

# Current Knowledge in Physiological and Genetic Mechanisms Underpinning Tolerances to Alkaline and Saline Subsoil Constraints of Broad Acre Cropping in Dryland Regions

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## 1. Introduction

Abiotic stresses are a serious problem to crop production under dryland conditions in arid and semi-arid regions of the world. These abiotic stresses include high and low temperature, water deficit, salinity, sodicity, alkalinity, acidity and ion deficiencies and toxicities. Many Australian agricultural soils accumulate salts under seasonal fluctuations and have multiple subsoil constraints such as alkalinity, acidity, sodicity, and toxic ions (Rengasamy, 2006). Of these, salinity and alkalinity are simultaneously found in soils of southern Australia (Nuttall *et al.*, 2003a; Nuttall *et al.*, 2003b). The simultaneous occurrence of multiple abiotic stresses may result in far greater productivity loss than any single abiotic or biotic factor.

Nearly 800 million ha of land throughout the world is salt affected either by salinity or associated with sodicity (FAO, 2009). The total area under salinity in Australia is estimated to be 32 million ha in arable and permanent cropping land (FAO, 2000). Transient or dryland salinity is probably the biggest factor causing salinity in Australia (Figure 1; Rengasamy 2002). Saline soils are generally defined as those having high concentrations of soluble salt with an electrical conductivity ( $EC_e$ ) of more than 4  $dSm^{-1}$ . Among the soluble salts, NaCl is the major component contributing to salinity (USSL, 2005).

Yields of important cereal, oilseed and forage crops are limited by soil salinity in broad acre dryland regions. Therefore, genetic crop improvement by conventional and non-conventional methods for salt tolerance is vital to maintain food production. The ability to grow and reproduce in saline soil differs widely between species, due to differences in the ability to control salt uptake from the soil and to compartmentalise it effectively at the cellular level (Munns & Tester, 2008).

Crops grown under dryland conditions on alkaline soils in south-eastern Australia are potentially limited by many factors, especially water supply and nutrition (Incerti & O'Leary, 1990). Alkaline soils are usually categorized by low availability of plant nutrients, high concentrations of  $HCO_3^-$  and  $CO_3^{2-}$ , and high pH (Marschner, 1995; Misra & Tyler, 1999). By definition, alkalinity is the concentration of soluble alkalis with the ability to neutralize acids (Bailey, 1996). Bicarbonate ( $HCO_3^-$ ) and carbonate ( $CO_3^{2-}$ ) are the principal

contributors to alkalinity, whereas hydroxide, borate, ammonia, organic bases, phosphates, and silicates are considered minor contributors (Petersen, 1996). This review will discuss how salinity and alkalinity affect plant growth and the different methods used to identify and improve tolerance in various crop species.

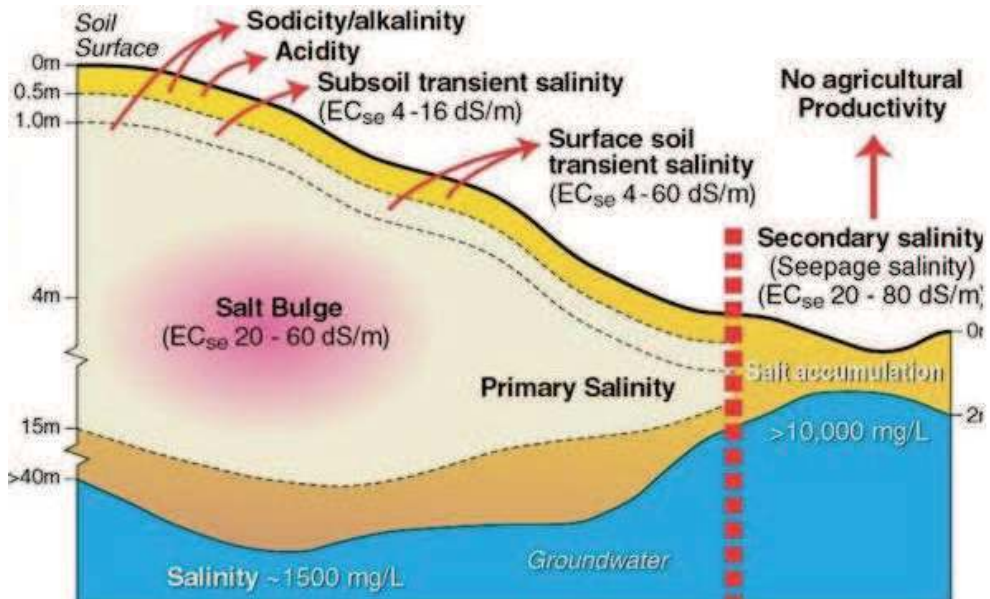


Fig. 1. Different types of salinity in Australian landscapes (after Rengasamy, 2002).

## 2. Salinity effects in plants

The most common effects of salinity on plant growth are smaller and fewer leaves, reduced plant height and poor yield (Kumar, 1995). At the physiological level, salinity imposes an osmotic stress that limits water uptake and ion toxicity can cause nutrition (N, Ca, K, P, Fe, Zn) deficiency and oxidative stress (Munns, 2002). Salinity can differentially affect the mineral nutrition of plants. Nutrient imbalances due to salinity diminish plant growth by affecting the availability, transport, and partitioning of nutrients. Nutrient deficiencies or imbalances result due to competition of Na and Cl with other nutrients such as K, Ca, Mg and  $\text{NO}_3$  (Hasegawa & Bressan, 2000; Hu & Schmidhalter, 1998; Hu & Schmidhalter, 2005; Munns, 2002; Netondo *et al.*, 2004). These nutrient imbalances due to salinity also cause reduction in plant growth. Plant responses to salinity can vary with the degree and duration of the stress imposed as well as the plant developmental stage (seedling, flowering, maturity) when the stress is applied (Munns, 1993). To identify truly tolerant germplasm, it is important to gain full information regarding the degree of salt tolerance at all growth stages of a crop species. Otherwise selection at one particular growth stage may result in plants that lose their tolerance at other stages.

