

Review

Plants' Challenges in a Salinized World: The Case of *Capsicum*

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Plants are sessile and are exposed to many environmental factors and are therefore under stress. In soils used for agriculture, salinity is a major abiotic factor that affects the growth, yield and quality of crops worldwide, mainly by the accumulation of Na^+ in plant tissues and resulting loss of water. Crop peppers of the genus *Capsicum* have a high sensitivity to stress induced by high concentrations of salts. Throughout their entire ontogeny, pepper plants are susceptible to salt stress, but damage to the seedling is more serious and results in growth retardation and even death of the seedling. Because of the economic importance of peppers, improved tolerance to salinity stress should be the primary target of research for different pepper species. For this reason, we examined the current state of research with regard to the effect of salt stress on plants of the genus *Capsicum*.

Key words: *Capsicum*, salinity, stress, sodium, pepper, transport.

INTRODUCTION

Salinization is the process by which soluble salts accumulate on the soil, either naturally or by anthropogenic practices. This phenomenon is a problem for agriculture worldwide and occurs in all climatic regions from the tropics to the Polar Regions (Hawighorst, 2007). Salinity is one of the most prominent abiotic factors affecting crop yields in arid and semiarid regions (Subramanyam et al., 2010). This type of stress generates some potential effects on plant tissues, including osmotic stress, ionic stress and oxidative stress (Munns and Tester, 2008; Alemán-Guillén, 2009). The osmotic stress is the result of relatively high concentrations of salts in the soil solution, which inhibits water intake by roots and thus leads to slower growth. Ionic stress is caused by the excessive accumulation of salts in the tissues of the plant, which gives rise to toxicity caused by high concentrations of ions and nutritional imbalances (Munns, 2005). Previous studies have reported that salt stress induces reactive oxygen species

(ROS) damage, mainly by oxidative damage to lipids, proteins and nucleic acids involved in metabolism and cellular homeostasis (Farhoudi et al., 2011).

Different plant species differ widely in their tolerance to salinity. From an ecological perspective, plants can be classified as halophytes or glycophytes according to their ability to grow in a saline environment. Furthermore, within the agronomic context, plant tolerance to salt refers to the ability of a plant to resist the effects of high concentrations of salts in the root zone or within the plant, with no or minimal reductions in growth or yield (Morales-García, 2008). The responses of plants to salinity include the production of osmolytes, osmotic adjustment, succulence, compartmentalization of ion-selective uptake and transport of ions, excretion of salts, specific gene expression, protein synthesis and stress-related enzyme activity (Flowers and Colmer, 2008; Subramanyam et al., 2010; Zribi et al., 2012).

Na^+ enters the root passively, from the external environment into the cytoplasm of the epidermal cells and root cortex. Passive entry of Na^+ to the roots is dependent on the concentration gradient and the potential difference across the plasma membrane (Alemán-Guillén, 2009). The transport of Na^+ consists of three components: the

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influx or inflow of Na^+ through nonselective cation channels (NSCCs), channels permeable to potassium (AKT1) and transporters with high and low affinity such as the KUP /HAK/KT family, LCT1 and HKTS; the compartmentalization of Na^+ to the vacuolar exchanger (Na^+/H^+) NHX1 and finally, in the case of the efflux or efflux of Na^+ , a signal transduction way known as salt overly sensitive (SOS), which will be explained later (Zhang et al., 2010).

Furthermore, to prevent the accumulation of Na^+ in the soil, the main solution has been the replacement of sensitive crops by others that are more salt-tolerant in areas affected by salinity. Soil washing (leaching) techniques has reduced this problem in some countries; however, the costs of such technology can be prohibitive. In addition, biotechnological tools, including quantitative trait locus (QTL) analysis and genetic engineering, have been used to obtain plants that grow in salt stress conditions (Leidi and Brown, 2002). Additionally, the physical and chemical fertility of soil may be increased while maintaining productivity through an adequate and balanced supply of mineral nutrients. Based on this last point, growing evidence suggests that the nutritional status of plants plays a critical role in increasing plant tolerance to environmental stress (Marschner, 1995).

