and plants tolerant to high salinity condition (Ventosa et al. 1998). It prevent the cell from plasmolysis under salt stress condition (Kempf and Bremer, 1998). During stress compatible solute induce change in dynamics properties of protein rather than going change in itself.

Compatible solute act as a stabilizer of biomolecule such as DNA, enzyme, membrane and whole cell. It also act salt antagonistic, stress protective agent. Increase freshness of food by stabilising component. These osmolyte helps in protein folding during salt stress hence make cell tolerant to above stress and could be useful to agriculture and xeriscaping (Roberts, 2005; Detkova and Boltyanskaya, 2007).

Ectoine and salinity tolerance

Trehalose, is a non reducing sugar, occurs in various type of organism such as bacteria, fungi,

archaea, invertebrate and plants. It is synthesized by two gene namely *ost A* and *ost B* (Elbein et al. 2003). Trehalose is a major constituent of insect shell of the Middle East (Richards et al. 2002). Trehalose also help in maintaining metabolic hemostasis and stress tolerance in many organism (Turan et al. 2012). It is also a constituent of various food stuff like bread, wine, beer, vinegar and honey. Trehalose is highly hydrophilic, chemically stable, non-hygroscopic with no internal hydrogen bond formation. As a carbon source for bacteria, it is also extensively used as compatible solute to avoid osmotic stress (Argüelles, 2000). It has also been utilized for freeze drying of molecule for cryopreservation. It is also used in long term storage of microbes as it help in retaining intact membrane structure (Empadinhas and Costa, 2008).

Glycine betaine and salinity tolerance

Glycine betaine (N, N, N- trimethyl glycine) is an ammonium compound synthesized by choline oxidase found in haemophilic archaeobacteria, bacteria, invertebrates, mammals and plants during dehydration and under adverse hyper osmotic environment (Kempf and Bremer, 1998; Turan et al. 2012; Hussain ET AL. 2013; Chen and Murata, 2002). Roessler et al.. 2001, has reported the first ABC type transporter gene cluster of compatible solute glycine betaine in *Methanosarcina*, a methanogen species.

Two gene *BetP* and *EctP* encoding secondary carrier for uptake of glycine betaine Na-coupled system which prefer ectoine to glycine betaine. These two gene encode high affinity secondary carrier (Peter et al. 1998; Boscari et al. 2002).

Na⁺ and H⁺ antiporters and salinity tolerance

Na⁺ and H⁺ is involved in various cell functioning whose extreme high and extreme low bring malfunctioning of cell (Padan et al. 2001). Cell adopt various mechanism for maintaining homeostatic balances by enhancement of K⁺ Uptake, removal of surplus Na⁺, sequestration of Na⁺ ions in to intracellular compartment such as vacuole and production of compatible solute in cytoplasm. In

prokaryote generally five classes of Na^+/H^+ antiporters is found (Krulwich et al. 2009), among them NhaA is the most extensively studied Na^+/H^+ antiporter present in plasma lemma and tonoplast of *E. coli* (Volkmar et al. 1998).

Plant has check methods to maintain the ion concentration at low level in cytoplasm. Membrane and its ion uptake transporter play a key role in maintaining low ionic concentration to mitigate ionic stress (Sairam and Tyagi, 2004). This complex network of ion exchange dynamics is controlled by carrier proteins, channels protein, various antiporters and symporters. Cellular regulation of Na^+/K^+ homeostasis is a major ditch attempt by the cell to counteract salt stress. Genes from arabidopsis such as NADPH oxidases Atrboh and AtrbohF activated in presence of reactive oxygen species helps in regulating Na^+/K^+ homeostasis under salt stress (Ma et al. 2012).