### 2.1 Mechanisms of salt tolerance

Plants are generally categorized as either halophytes or glycophytes. Halophytes grow and survive best where salt concentration is 200 mM or more (Flowers & Colmer, 2008). Conversely, glycophytes cannot survive under high saline conditions (Figure 2). A major difference between halophytes and glycophytes is the ability of halophytes to survive salt shock (Braun *et al.*, 1986; Casas *et al.*, 1991; Hassidim *et al.*, 1990).

Several mechanisms for tolerance operate in both halophytes and glycophytes and the differences are presented in Figure 3. However, the main adaptive strategies of salt-tolerant glycophytic plants exposed to salinity are: 1) avoidance through ion exclusion, potentially as a result of low membrane ion permeability; 2) tolerance, through ion inclusion and possible compartmentalisation; and 3) osmotic stress tolerance, which enables the plant to remain functional despite internal ionic stress (Blumwald *et al.*, 2004; Munns, 2005; Munns & Tester, 2008).

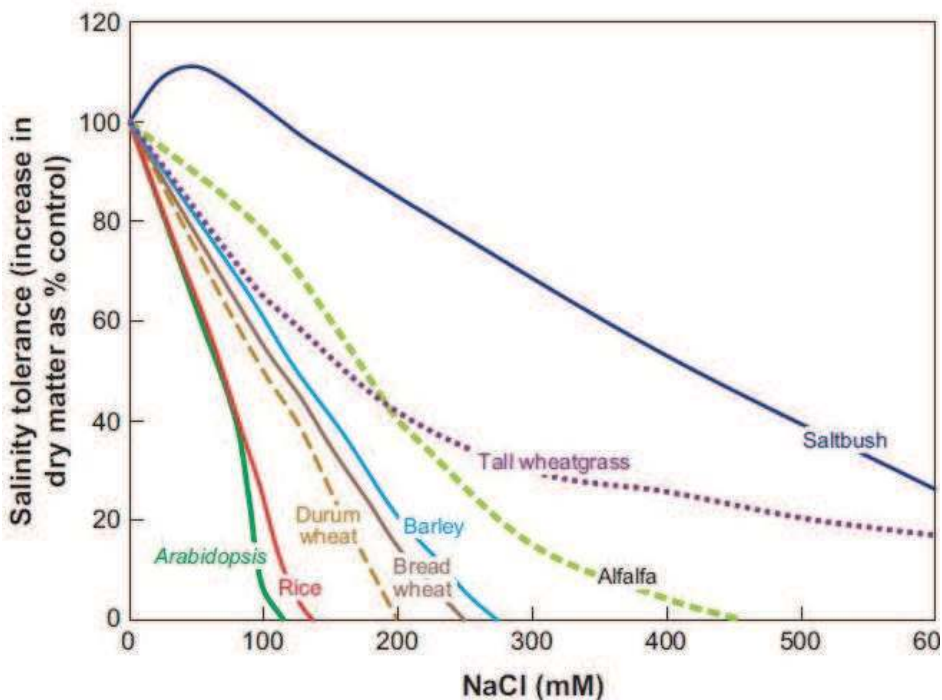


Fig. 2. Response of glycophytes and halophytes to varying concentrations of NaCl after 3 weeks of treatment (after Munns & Tester, 2008).

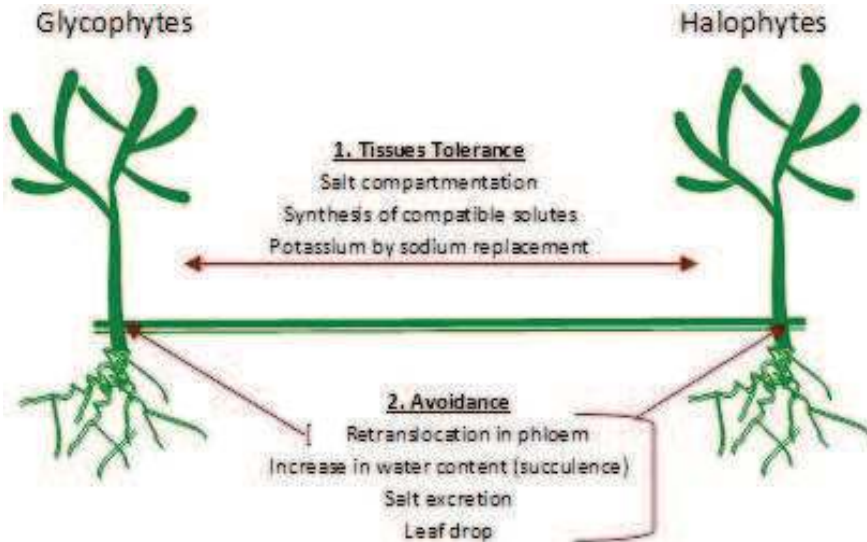


Fig. 3. Adaptive strategies for salt tolerance in plants.