Peppers are among the most important vegetables, and they are valued for their savor, flavor and pungency. In addition to their importance as a vegetable, peppers have received attention recently for their potential as a nutraceutical. Although they are typically used for seasoning, peppers have been recognized as an important source of vitamin C. Compared with other horticultural crops, pepper plants have a high sensitivity to high salt concentrations (Subramanyam et al., 2010). Therefore, this paper reports a compilation of studies of the genus *Capsicum* and salinity, with regard to the effect that this abiotic stress has on vegetative growth and plant production; some general aspects of salinity in plants are also addressed.

STRESS IN PLANTS

Plants are sessile organisms that are constantly subjected to a wide range of biotic and abiotic factors (Walley et al., 2007). Factors such as water and nutrient deficiency, adverse weather conditions (extreme temperatures), disease caused by pathogens and damage caused by insects affect plant growth and cause losses in crop production worldwide (Jackson, 1986; Walley et al., 2007). When a plant is subjected to biotic or abiotic factors, it is referred to as being stressed or in stress. The term stress can be defined as an impairment that adversely affects growth, so it is accepted that maximum performance can only be obtained if the stress of the plant is kept to a minimum (Jackson, 1986). As a result of stress, plants may undergo physiological and

anatomical changes that may be caused by single or multiple stressors.

Tolerance or susceptibility to abiotic stress is complex because stress can occur at various stages during plant development, and often more than one type of stress affects the plant at the same time (Jackson, 1986; Schulze et al., 2002; Chinnusamy et al., 2003). When environmental conditions change or external changes occur, plants can detect these changes. After this initial perception, a signal is transmitted via a signal transduction cascade to amplify the signal, which triggers an adaptive response that is critical to allow the plant to tolerate stress (Chinnusamy et al., 2003; Vij and Tyagi, 2007). Therefore, some studies on physiological, biochemical and molecular stress tolerance have been conducted to determine the intrinsic mechanisms developed during evolution to combat abiotic stress in plants (Vij and Tyagi, 2007.)

Drought, extreme temperatures and salinity are the main environmental factors that significantly affect crop productivity, and we will focus mainly on general aspects of salinity stress in plants.

SALINITY STRESS

In soils for agriculture, salinity is a major abiotic factor that affects the growth, yield and quality of crops worldwide. Damage caused by salinity limits the production of plant foods and may even cause the death of plants (Hasegawa et al., 2000; Zhu, 2001; Cramer, 2002; Flowers, 2004; Foolad, 2004; Aktas et al., 2005). This problem is especially important in arid and semiarid regions. Worldwide, over 800 million ha are affected by salinity, which is more than 6% of the total land area (Munns and Tester, 2008). It is estimated that of the 230 million ha of arable land, 45 million (20%) are affected by salinity (FAO, 2008). Furthermore, with increasing salinity in recent years, it is expected that by 2050, over 50% of the land available for agriculture will be lost because of salinity (Flowers, 2004). In Mexico, 6.8 million ha of soils are salt-affected (Ortiz, 1992); however, it is possible that in recent years, this area has increased.

Salinity and saline soils

Salinity is generally defined as the presence of excessive amounts of ions of soluble salts that prevents or affects the functions necessary for the normal growth of plants (Jéréme-Diedhiou, 2006). The major ions contributing to salinity include the chloride (Cl^-), calcium (Ca^{+2}), magnesium (Mg^{+2}), sulfate (SO_4^{-2}), carbonate (CO_3^{-2} and HCO_3^-) and sodium (Na^+) ions, and the latter is the most abundant ion in soil (Alemán-Guillén, 2009). Salinity is often expressed as concentration (mM) or electrical conductivity (EC). Therefore, saline soils have an EC

value greater than 4 dS^{-1} , which is equivalent to 40 mM NaCl, an exchangeable sodium percentage (ESP) less than 15% and a pH below 8.5, which generates an osmotic pressure of approximately 0.2 MPa (Waisel, 1972; Jérémie-Diedhiou, 2006, Munns and Tester, 2008).

Salinization is a problem for agriculture worldwide and occurs in all climatic regions from the tropics to the Polar Regions, although it is particularly important in arid and semiarid regions. Salinity occurs on all populated continents (Hawighorst, 2007). Salinization is the process by which water-soluble salts accumulate in the soil and may occur naturally or because of conditions resulting from farming practices.