High potassium level is required by plant cell in order to carry out cytoplasmic enzyme activity. A concentration of 100 mM is considered ideal for cellular enzymatic activity. Vacuole act as a storage reservoir of K^+ ion in plant cell and act as a key component in maintaining plant cell turgor pressure. During salt stress K^+ is transported against the concentration gradient with the help of K^+ transporter and membrane channels. During low concentration of extra cellular K^+ , high affinity K^+ uptake is mediated by K^+ transporter where as low affinity uptake is carried out by K^+ transporter. The uptake of K^+ ion depend on the concentration dynamics of soil. In order to maintain plant cell hemostasis Na^+ ion concentration are low in the cytosol. During saline condition in soil there is increase in soil Na^+ concentration which competes with uptake of K^+ ion for the transporter as both the ion shares common transport mechanism, there by decreasing the K^+ ion uptake ((Sairam and Tyagi, 2004; Munns and Tester, 2008).

Polyamines and Salinity tolerance

Polyamines are polycationic molecules which play role in growth and development such as cell proliferation, somatic embryogenesis, dormancy breaking of tubers, development of fruit and

senescence (Knott et al. 2007; Gupta et al. 2013; Panicot et al. 2012 and Galston et al. 1997). It is synthesised against abiotic stress tolerance such as salinity in plants. Increase in concentration of polyamines are highly linked with stress tolerance in plants (Gupta et al. 2013; Yang et al. 2007; Groppa and Benavides, 2008; Kovács et al. 2010). Diamine putrescine (PUT), triamine spermidine (SPD), and tetra-amine spermine are most commonly found polyamines in plants (Alcázar et al. 2011; Martin-Tanguy, 2001; Kuznetsov and Shevyakova, 2007; Hussain et al. 2011; Shu et al. 2012).

Nitric Oxide and Salinity Tolerance

NO is well known to be involved in different plant growth and developmental processes like flowering, stomatal closure and respiration, cell death and stress response (Delledonne et al. 1998; Lamattina et al. 2013; Besson-Bard et al. 2008; Zhao et al. 2009; Crawford, 2005). It is also involved in regulation of gene expression under variant redox potential. It reacts with lipid radicals and helps in removal of superoxide radical by formation of peroxynitrite that can be neutralized by cellular processes. The antioxidant enzymes such as SOD, CAT, GPX, APX and GR are activated by NO. It has been reported that exogenous application of NO on plant is able to mitigate stress (Sung and Hong, 2010; Hossain et al. 2010; Xiong et al. 2010). An application of Sodium nitroprusside (SNP) at concentrations between 0.1 and 800 μ M, act as NO donor and show increase in seed germination and root growth in *Lupinus* lettuce subjected to salt stress (Kopyra and Gwóźdz, 2003).

Stress Induced Reactive Oxygen Species

Salt stress induces production of reactive oxygen species. These species are highly reactive and damage cellular DNA, RNA, protein and lipid leading to cell death. In stress tolerant plant these ROS induced gene expression of certain set of genes such as catalase, peroxidase, superoxide dismutase etc to detoxify reactive oxygen species. Plant requires some amount of ROS to initiate gene expression for their vital function and different concentration of ROS will bring different kind of

changes in physiology of plant. These type of delicate imbalance in ROS will is optimized by efficient working of internal defence system of plant called an antioxidant system. This system may comprise of enzymatic and non enzymatic components. Down regulation of antioxidant system may lead to cell death due to damage of cellular component by distotered ion hemostasis hence anamolies in metabolism (Ahanger et al. 2017).

Therefore proper mineral nutrition is required during agricultural practices to ensure no stress condition. Potassium is a key macro elements involved in proper regulation of hemostasis and report in accumulation of of osmolyte and regulation of antioxidant mechanism. Suboptimal soil level of K soil status is very important in preventing salt stress.

Conclusion

In a changing climatic senerio it is important to develop engineered microbes and plants tolerant to salt stress condition. With above view , there is need to emphasise on halophillic plant and microbes. There is also need to understand the genetic mechanism underlying stress tolerance to utilize the gene resource in direction to enhance the quality and quantity of product under continous stress condition. There is also need to change agricultural practices in order to mitigate stress due to salts present in soil. Plant and microbes produces variety of compatible solutes which help in maintaining optimal cell hemostasis during salt stress can prove to be promising solution. Identification of genes is the primary diagnosis for molecular studies involved in the accumulation of compatible solutes.