## 2.2 Ion exclusion

Na avoidance is an essential salt tolerance mechanism that operates in several glycophytes such as; wheat (Munns, 2005; Munns & Tester, 2008), *Arabidopsis* (Møller *et al.*, 2009; Møller & Tester, 2007), *B. napus* and *B. juncea* (Ashraf & McNeilly, 2004; Ashraf *et al.* 2001). To achieve this type of tolerance, plant roots must exclude most of the Na and Cl dissolved in the soil solution, and escape from gradual build-up of salt in shoots to toxic levels (Munns, 2005). Salinity tolerance in cereals is largely contributed by Na exclusion. As plants transpire about 50 times more water than they retain in their leaves, exclusion of 98% (1/50) of the salt in the soil solution results in stable leaf Na concentration (Munns, 2005). For instance, bread wheat excluded > 98% of the Na in the soil solution, and consequently Na concentration build-up in leaves remained less than 50 mM (Husain *et al.*, 2004). Barley, on the other hand, excluded < 98% of the Na in the soil solution, and the concentrations reached up to 500 mM (Rawson *et al.*, 1988).

Salinity tolerance in *B. juncea* however, is achieved through partial exclusion (Ashraf & McNeilly, 2004; Ashraf *et al.*, 2001). In some members of the Brassicaceae including *Arabidopsis*, salinity tolerance appears to be mainly achieved by tissue tolerance to accumulated Na rather than by exclusion of Na from the shoot (Møller & Tester, 2007). In two *B. napus* genotypes, the genotype with the higher Na accumulation in the shoot was more Na tolerant and also accumulated increased amounts of K and proline (Huang & Redman, 1995). These results imply that tissue tolerance to Na and tolerance to osmotic stress are more important than Na exclusion from the shoot in members of the Brassicaceae.

## 2.3 Ion compartmentation

Salt tolerance by compartmentation is very important mechanism that operates in many glycophytes such as *Arabidopsis* (Møller *et al.*, 2009; Møller & Tester, 2007), wheat and barley (Munns, 2005; Munns *et al.*, 1995; Munns & Tester, 2008), and *B. juncea* (Ashraf & McNeilly,

2004; Kumar *et al.*, 2009). Ion specific effects of salinity primarily result in accumulation of higher levels of sodium (Na), usually in the older leaves. Entry of Na into the cell is due to the similarity in hydrated ionic radii between Na and K that makes it difficult for the transporters to distinguish between these two ions (Blumwald *et al.*, 2000). To avoid deleterious Na toxicity in the cytoplasm, it must be compartmentalised into cell vacuoles (Munns & Tester, 2008). This allows maintenance of optimum cellular levels of K and calcium (Ca) as well as Na exclusion by the plant. These two activities are known to operate at the plasma membrane and tonoplast levels, as integral components of the ion transport network. This is one of the key physiological criteria of plant salt tolerance, to maintain optimal K/Na ratio in the cytosol (Singla-Pareek *et al.*, 2003; Singla-Pareek *et al.*, 2008; Tester & Davenport, 2003). A higher K/Na ratio essentially indicates that a plant has not only excluded Na to some extent but has also maintained a healthy level of K for normal metabolic activities and injury avoidance under salinity. Hence, manipulation of the salt overly sensitive (SOS) pathway, Na/H antiporters and/or K transmembrane transporters that are involved in ion homeostasis may be the target of future strategies for salt tolerance improvement in a range of crops including canola quality *B. juncea* (Benke *et al.*, 2010; Blumwald *et al.*, 2004).

The role of transport proteins such as antiporters, ion channels, ABC-type transporters, Na and K transporters, plasma membrane and vacuolar ATPases is fundamental for salt tolerance in Na<sup>+</sup> exclusion, ion homeostasis, and compartmentalization of solutes and amino acids under stress (Apse *et al.*, 2003; Takahashi *et al.*, 2009). The over-expression of vacuolar Na/H antiporter in *B. napus* greatly diminished the salt-induced oxidative stress in the vacuoles, highlighting the importance of Na homeostasis during salt stress tolerance (Ruiz & Blumwald, 2002; Zhang *et al.*, 2001b). The *Arabidopsis thaliana* vacuolar alkali cation transporter AtNHX1 has been shown to increase salt tolerance in transgenic plants through the intracellular compartmentation of Na (Apse *et al.*, 1999; Hernández *et al.*, 2009; Venema *et al.*, 2002).

#### 2.4 Osmotic adjustment

Salinity is a common feature of arid and semiarid lands, and plants have evolved mechanisms to tolerate the low soil water potential caused by salinity, as well as by drought, and so some level of tolerance to osmotic stress is a feature of most glycophytes and halophytes (Munns & Tester, 2008). Osmotic adjustment in plants exposed to salt stress helps to maintain turgor pressure, which consequently helps plant to achieve tolerance under saline conditions (Ashraf & McNeilly, 2004). A significant genetic variation within species may exist in the osmotic response under saline stress; however this has not yet been documented (Munns & Tester, 2008). In salt sensitive plants, low water potential caused by salinity stress leads to cell membrane damage causing ion toxicity and cell injury (Chen & Murata, 2002; Sreenivasulu *et al.*, 2000). This primarily results in smaller leaves and reduction in leaf area in many crops. Reduction in leaf area development and relative root growth might decrease the water use by the plant, which allows it to preserve soil moisture and avoid an escalation in the salt concentration in the soil (Munns & Tester, 2008). Osmotic adjustment occurs in plants subjected to saline stress, but particularly to a large extent in salt-tolerant Brassica species (Ashraf & McNeilly, 2004).

This is primarily due to accumulation of different types of organic osmotica such as soluble sugars, free amino acids and free proline in most of the salt-tolerant Brassica species (Ashraf & Akram, 2009; Ashraf & McNeilly, 2004). The relative importance of variation in osmotic tolerance remains unknown for most crop species, due to inherent difficulties in quantifying this parameter. However, a close association is likely exists between osmotic tolerance and

tissue tolerance of  $\text{Na}^+$ , because genotypes that tolerate high internal  $\text{Na}^+$  concentrations in leaves by compartmentalizing it in the vacuole may also be more tolerant of the osmotic stress owing to their elevated osmotic adjustment (Munns & Tester, 2008). However, this theory needs further investigation.

