CHAPTER 6

General discussion

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Growth and survival of halophytes depend on adaptation to low osmotic potential and high salt concentrations in sea water. Since *Bruguiera cylindrica* is a facultative mangrove, it was possible to grow salt-acclimated as well as non-salt acclimated plants, which provided a good experimental system to try and understand various aspects of salt adaptation in this species.

The osmolarity of the salt-acclimated plants was about 1.5 fold that of the non-acclimated plants and could be attributed mainly to accumulation of Na^+ ions, while K^+ and Ca^{2+} levels were similar. Since stress is known to lead to Ca^{2+} influx into the cytosol, where it acts as a signaling molecule, the additional Ca^{2+} needs to be removed and homeostasis restored, since cytosolic Ca^{2+} accumulation is know to have harmful effects (Plieth, 2005). We discovered a high capacity Ca^{2+}/H^+ antiporter in the plasma membrane of *B. cylindrica* that probably plays a role in Ca^{2+} efflux from the cytosol (Chapter 4). Maintenance of Ca^{2+} homeostasis would be a crucial aspect of salt adaptation in mangroves since ionic perturbations are likely to arise due to accumulation of Na^+ ions.

An important metabolic consequence of salt exposure is an increase in the respiration rate and this was seen to be concentration dependent in leaves of *B. cylindrica*. However the surge in respiration rate was short-lived and after an hour the leaves showed a drop in respiration rates to original levels irrespective of the salt-concentrations they were exposed to. Since respiratory energy is required for providing the driving force for ion transport, we attempted to study whether blocking Na^+ transport could reduce the respiratory rate. This was seen to be the case and we could find a possible explanation for the salt-induced respiration (Chapter 3). Additionally this effect was seen to be specific to

Na^+ and K^+ ions, indicating that activity of specific ion transporters were involved. However, the increase in respiratory rate was seen to have a non-phosphorylating, SHAM sensitive component, in addition to the phosphorylating, KCN sensitive component, indicating that the alternative oxidase was recruited in mitochondrial electron transport, besides cytochrome c oxidase. The alternative oxidase is known to carry out futile electron transport in plant mitochondria, in that most of the energy is released as heat rather than being used for oxidative phosphorylation. The AOX pathway has been shown to prevent generation of reactive oxygen species that could arise due to metabolic dysfunction under stress conditions (Clifton *et al*., 2005; Arnholdt-Schmitt *et al*., 2006). In *B. cylindrica*, we showed that the AOX pathway was engaged in leaves of non-acclimated plants, indicating that the functioning of mitochondria of non-acclimated plants was probably affected on salt exposure and that these plants resorted to using the AOX pathway to prevent accumulation of reactive oxygen species. We were therefore able to ascribe two possible causes for the higher respiratory rates seen in this mangrove on exposure to high salt – (a) for sequestering Na^+ ions through energizing the ion transporters via ATPases and (b) for preventing accumulation of reactive oxygen species by recruiting the AOX pathway. Though 4 M NaCl was a very high concentration to use, since such a high salt concentration is seldom encountered by the mangrove, it enabled us to discover the probable mechanisms that a plant is likely to use for salt adaptation.

By comparing the responses of salt-acclimated and non-acclimated plants of *B. cylindrica* to subsequent dehydration stress or osmotic shock, we were able to ascribe the better performance of salt-acclimated plants to the phenomenon of priming. Priming is seen to improve tolerance of a variety of plants to subsequent stress conditions and salt or halopriming has been reported to improve the ability of the primed plants to tolerate different types of abiotic stresses (Knight *et al*., 1998; Sivritepe *et al*., 2003; Cuartero *et al*., 2006). While the beneficial effects of priming have been studied in glycophytes, we hypothesized that similar priming effects may play a role in enabling this mangrove to cope with seasonal variations in salt concentration that are known to occur in their natural habitat. Salt priming could therefore be the cause underlying the improved osmotic adjustment and antioxidant metabolism that was seen in the salt-acclimated plants as compared to the non-acclimated plants, which enabled the former to withstand dehydration stress or a severe osmotic stress better than the latter (Chapter 2). The mechanism of priming is thought to involve accumulation of transcripts of protective proteins probably through chromatin remodeling (Bruce *et al*., 2007). Some evidence of this mechanism was seen when we compared the transcript levels of three ion transporters and a p-type H^+-ATPase, where the salt-acclimated plants showed higher expression levels than the non-acclimated plants (Chapter 5).

Bruguiera cylindrica is a mangrove that is well adapted to the salinity prevailing in coastal waters of the sea-shore, which is its natural habitat. We hypothesized that salt-acclimated plants of *Bruguiera cylindrica* (which would be the physiological condition of the plants growing in their natural habitat) were better equipped to cope with variation in the external salt concentration due to the priming effect of salt. In our experiments these salt-acclimated plants showed no apparent stress symptoms even when subjected to ten-fold higher salt concentration or to dehydration stress.

When grown in absence of salt, the mangrove was unable to acclimate to salt and exhibited a stressed phenotype like glycophytes. This indicated that salt tolerance was not an outcome of the presence of suitable genes in the genome, but depended on the regulation of their expression. Various researchers have claimed enhancement of salt tolerance through overexpression of genes that act on mechanisms involved in the process of tolerance (Flowers, 2004). However, it has not been possible to obtain transgenics with sufficient tolerance level from an agronomic point of view, probably due to the lack of knowledge about the regulatory mechanisms operating in the plant that control their expression. On the other hand, functional genomic studies have revealed the induction of a large number of genes involved in the process of salt tolerance, but it is difficult to distinguish between the genes contributing to salt tolerance and those induced in response to non-specific effects of saline treatments (Munns, 2005).

Halophytes are interesting model systems for studying salt-adaptation mechanisms. These species are envisaged to use the same range of transporters and regulatory networks seen in other, less tolerant, species but with different set points (Flowers and Colmer, 2008). Understanding the set points for the different salt tolerant mechanisms in halophytes would be vital for improving salt tolerance in commercially important crop species.