References

1. Ahanger, M. A., Tomar, N. S., Tittal, M., Argal, S., & Agarwal, R. M. (2017). Plant growth under water/salt stress: ROS production; antioxidants and significance of added potassium under such conditions. *Physiology and Molecular Biology of Plants*, 23(4), 731-744.
2. Alcázar, R., Cuevas, J. C., Planas, J., Zarza, X., Bortolotti, C., Carrasco, P., & Altabella, T. (2011). Integration of polyamines in the cold acclimation response. *Plant Science*, 180(1), 31-38.

3. Argüelles, J. C. (2000). Physiological roles of trehalose in bacteria and yeasts: a comparative analysis. *Archives of microbiology*, 174(4), 217-224.
4. Behera, B.K., Das, P., Maharana, J., Singh, N.S., De, B.C., Jana, A.K., Meena, D.K., Sharma, A.P. (2013a.) Transcriptome Analysis of marine bacterium, *Bacillus cereus* in response to elevated salt stress. In: Abstracts of 100th the Indian Science Congress, held at Kolkata during 3 7th January.
5. Behera, B.K., Das, P., Maharana, J., De, B.C., Pramanik, S., Meena, D.K., Samarjit, N.S., Sahu, T.K., Rao, A.R., Jana, A.K., Sharma, A.P. (2013b). De novo whole transcriptome analysis of P-30 (*Staphylococcus epidermidis*), Gene Bank accession (JZ1989-JZ199140).
6. Behera, B. K., Das, P., Maharana, J., Meena, D. K., Sahu, T. K., Rao, A. R., & Sharma, A. P. (2015). Functional screening and molecular characterization of halophilic and halotolerant bacteria by 16S rRNA gene sequence analysis. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, 85(4), 957-964.
7. Behera, B. K., Das, P., Maharana, J., Paria, P., Mandal, S. N., Meena, D. K., & Vellarikkal, S. K. (2015). Draft genome sequence of the extremely halophilic bacterium *Halomonas salina* strain CIFRI1, isolated from the east coast of India. *Genome Announc.*, 3(1), e01321-14.
8. Besson-Bard, Angélique, Alain Pugin, and David Wendehenne. "New insights into nitric oxide signaling in plants." *Annu. Rev. Plant Biol.* 59 (2008): 21-39.
9. Boscardi, A., Mandon, K., Dupont, L., Poggi, M. C., & Le Rudulier, D. (2002). BetS is a major glycine betaine/proline betaine transporter required for early osmotic adjustment in *Sinorhizobium meliloti*. *Journal of bacteriology*, 184(10), 2654-2663.
10. Chen, T. H., & Murata, N. (2002). Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. *Current opinion in plant biology*, 5(3), 250-257.
11. Crawford, N. M. (2005). Mechanisms for nitric oxide synthesis in plants. *Journal of experimental botany*, 57(3), 471-478.
12. C. H. Sung and J. K. Hong, "Sodium nitroprusside mediates seedling development and attenuation of oxidative stresses in Chinese cabbage," *Plant Biotechnology Reports*, vol.4,no.4,pp. 243–251,2010.
13. Das, P., Behera, B. K., Meena, D. K., Azmi, S. A., Chatterjee, S., Meena, K., & Sharma, A. P. (2015). Salt stress tolerant genes in halophilic and halotolerant bacteria: Paradigm for salt stress adaptation and osmoprotection. *Int. J. Curr. Microbiol. App. Sci*, 4(1), 642-658.
14. Delledonne, M., Xia, Y., Dixon, R. A., & Lamb, C. (1998). Nitric oxide functions as a signal in plant disease resistance. *Nature*, 394(6693), 585.