## 2.5 Molecular control of salt tolerance

The adaptive physiological and biochemical responses of a plant to salinity are controlled by genes that encode salt tolerance mechanisms (Casas *et al.*, 1992). Since salinity tolerance is a complex trait, it is most likely controlled by interactions of hundreds of salt responsive genes (Sahi *et al.*, 2006; Winicov, 1998). Plants recognise a salinity stress and condition adaptive response mechanisms (Hasegawa & Bressan, 2000). Reported responses involve many molecular processes such as ion homeostasis (membrane proteins involved in ionic transport), osmotic adjustment and water regime regulation (osmolytes), as well as scavenging of toxic compounds (enzymes; Benke *et al.*, 2010; Blumwald *et al.*, 2004). The regulatory molecules conditioning these responses have been found to be cellular signal pathway components and transducers of long distance response co-ordination such as hormones, mediators, transcription factors and regulatory genes (Mishra *et al.*, 2006). The expression of such genetic regulators during plant stress has been studied at the transcriptional level (Fernandez *et al.*, 2008; Hasegawa & Bressan, 2000). Consequently, abiotic stress-inducible genes have been classified into two categories; 1) those that directly protect against environmental stress; and 2) those that regulate gene expression and signal transduction against stress response (Hasegawa & Bressan, 2000; Kawaura *et al.*, 2008; Mishra *et al.*, 2006; Popova *et al.*, 2008; Ueda *et al.*, 2002). Some of the major genes/proteins that are activated under salinity might be involved in tolerance (Table 1). Hence, it is imperative to analyse the functions of stress-inducible genes for amplification of the molecular mechanisms of stress tolerance in plants.

Salt tolerance is attained through three interrelated characteristics; the foremost, salt injury must be avoided or alleviated. Second, homeostatic conditions must be re-established in the new stressful environment. Third, growth must resume, even if at a reduced rate (Fig. 4; Zhu, 2001).

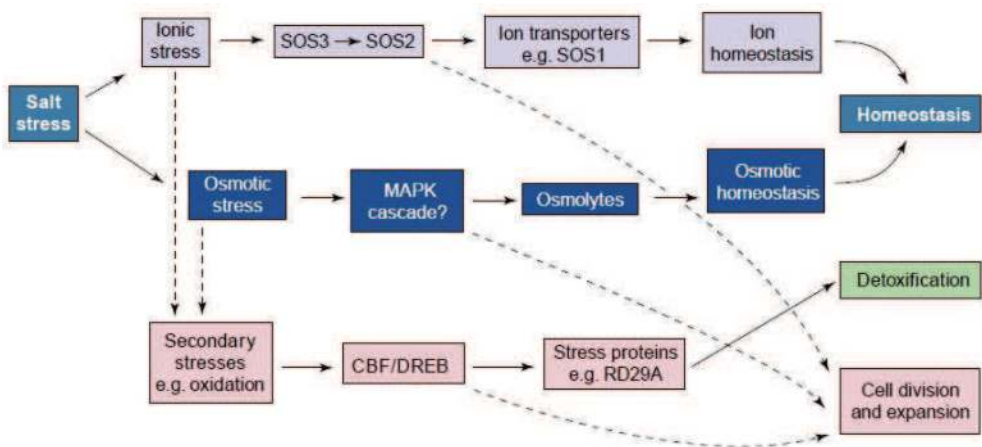


Fig. 4. Three avenues of salt tolerance in plants (after Zhu, 2001).

Functionality class	Possible role in stress	References
Signalling molecules	Stress signal transduction and gene expression	Cardinale et al. 2002; Pardo et al. 1998; Saijo et al. 2000; Ulm et al. 2002
Transcriptional and post-transcriptional machinery	Transcriptional regulation of stress gene expression, transcript stability, turnover, processing	Cooper et al. 2003; Lee et al. 2001; Park et al. 2001; Sanan-Mishra et al. 2005
Translational machinery	Stress-regulated protein translation, selective translation, transport, localization	Wood et al. 2000; Wood and Oliver 1999
Protein folding	Maintenance of protein structures, protein folding, preventing protein denaturation, Protein sorting, targeting	Sun et al. 2001
Protein turnover	Regulation of protein metabolism, targeted protein degradation in response to stress	Khedr et al. 2003; Moon et al. 2004
Osmoprotectants	Osmotic adjustment, protection of cellular structures and macromolecules	Nomura et al. 1998; Tarczynski et al. 1993
Transport protein	Ion homeostasis during stress, compartmentalization of solutes and amino acids	Apse et al. 1999; Gisbert et al. 2000; Shi et al. 2000; Zhang and Blumwald 2001; Zhang et al. 2001a
ROS scavengers, cell death, senescence and ageing	Detoxification of free oxygen radicals, cell death, hypersensitive response	Reddy and Sopory 1999; Roxas et al. 1997
Metal-binding proteins	Affecting cellular metabolism, metal ion homeostasis, acting as cofactors for critical reactions, signaling, metal toxicity, secondary stress responses, oxidative stress	Kawasaki et al. 2001; Sahi et al. 2003
Photosynthesis	Regulation of photosynthesis	Kawasaki et al. 2001; Sahi et al. 2003
Defense-related proteins	Protection against biotic stress including viral, bacterial and fungal infestation	Cheong et al. 2002; Dombrowski 2003; Reymond et al. 2000
Hormone-related proteins	Hormonal homeostasis and gene expression	Kalifa et al. 2004
General metabolism	Overall cellular function, housekeeping metabolic pathways carbohydrate, fatty acid and protein synthesis and modifications membrane fluidity, nitrogen metabolism, carbon and nitrogen fixation	Hoshida et al. 2000; Jeong et al. 2002

Table 1. Major categories of genes/proteins related to salt-stress responses/tolerances in plants (After Sahi *et al.* 2006).