The factors that increase soil salinity include low rainfall, evaporation of water from the soil surface, continuous erosion of rock minerals, irrigation with salt water, sea water input to freshwater bodies and poor farming practices. The accumulation of salts because of irrigation has an effect on agriculture (Foolad, 2004; Göl, 2006). Water sources in the ground contain 30 gL^{-1} of NaCl (equivalent to approximately 500 mM), which is why the earth is considered to be a planet of salt (Flowers, 2004; Foolad, 2004). In addition, high rates of evapotranspiration of water rise increase the accumulation of salts in the soil, and this problem is compounded if irrigation is performed with water that has a high concentration of solutes and if the soil drainage is deficient (Leidi and Brown, 2002; Alemán-Guillén, 2009). The soil may also become saline because of salts carried by the wind and clouds from the oceans (Zafar, 2008), as well as fertilizers used in agriculture (Leidi and Brown, 2002). Other factors that interact with salinity include humidity, temperature, light and soil fertility (Foolad, 2004; Göl, 2006).

PLANTS AND SALINITY

Different plant species differ widely in their tolerance to salinity. This tolerance can be defined as the ability of plants to survive and maintain their growth under saline conditions; however, this definition may change depending on the ecological or agronomic context (Göl, 2006).

From an ecological perspective, plants can be classified as halophytes or glycophytes according to their ability to grow in a saline environment. Halophytes are considered to be the native flora of saline soils, survive and complete their life cycle at high salt concentrations; they have been called salt-loving or salt-water plants (Ruan et al., 2010). For optimal growth, halophyte plants require high salt concentrations, and they can grow in soils with NaCl present at concentrations of over 400 mM (Flowers et al., 1977; Hawighorst, 2007). Glycophyte plants have a relatively limited capacity to accommodate the presence of salts and their development is greatly reduced at relatively low levels of salts (Alemán-Guillén,

2009). Glycophytes are thus defined as plants that are sensitive to salinity (Flowers, 2004; Göl, 2006) or tolerant to low salt concentrations (Maas and Nieman, 1978; Zafar, 2008).

Halophytes can grow in extremely saline environments because of their anatomical, morphological, physiological and biochemical adaptations (Flowers and Yeo, 1986; Zafar, 2008), which include compartmentalization, production of osmolytes, responses in germination, osmotic adjustment, enzyme responses, genetic control and selective transport of ions, among others (Baydara, 2006). The ability of halophytes to grow under salinity stress has permitting the identifications of the mechanisms underlying tolerance to this stress (Flowers, 2004, Ruan et al., 2010). Glycophytes do not appear to possess mechanisms of adaptation to the adverse effects of salinity because they evolved in soils with a low content of NaCl (Greenway and Munns, 1980; Attumi, 2007).

Furthermore, within the agronomic context, plant tolerance to salts is referred to as the ability of the plant to resist the effects of high concentrations of salts in the root zone or within the plant, with minimal or no reductions in growth or yield (Morales-Garcia, 2008). Based on this context, plants have been classified as sensitive, moderately sensitive, moderately tolerant and salt tolerant. This classification is based on the relative performance of a crop as a continuous function of soil salinity (FAO, 2003). In addition, the sensitivity of crops to soil salinity generally changes from one stage vegetative development to another. Depending on the species, plants may be more sensitive during the early stages of development, that is, during the reproductive stages. In general, germination is the stage that is most tolerant to salinity (Alemán-Guillén, 2009).

Effect of salinity stress in plants

The main requirements of plants to complete their life cycle are mineral nutrients (elements) and the energy of sunlight. Certain elements are called essential nutrients (minerals) because they are necessary for the growth and development of plants. These elements are important in many biological processes, and each has its own functions in the cell; however, although these nutrients are essential for plant survival, their excess or deficiency in the soil under stress conditions adversely affects most plants (Xiong and Zhu, 2002; Baydara, 2006; Göl, 2006).