15. Detkova, E. N., & Boltyanskaya, Y. V. (2007). Osmoadaptation of haloalkaliphilic bacteria: role of osmoregulators and their possible practical application. *Microbiology*, 76(5), 511-522.
16. Elbein, A. D., Pan, Y. T., Pastuszak, I., & Carroll, D. (2003). New insights on trehalose: a multifunctional molecule. *Glycobiology*, 13(4), 17R-27R.
17. Empadinhas, N., & da Costa, M. S. (2008). Osmoadaptation mechanisms in prokaryotes: distribution of compatible solutes. *Int Microbiol*, 11(3), 151-161.
18. Galston, A. W., Kaur-Sawhney, R., Altabella, T., & Tiburcio, A. F. (1997). Plant polyamines in reproductive activity and response to abiotic stress. *Botanica Acta*, 110(3), 197-207.
19. Groppa, M. D., & Benavides, M. P. (2008). Polyamines and abiotic stress: recent advances. *Amino acids*, 34(1), 35.
20. Gupta, K., Dey, A., & Gupta, B. (2013). Plant polyamines in abiotic stress responses. *Acta physiologiae plantarum*, 35(7), 2015-2036.
21. Gupta, K., Dey, A., & Gupta, B. (2013). Polyamines and their role in plant osmotic stress tolerance. *Climate Change and Plant Abiotic Stress Tolerance*, 1053-1072.
22. Hossain, K. K., Itoh, R. D., Yoshimura, G., Tokuda, G., Oku, H., Cohen, M. F., & Yamasaki, H. (2010). Effects of nitric oxide scavengers on thermoinhibition of seed germination in *Arabidopsis thaliana*. *Russian journal of plant physiology*, 57(2), 222-232
23. Hussain, S. S., Ali, M., Ahmad, M., & Siddique, K. H. (2011). Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. *Biotechnology advances*, 29(3), 300-311.
24. Hussain Wani, S., Brajendra Singh, N., Haribhushan, A., & Iqbal Mir, J. (2013). Compatible solute engineering in plants for abiotic stress tolerance-role of glycine betaine. *Current genomics*, 14(3), 157-165.
25. Jenkins, J. N., Parrott, W. L., McCarty Jr, J. C., Barton, K. A., & Umbeck, P. F. (1991). Field test of transgenic cottons containing a *Bacillus thuringiensis* gene. *Technical bulletin-Mississippi Agricultural and Forestry Experiment Station (USA)*.
26. Kempf, B., & Bremer, E. (1998). Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. *Archives of microbiology*, 170(5), 319-330.
27. Knott, J. M., Römer, P., & Sumper, M. (2007). Putative spermine synthases from *Thalassiosira pseudonana* and *Arabidopsis thaliana* synthesize thermospermine rather than spermine. *FEBS letters*, 581(16), 3081-3086.
28. Kopyra, M., & Gwózdź, E. A. (2003). Nitric oxide stimulates seed germination and counteracts the inhibitory effect of heavy metals and salinity on root growth of *Lupinus luteus*. *Plant Physiology and Biochemistry*, 41(11-12), 1011-1017.