The mitogen-activated protein kinases (MAP kinases), a specific class of serine/threonine protein kinases, play a central role in the transduction of various extra- and intracellular signals for cell division and stress responses in plants. Most of the abiotic stresses; salinity, cold, drought and oxidative stress can induce defence responses in plants through MAP kinase pathways such as osmoregulation, cell growth and differentiation (Mishra *et al.*, 2006; Pitzschke *et al.*, 2009). AtMKK3, AtMPK1, and AtMPK2 genes induced by ABA signalling amplified through MAP kinase-1 or MAP kinase-2 might increase salt tolerance in Arabidopsis (Hwa & Yang, 2008). Similarly, expression of active MKK9 protein enhanced salt tolerance and its loss increased sensitivity of transgenic Arabidopsis seedlings, emphasizing the significant role of MKK9 in salt stress response (Xu *et al.*, 2008b). Reactive oxygen species (ROS) scavengers such as peroxidases and glutathione are known to play a significant role in plant salt tolerance by reducing oxidative damage. For instance, in wild *B. napus*, glutathione synthesis was induced during salt stress, suggesting a possible protective mechanism against salt-induced oxidative damage (Ruiz & Blumwald, 2002).

In another study, genetic manipulation of carotenoid biosynthesis through over-expression of phytoene synthase gene SePSY in transgenic Arabidopsis increased the photosynthesis rate from 92% to 132% under 100 mM NaCl stress. The transgenic plants also displayed higher activities of superoxide dismutase (SOD) and peroxidase (POD) and lower concentrations of H<sub>2</sub>O<sub>2</sub> and malondialdehyde (MDA) than the wild plants (Han *et al.* 2008). Therefore, it is important to understand the genetics of detoxification of free oxygen radicals in order to enhance crop salt tolerance.

## 2.6 Methods for improving crop salinity tolerance

Several methods such as germplasm selection, marker assisted selection, transcriptional profiling, metabolomics, proteomics and transgenics have been successfully used for crop salinity improvement. This chapter will only discuss gene expression analysis for salinity tolerance with a main focus on microarrays.

### 2.6.1 Gene expression profiling for crop improvement

Messenger RNAs that are differentially transcribed between tolerant and sensitive genotypes under a particular stress may be targets for selection for future crop improvement. However, the expression of genes involved in stress responses is highly affected by the environment in which they are located, and thus care must be taken to accurately represent the stress environment conditions when seeking differentially transcribed gene responses. The responses may also differ among plant growth stages and among genotypes (Ashraf & McNeilly, 2004; Munns, 2005; Munns & Tester, 2008).

In order to determine key genes that are differentially transcribed for metabolic regulation under stress environments and among genotypes, several techniques have been developed. These rapidly provide gene-specific or genome wide expression patterns with high accuracy through biological and technical replication (Kuhn, 2001). Moreover, the information generated can be integrated within functional genomic processes to aid in understanding relationships between gene expressions and observed phenotypes.

### 2.6.2 Types of gene expression techniques

The last decade has produced several dynamic transcriptional technologies for measuring and interpreting single and multiple gene expressions. These have facilitated the analysis of mRNA



from selected cells/tissues to generate multi-dimensional measurements of differentially expressed genes. Techniques that assess gene expression are grouped into two categories; open and closed systems, based on their architecture. The open system techniques such as AFLP (Amplified Fragment Length Polymorphism), SAGE (Serial Analysis of Gene Expression), MPSS (Massively Parallel Signature Sequencing), and Real-time RT-PCR can permit the discovery of novel genes; however they might not cover the whole genome (Cheng *et al.*, 2008; Drea *et al.*, 2009; Nakano *et al.*, 2006; Sreenivasulu *et al.*, 2010). On the other hand, closed system techniques such as microarrays rely on already annotated information; therefore they can be used to study several thousand genes from a single experiment (Lee *et al.*, 2005; Seki *et al.*, 2002). Due to the flexibility of microarrays to permit the study of multiple stress situations such as salinity, drought and cold in a single experiment, this technique has become a method of choice for many for assessing differential genes expression studies in molecular biology (Dai *et al.*, 2007; Nakashima *et al.*, 2009; Seki *et al.*, 2002).

### 2.6.3 Microarray analysis for salt tolerance

Microarrays utilize the preferential binding of complementary single-stranded nucleic acid sequences. Instead of working on individual genes, the aim of a microarray experiments is to examine the profiles of expression of thousands of genes in a single experiment. Microarrays have been extensively used to study global gene expression profiling of plant responses to abiotic and biotic stresses. Studies on gene expression for abiotic stress include; salinity, drought and cold tolerance in *Arabidopsis* (Do-Young *et al.*, 2010; Lee *et al.*, 2005; Seki *et al.*, 2002; Seki *et al.*, 2010; Zhenxian *et al.*, 2010), rice (Huang *et al.*, 2008; Walia *et al.*, 2009), wheat (Huang *et al.*, 2008; Kawaura *et al.*, 2008), *B. napus* (Dalal *et al.*, 2009).