For many plants, sodium (Na^+) is not an essential nutrient. However, some plants, such as Na^+ C4/CAM, require Na^+ for their photosynthetic pathway. In addition, the growth of some plants is increased at low concentrations of Na^+ in the soil (Flowers et al., 1977; Marschner, 1995). It is generally agreed that Na^+ can be toxic or beneficial to plants and Na^+ has even been considered as a functional nutrient (Subbarao et al., 2003). However, Na^+ has been studied more with regard

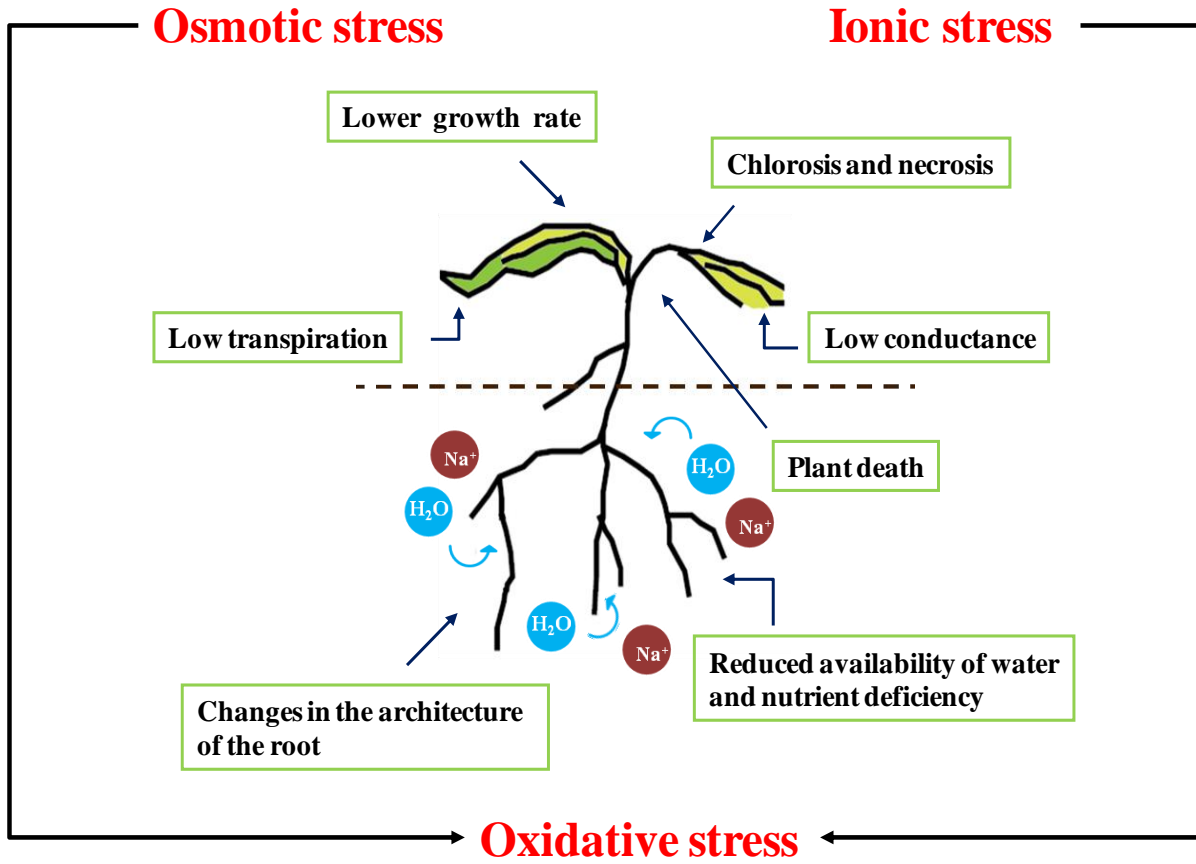


Figure 1. General effects of Na^+ toxicity in plants under saline conditions

to its negative effect when present at high concentrations (salt stress) than as a beneficial or essential element (Pilon-Smits et al., 2009).