29. Kovács, Z., Simon-Sarkadi, L., Szűcs, A., & Kocsy, G. (2010). Differential effects of cold, osmotic stress and abscisic acid on polyamine accumulation in wheat. *Amino acids*, 38(2), 623-631.
30. Krulwich, T. A., Hicks, D. B., & Ito, M. (2009). Cation/proton antiporter complements of bacteria: why so large and diverse?. *Molecular microbiology*, 74(2), 257-260.
31. Kushner, D. J. (1988). What is the "true" internal environment of halophilic and other bacteria?. *Canadian journal of microbiology*, 34(4), 482-486.
32. Lamattina, L., García-Mata, C., Graziano, M., & Pagnussat, G. (2003). Nitric oxide: the versatility of an extensive signal molecule. *Annual Review of Plant Biology*, 54(1), 109-136.
33. L. Ma, H. Zhang, L. Sun et al. (2012.) NADPH oxidase Atrboh D and AtrbohF function in ROS-dependent regulation of Na⁺/K⁺ homeostasis in Arabidopsis under salt stress. *Journal of Experimental Botany*, 63(1), 305–317.
34. Margesin, R., & Schinner, F. (2001). Potential of halotolerant and halophilic microorganisms for biotechnology. *Extremophiles*, 5(2), 73-83.
35. Martin-Tanguy, J. (2001). Metabolism and function of polyamines in plants: recent development (new approaches). *Plant Growth Regulation*, 34(1), 135-148.
36. Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59, 651-681.
37. Padan, E., Venturi, M., Gerchman, Y., & Dover, N. (2001). Na⁺/H⁺ antiporters. *Biochimica et biophysica acta (BBA)-Bioenergetics*, 1505(1), 144-157.
38. Panicot, M., Minguet, E. G., Ferrando, A., Alcázar, R., Blázquez, M. A., Carbonell, J., & Tiburcio, A. F. (2002). A polyamine metabolon involving aminopropyl transferase complexes in Arabidopsis. *The Plant Cell*, 14(10), 2539-2551.
39. Peter, H., Weil, B., Burkovski, A., Krämer, R., & Morbach, S. (1998). *Corynebacterium glutamicum* is equipped with four secondary carriers for compatible solutes: identification, sequencing, and characterization of the proline/ectoine uptake system, ProP, and the ectoine/proline/glycine betaine carrier, EctP. *Journal of bacteriology*, 180(22), 6005-6012.
40. Richards, A. B., Krakowka, S., Dexter, L. B., Schmid, H., Wolterbeek, A. P. M., Waalkens-Berendsen, D. H., ... & Kurimoto, M. (2002). Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. *Food and chemical toxicology*, 40(7), 871-898.
41. Roberts, M. F. (2005). Organic compatible solutes of halotolerant and halophilic microorganisms. *Saline systems*, 1(1), 5.
42. Roessler, M., Pflüger, K., Flach, H., Lienard, T., Gottschalk, G., & Müller, V. (2002).

- Identification of a salt-induced primary transporter for glycine betaine in the methanogen *Methanosarcina mazei* Gö1. *Appl. Environ. Microbiol.*, 68(5), 2133-2139.
43. Sairam, R. K., & Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current science*, 407-421.
 44. Shu, S., Guo, S. R., & Yuan, L. Y. (2012). A review: polyamines and photosynthesis. *Advances in Photosynthesis-Fundamental Aspects InTech*, 439-464.
 45. Solomon, E., Berg, L., Martin, D. 2002. Biology, 6th edn. Brooks/Cole Publishing.
 46. Turan, S., Cornish, K., & Kumar, S. (2012). Salinity tolerance in plants: breeding and genetic engineering. *Australian Journal of Crop Science*, 6(9), 1337.
 47. Ventosa, A., Nieto, J.J., Oren. (1998). A. Biology of moderately halophilic aerobic bacteria. *Microbiol. Mol. Biol. Rev.*, 62, 504-544.
 48. V. V. Kuznetsov and N. I. Shevyakova. (2007) Polyamines and stress tolerance of plants. *PlantStress*, 1, 50–71.
 49. Volkmar, K. M., Hu, Y., & Steppuhn, H. (1998). Physiological responses of plants to salinity: a review. *Canadian journal of plant science*, 78(1), 19-27.
 50. Wood, J. M. (2011). Bacterial osmoregulation: a paradigm for the study of cellular homeostasis. *Annual review of microbiology*, 65, 215-238.
 51. Xiong, J., Fu, G., Tao, L., & Zhu, C. (2010). Roles of nitric oxide in alleviating heavy metal toxicity in plants. *Archives of biochemistry and biophysics*, 497(1-2), 13-20.
 52. Yang, J., Zhang, J., Liu, K., Wang, Z., & Liu, L. (2007). Involvement of polyamines in the drought resistance of rice. *Journal of Experimental Botany*, 58(6), 1545-1555.
 53. Yeo, A. (1998). Predicting the interaction between the effects of salinity and climate change on crop plants. *Scientia Horticulturae*, 78(1-4), 159-174.
 54. Zhao, M. G., Chen, L., Zhang, L. L., & Zhang, W. H. (2009). Nitric reductase-dependent nitric oxide production is involved in cold acclimation and freezing tolerance in *Arabidopsis*. *Plant physiology*, 151(2), 755-767.