Several types of microarray platforms are available for gene expression studies: those that are spotted with known sequences comprised of cDNA or oligonucleotides, and those manufactured by Agilent and Affymetrix using GeneChip® technologies, which involve synthesis of oligonucleotides directly onto the microarray support. The cDNA microarray is a fabrication of spotted PCR products resulting from direct amplification of genomic DNA by using ESTs or gene specific primers (Alba *et al.*, 2004; Scott *et al.*, 2009). A number of cDNA microarrays have been developed for a variety of plant species such as *Arabidopsis*, rice, maize, petunia and lima bean (Vij & Tyagi, 2007). These have been used to study gene regulation at different developmental stages and in response to both abiotic and biotic stresses. Seki *et al.*, (2002) developed a full length cDNA microarray in *Arabidopsis* to identify genes transcribed in response to cold, drought and salinity, to examine differences in cross-talk between signalling cascades. Currently there are no specific EST-enriched or cDNA gene libraries from *B. juncea* in response to the abiotic stresses of high salinity, alkalinity and/or boron. However, limited information is reported for genes involved in stress tolerance in the Brassicaceae. For example, the cDNAs of the BjdHN2 and BjdHN3 genes from *B. juncea*, a novel subclass of dehydrin genes conferred salt and freezing tolerance in transgenic yeast (Xu *et al.*, 2008a). Similarly, Wang *et al.* (2003) cloned a new Na<sup>+</sup>/H<sup>+</sup> vacuolar antiporter gene from *B. napus* using a full-length cDNA. The designated vacuolar antiporter gene BnNHX1 was found to be salt-inducible and its transcript level was abundant after 24 hours treatment with 200 mM sodium chloride shock treatment.

## 3. Alkalinity

Alkaline soils are usually categorized by low availability of plant nutrients, high concentrations of HCO<sub>3</sub><sup>-</sup>, CO<sub>3</sub><sup>2-</sup> and high pH (Marschner, 1995; Misra & Tyler, 1999). By

definition alkalinity is the concentration of soluble alkalis with the ability to neutralize acids (Bailey 1996). Bicarbonate ( $\text{HCO}_3^-$ ) and carbonate ( $\text{CO}_3^{2-}$ ) are the principal contributors to alkalinity, whereas hydroxide, borate, ammonia, organic bases, phosphates, and silicates are considered minor contributors (Petersen, 1996). Although alkaline soils have high pH (more  $\text{OH}^-$  than  $\text{H}^+$ ), the  $\text{OH}^-$  ions contribute to alkalinity only at  $> \text{pH } 11$ . Below this pH, alkalinity is mainly caused by  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  ions. Hence, the predominant form of carbonates is determined by the soil pH (Whipker *et al.*, 1996). The carbonate system consists mostly of  $\text{HCO}_3^-$  at pH 8.34. As pH increases due to the availability of atmospheric  $\text{CO}_2$  in the system, the proportion of  $\text{CO}_3^{2-}$  increases and  $\text{HCO}_3^-$  declines (Figure 5; Lindsay, 1979). Hence crop growth is mainly inhibited by  $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$  ions rather than  $\text{OH}^-$  ions in alkaline soils. This has been demonstrated by growing maize plants in solution at pH 8.0 with the buffer HEPES, without  $\text{HCO}_3^-$ . The high pH due to HEPES buffer did not cause any reduction of root and shoot elongation (Lee & Woolhouse, 1969; Romera *et al.*, 1992).

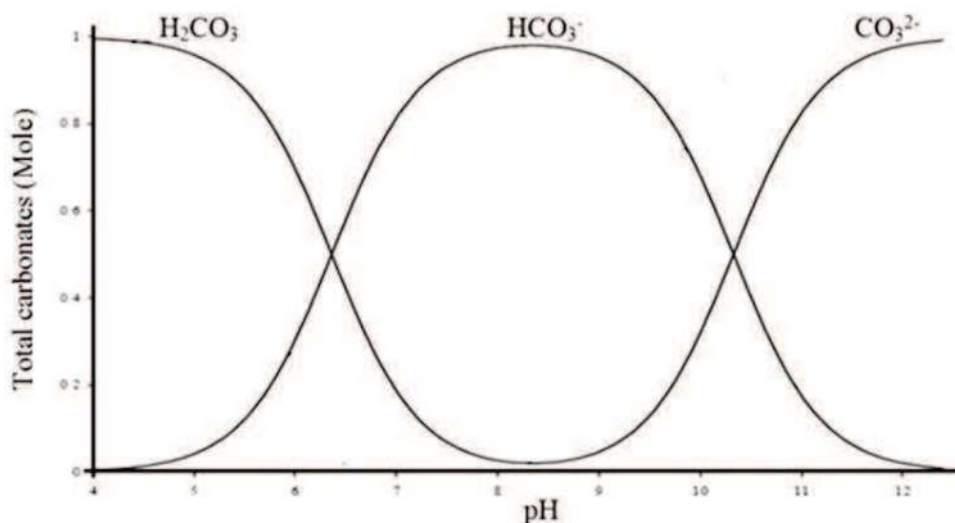


Fig. 5. Three carbonate species at different pH (after Lindsay, 1979)

### 3.1 Nutrient availability in alkaline soils

Several essential micro nutrients such as Fe, Zn and Mn become less available to plants under alkaline stress (Guardia & Alcántara, 2002; Valdez-Aguilar & Reed, 2008; 2010). Nitrogen and Phosphorus deficiencies are also caused by alkalinity. Bicarbonate can significantly decrease Fe uptake, accumulation and/or raise internal Fe precipitation (Fernández-Falcón *et al.*, 1986; Bertoni *et al.*, 1992; Alhendawi *et al.*, 1997; Norvell & Adams, 2006). The alkalization of root tissues due to  $\text{HCO}_3^-$  can either inhibit Fe acquisition or cause Fe to precipitate in the root apoplasm (Fernández Falcón *et al.*, 1986; Bertoni *et al.*, 1992 and Römheld, 2000). Alhendawi *et al.* (1997) found that Fe concentrations in roots of barley, maize and sorghum were significantly reduced when grown in solutions containing 5 to 20 mM  $\text{HCO}_3^-$ .