Effects of Na^+ toxicity in plants

Salinity imposes two types of stress in plant tissues: osmotic stress and ionic stress. Osmotic stress is caused by the low water potential of solutions with high concentrations of salts, which makes it difficult to water the plant (Hawighorst, 2007). Ionic stress is caused by excessive accumulation of salt in the tissues of the plant, which gives rise to toxicity through high concentrations of ions and nutritional imbalances. In addition, these primary effects (osmotic stress and ionic stress) often produce a secondary effect: oxidative stress (Figure 1) (Selcuk, 2004; Munns, 2005; Munns et al., 2005; Hawighorst, 2007; Munns and Tester, 2008; Alemán-Guillén, 2009).

In saline conditions, the ability of plants to absorb water is reduced because the water potential of the medium decreases with increasing salt concentrations in the external solution; for this reason, the plants can not absorb water. To maintain the uptake of water, plants need to maintain an internal water potential that is below the potential of the external solution (Hawighorst, 2007).

As a result of osmotic stress, plants lose turgidity, which decreases the rate of cell expansion (Xiong and Zhu, 2002). This effect decreases the growth of leaves and roots, even when the concentrations of ions (Na^+ and Cl^-) in the cells are below toxic concentrations (Hawighorst, 2007). In addition, stomatal conductance, photosynthesis, nutrient uptake and the growth of mycorrhizal fungi may be inhibited by the osmotic effect (Xiong and Zhu, 2002; Munns, 1993, Tester and Davenport, 2003).

One of the most important effects of high concentrations of Na^+ is the displacement of K^+ from target sites within the cell because both ions show great chemical similarity. The first point of interaction between Na^+ and K^+ is the entry of these ions to the root symplast. However, high concentrations of Na^+ inhibit directly the transport systems for K^+ . Furthermore, Na^+ causes depolarization of the membrane electrical potential, which decreases the absorption of K^+ . Accordingly, in salt stress conditions, K^+ deficiency may occur as has been observed in corn, melon and pepper (Botella et al., 1997; Kaya and Higgs, 2003; Kaya et al., 2007; Alemán-Guillén, 2009).

When Na^+ accumulates in leaves at concentrations toxic to the cell, a specific ion effect may occur (Munns, 2005). If Na^+ is transported into the leaves, especially in mature leaves, an increase in the concentration of Na^+

and a decrease in the concentration of K^+ / Na^+ will occur because of the interactions between these ions. A low cytosolic concentration of K^+ / Na^+ inhibited enzyme activity in the cytoplasm because more than 50 enzymes are activated by K^+ (Hawighorst, 2007). Similarly, protein synthesis is altered by high concentrations of Na^+ in the cytoplasm (Blaha et al., 2000). In the cytoplasm, high concentrations of K^+ are required for tRNA binding to ribosomes; however, Na^+ can affect negatively this process (Hawighorst, 2007).

In addition to osmotic stress and ionic stress, salinity stress and other environmental factors can cause secondary stress, that is, oxidative stress (J  r  mie-Diedhiou, 2006). Oxidative stress is caused by the formation of hydrogen peroxide and reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radical (OH^\cdot) and singlet oxygen (1O_2). ROS are usually generated by normal activities of the cell as a byproduct of aerobic metabolism, photorespiration and β -oxidation of fatty acids through electron transport, redox cascades and metabolic processes. Plant damage caused by ROS inactivates enzymes, damages nucleic acid structures, delays the synthesis of proteins, and causes lipid peroxidation and membrane damage (Amooaghaie, 2011). ROS production is increased under the influence of unfavorable environmental cues (Bhattacharjee, 2012).

Previous studies have reported that salt stress induces the formation of ROS that impair metabolism and cell homeostasis (Farhoudi et al., 2011), and NaCl can quickly activate nicotinamide adenine dinucleotide phosphate (NADPH) oxidase for the production of ROS. ROS have a dual role in the homeostasis of K^+ , as they mediate the efflux of K^+ and programmed cell death (PCD); both of these processes are induced by salinity (Demidchik et al., 2010). ROS regulate Na^+/K^+ balance (inverse of the K^+/Na^+ ratio) by increasing cytoplasmic Ca^{2+} and through K^+ homeostasis (Mori and Schroeder, 2004; Demidchik et al., 2010, Ma et al., 2012). This role of ROS in the homeostasis of K^+ is of interest because cultivars tolerant to salinity have the ability to retain K^+ in their roots and prevent the outflow induced by ROS and salinity (Chen et al., 2005, 2007a, b).