Iron (Fe) chlorosis is a major problem for crops grown in calcareous soils. Soluble bicarbonate has been documented as a contributor to iron (Fe) deficiency and lime-induced chlorosis of crops growing on calcareous soils (Wadleigh & Brown, 1952; Brown, 1978;

Coulombe *et al.*, 1984; Marschner, 1995). In bicarbonate buffer, the pH is in the alkaline range and reduces the uptake or utilization of Fe, leading to deficiency that results in leaf chlorosis (Chaney *et al.*, 1992; Parker & Norvell, 1999; Brand *et al.*, 2002; Lucena, 2000 Norvell & Adams, 2006; Valdez-Aguilar & Reed, 2008; 2010).

Besides Fe, other nutrients that become deficient at high pH include calcium (Ca), copper (Cu), phosphorus (P), and zinc (Zn) (Figure 6; Al-Karaki & Al-Omouh, 2002; Chaves *et al.*, 2006; Naidu & Rengasamy, 1993; Valdez-Aguilar & Reed, 2010). Plant response to alkalinity for nutrient uptake varies from crop to crop. For example, Solaiman *et al.* (2007) found that canola genotypes maintained higher uptake of P and accumulated greater biomass on alkaline soils compared to wheat genotypes. The better growth and P content of the canola genotypes compared to the wheat genotypes was due to the greater root length, leading to exploitation of greater soil volume. However on alkaline soils, P may be rapidly fixed into non plant-labile pools, by precipitation of Ca-P compounds not accessible to plant roots (Bertrand *et al.*, 2006). In a recent study, Valdez-Aguilar & Reed (2008) found that N, K, Ca, Mg and Fe concentrations were higher in roots than shoots of  $\text{HCO}_3^-$  treated tomato plants.

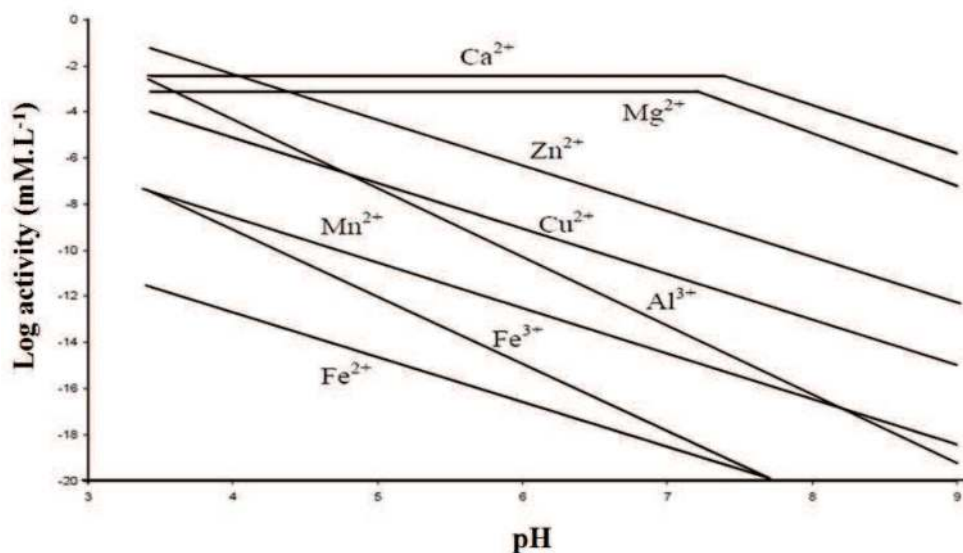


Fig. 6. Effect of increasing pH on availability of various nutrients (after Lindsay 1979).

### 3.2 Effect of alkalinity on plant growth

Plant growth is significantly reduced by alkaline stress mainly contributed by decreased shoot growth with smaller leaves and reduced leaf area as well as reduced root growth and elongation (Pearce *et al.*, 1999). Plants show minor to severe stunting of growth depending on  $\text{HCO}_3^-$  concentration in the soil solution. Growth of many commercial crops such as bean (Valdez-Aguilar & Reed, 2008; 2010), cucumber (Rouphael *et al.*, 2010), wheat (Yang *et al.*, 2008c), sorghum, maize barley (Alhendawi *et al.*, 1997; Yang *et al.*, 2009), soybean (Rogovska *et al.*, 2009), sunflower (Alcántara *et al.*, 1988; Shi & Sheng, 2005), tomato (Bailey & Hammer, 1986; Bialczyk & Lechowsk, 1995; Bialczyk *et al.*, 2004; Navarro *et al.*, 2000), pea (Zribi & Gharsalli, 2002), and rice (Hajiboland *et al.*, 2005; Yang *et al.*, 1994) are reported to be

considerably affected by  $\text{HCO}_3^-$ . Toxic concentrations of bicarbonate can diminish leaf area, leaf length and leaf width, consequently shoot biomass is decreased. This may be due to decreased photosynthetic rate and stomatal conductance in bicarbonate-induced leaf chlorosis (Bie *et al.*, 2004). The reduction in photosynthetic rate is due to impaired chlorophyll synthesis as a result of low translocation of Fe (Bavaresco *et al.*, 1999).

Increasing concentrations of bicarbonate inhibit root growth, which varies with crop species and bicarbonate concentration. Higher  $\text{HCO}_3^-$  concentrations can inhibit root respiration and may result in reduced root growth (Bingham & Stevenson, 1993; Alhendawi *et al.*, 1997). This inhibitory effect may also be related to high accumulation and compartmentation of organic acids such as malate and citrate in root cells (Lee & Woolhouse, 1969; Yang *et al.*, 1994). A bicarbonate-induced build-up of surplus organic acids, particularly malate, in the elongation zone appeared to be related to inhibition of root elongation by bicarbonate in calcifuge plant species (Lee & Woolhouse, 1969). Abscisic acid (ABA), an important stress-induced hormone, is produced in the roots and leaves, transferred from the roots to the leaves in the xylem and from the leaves back to the roots in the phloem (Wolf *et al.*, 1990). Excessive ABA inhibits shoot growth, but increases root growth especially under stress conditions, thus sustaining root growth in drying soils (Saab *et al.*, 1990). In alkaline soils, ABA may leak from the roots to the soil despite being released into the xylem, thereby causing root inhibition as water becomes less available (Daeter *et al.*, 1993; Slovik *et al.*, 1995). Crop species such as corn that can retain root ABA in the face of its tendency to leach into the more alkaline compartment are better able to tolerate these harmful stresses (Degenhardt, 2000).

Crops such as maize, sorghum, and barley have also shown depressed root growth at elevated levels of bicarbonate stress (Alhendawi *et al.*, 1997). Whereas some other crops such as sugar beet, sunflower, pea, and rice are considered better able to tolerate bicarbonate stress (Alcántara *et al.*, 1988; Campbell & Nishio, 2000; Yang *et al.*, 1994; Zribi & Gharsalli, 2002). For instance, root thickness, and lateral root production of sugar beet were increased after three days of Fe deficiency and  $\text{HCO}_3^-$  treatments (Campbell & Nishio, 2000).

### 3.3 Molecular responses to alkalinity stress

Most of the studies to date have focussed on the physiological impacts of alkalinity stress. Recently, Yang *et al.*, (2008) constitutively expressed the high affinity bicarbonate transporter gene "IctB" from *Cyanobacterium* in rice. Under low  $\text{CO}_2$  or alkaline water conditions, cyanobacteria use bicarbonate transporters to pump in bicarbonate as a major carbon source to survive under unfavourable growth conditions. All transgenic rice lines expressing the transporter exhibited enhanced photosynthetic capacity, growth and grain yield (Yang *et al.*, 2008).

As previously mentioned, Fe deficiency is one of the predicted outcomes of alkalinity, therefore an understanding of how plants acquire this ion under stress is needed. Plants have developed two discrete iron uptake strategies by the roots (Marschner *et al.*, 1987). For most plants, including dicots and non-graminaceous monocots, ferrous ion Fe (II) transport from soil into root cells takes place via a transporter after reduction from ferric ion Fe (III) on the plasma membrane (Eide *et al.*, 1996; Robinson *et al.*, 1999; strategy I). However some graminaceous plants synthesise and release iron-chelating phytosiderophores, hence have a specific iron uptake system, the Fe (III)-phytosiderophore complex (Romheld & Marschner, 1986; strategy II).

Barley is the most tolerant species to iron deficiency among the graminaceous plants and Murata *et al.* (2006) identified an iron-phytosiderophore transporter "HvYS1" gene which has

72.7% similarity with ZmYS1, the first protein identified as an iron(III)-phytosiderophore transporter in maize. The expression of this gene is linked to iron deficient conditions and is expressed in the epidermal root cells. The localization and substrate specificity of HvYS1 is different from those of ZmYS1, indicating that HvYS1 is a specific transporter involved in primary iron acquisition from soil in barley roots (Namba & Murata, 2010).

#### 4. Conclusions

Further research is required to determine the key genes and molecular pathways that underpin the best tolerance responses of our elite crop genotypes to the common abiotic soil constraints including salinity and alkalinity. Once uncovered and assessed to be stably expressed under varying background environments and genomes, these genetic mechanisms may become central to future tolerance breeding programs through advanced selection methods. Also, full characterisation of shared molecular mechanisms to multiple stresses may uncover strategic selection tools for breeding cultivars that are tolerant to stresses that occur simultaneously.

For example, when alkalinity is combined with salinity in the same soil environment, the negative impact on plant growth is significantly increased (Li *et al.*, 2010; Shi & Sheng, 2005). Saline soils containing  $\text{CO}_3^{2-}$  and/or  $\text{HCO}_3^-$  can cause injury to plants through high salts as well as through carbonates and bicarbonate (Shi & Sheng, 2005). The combined stress (alkaline salinity) leads to Na toxicity due to high concentrations of salt and deficiencies of Fe and Zn. Therefore, future genetic studies and screening for selective breeding should incorporate the interactive nature and impacts of multiple concurrent stresses.

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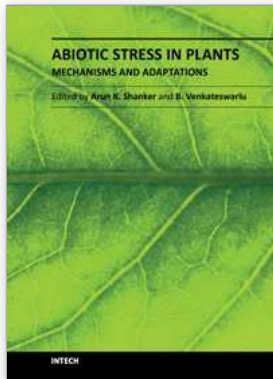
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## **Abiotic Stress in Plants - Mechanisms and Adaptations**

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World population is growing at an alarming rate and is anticipated to reach about six billion by the end of year 2050. On the other hand, agricultural productivity is not increasing at a required rate to keep up with the food demand. The reasons for this are water shortages, depleting soil fertility and mainly various abiotic stresses. The fast pace at which developments and novel findings that are recently taking place in the cutting edge areas of molecular biology and basic genetics, have reinforced and augmented the efficiency of science outputs in dealing with plant abiotic stresses. In depth understanding of the stresses and their effects on plants is of paramount importance to evolve effective strategies to counter them. This book is broadly divided into sections on the stresses, their mechanisms and tolerance, genetics and adaptation, and focuses on the mechanic aspects in addition to touching some adaptation features. The chief objective of the book hence is to deliver state of the art information for comprehending the nature of abiotic stress in plants. We attempted here to present a judicious mixture of outlooks in order to interest workers in all areas of plant sciences.

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