Similarly, ROS have emerged as an important signal in the activation of PCD in plants (Shabala, 2009). PCD is an important physiological process that occurs in plants and animals also known as apoptosis (van Doorn, 2011). In plants, PCD is essential for growth and development and plays an important role in the response to stress (biotic and abiotic) (Joseph and Jini, 2010). Salinity is the main environmental factor that causes PCD in plant cells. Prolonged salt stress (24 h) leads to the degradation of organelles by autophagy (a special form of PCD) (Affenzeller et al., 2009, van Doorn, 2011).

It has also been suggested that the main reason that salinity induces PCD is the efflux of K^+ and the decrease of this ion in the cytoplasm (Shabala, 2009). As mentioned earlier, the efflux of K^+ can also be induced by

ROS. In contrast, changes in cytosolic K^+/Na^+ can be crucial to trigger PCD in cells (Joseph and Jini, 2010). In addition, Shabala (2009) mentioned that PCD in cells treated with salinity could be prevented by decreasing ROS levels through antioxidant mechanisms.

In plants, the complex antioxidant defense mechanisms include enzymes and nonenzymatic compounds of low molecular weight (Noctor and Foyer, 1998). The antioxidant enzymes include catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), and ascorbate peroxidase (APX), among others; these enzymes can remove ROS and maintain homeostasis between ROS production and degradation (Amooaghaie, 2011; Gill and Tuteja, 2010). In contrast, scavengers of ROS antioxidant compounds include ascorbic acid, glutathione and pigments such as carotenoids (Gill and Tuteja, 2010).

General effects caused by salinity

Salinity generally affects the growth rate and results in plants with smaller leaves. Salinity also changes the structure of the roots by reducing their length and mass to become thinner or thicker. Additionally, this type of stress produces changes in leaf color (chlorosis) and even necrosis, which leads to the deterioration of the leaves and thus to inhibition of photosynthesis. In general, the reduction in the growth of the plant under salt stress can lead to death of the plant (Figure 1) (Munns, 2005).

TRANSPORT OF Na^+ IN PLANTS

Na^+ enters the root passively, from the external environment into the cytoplasm of epidermal cells and root cortex. The passive entry of Na^+ to the root depends on the concentration gradient and potential difference across the plasma membrane (Cheeseman, 1982; Maathuis and Amtmann, 1999; Apse and Blumwald, 2007; Hawighorst, 2007). Additionally, the exclusion of Na^+ is an important mechanism of salinity tolerance in plants. Plants exclude between 94 to 98% of the Na^+ that enters the cell; however, the efflux of Na^+ is insufficient to counteract the negative effects on plant growth (Maathuis and Amtmann, 1999; Hawighorst, 2007).

Root Uptake of Na^+

Epidermal cells constitute the first barrier to the movement of ions in the root (Plett and Moller, 2010). This barrier allows most plants to exclude approximately 98% of salts in soil solution and allows only 2% of the salts to enter the xylem (Munns et al., 2005). Ions or solutes can travel from the epidermis to the xylem via symplastic effects, through introduction into cells of the root and movement

from cell to cell through plasmodesmata or by apoplastic processes, that is, without passing through the plasma membrane (White et al., 2002).

In many plant species, various molecular mechanisms are involved in Na^+ absorption in plants. It has been argued that nonselective cation channels (NSCC) family members or voltage independent channels (VICs), low-affinity cationic transporters (LCT1), transporters of K^+ high affinity (HKTs), KUP/HAK/KT (transporters of high-affinity K^+), AKT1 (Shaker type K^+ channel) and cation chloride cotransporters (CCC) may be candidates for the uptake of Na^+ in higher plants (Zhang et al., 2010). However, the role of each type of transport can vary among species and growing conditions (Apse and Blumwald, 2007). Figure 2 shows the candidate channels and conveyors involved in the transport of Na^+ in plants.

Efflux and Compartmentalization of Na^+

For plant cells, it is crucial to maintain a low cytosolic concentration of Na^+ under saline conditions. One way to establish a low cytosolic concentration of Na^+ is to incorporate it into the vacuoles of the cells. This process is catalyzed by the vacuolar exchanger Na^+/H^+ , the expression of which is increased by the addition of Na^+ (Blumwald et al., 2000). Vacuolar Na^+/H^+ exchangers are members of the family of NHX transporters located in the tonoplast membrane (Apse et al., 2003). It has been reported that the overexpression of the vacuolar exchanger Na^+/H^+ confers increased tolerance to salt stress in *Arabidopsis* and tomato by increasing the vacuolar compartmentalization of Na^+ (Apse et al., 1999; Zhang and Blumwald, 2001). In addition, the *Arabidopsis* protein NHX1 has the ability to transport both Na^+ and K^+ and thus may contribute to cell and tissue osmotic balance under all conditions of plant growth (Venema et al., 2002). Another way to establish a low concentration of Na^+ in the cytosol is through the exclusion of Na^+ from the cell. Na^+ efflux from cells is a process that is catalyzed by the plasma membrane Na^+/H^+ exchanger (Blumwald et al., 2000). This efflux occurs against the electrochemical gradient.

In *Arabidopsis*, the exclusion of Na^+ to maintain ionic homeostasis is mainly mediated by the SOS cascade of signals, which consists of three main components: SOS1, SOS2 and SOS3 (Yang et al., 2009). SOS1 encodes a Na^+/H^+ transmembrane interchanger that play a critical role in the extrusion and control of Na^+ transport over the long distance between shoots and roots (Shi et al., 2000; 2002a). SOS2 encodes a Ser/Thr kinase, and SOS3 encodes a Ca^{2+} binding protein (EF-hand) that acts as a calcium sensor for salt tolerance (Liu and Zhu, 1998; Yang et al., 2009). Under conditions of salinity, an SOS3, SOS2 interacts with and is activated by SOS3 to form the complex kinase SOS2/SOS3, which will increase in the cytosolic Ca^{2+} concentration is sensed by subsequently

phosphorylate and activate SOS1. Phosphorylation of SOS1 increases the activity of Na^+/H^+ (Maathuis and Amtmann, 1999) to allow the efflux of Na^+ with a probable stoichiometry $\text{Na}^+:\text{H}^+$ (1:1) and ultimately drives proton translocation by plasmatic ATPase (Malagoli et al., 2008). The SOS2/SOS3 complex also regulates NHX1 and HKTs.

STRATEGIES TO ADDRESS SALINITY STRESS

Because of the increasing world population and increasing demand for food, sustainable land use and efficient management of resources are the challenges faced by the agricultural industry to meet demand and improve food production, especially in stressful conditions. According to Epstein et al. (1980) and Pitman and Lauchli (2002), two types of methods can be applied to address the negative effects of salinity on agriculture. One of these methods is soil remediation through the use of engineering environment. The second method is the generation of salt-tolerant crops through genetic engineering.

Many techniques can be used to recover saline from soils. One of these methods is the leaching of salts from the soil, either naturally by rainfall or by washing (drainage) of the soil. We have used the addition of calcium sulfate (CaSO_4) and sulfuric acid (H_2SO_4) to displace Na^+ and replace it with Ca^{2+} to improve the structure and chemical composition of soil. To solve the problems caused by salinity, sensitive crops can be replaced with more tolerant crops, such as beet, barley and cotton (Zafar, 2008; Shannon et al., 1984).

Biotechnological tools, including QTL analysis and genetic engineering, have also been applied. Assisted selection based on QTL analysis has resulted in varieties or lines tolerant to salt stress conditions based on their capacity for absorption, ion selectivity and compartmentalization. Furthermore, the transformation of plants with genes isolated from other organisms permits the expression of characteristics related to a higher tolerance to salt stress, such as the activity of ion transporters, synthesis of compatible solutes (osmolytes) and expression of enzymes involved in clearing ROS (Leidi and Brown, 2002). These biotechnology tools have focused on preventing the accumulation of Na^+ and preventing its toxic effects.

Theoretically, three mechanisms can be used to prevent excessive accumulation of Na^+ in plants and its toxic effects. First, the entry of Na^+ into cells of the plant can be reduced when the transporters involved in the influx of Na^+ are identified. Second, the Na^+ that enters the cell can be transported and stored in vacuoles. Third, the Na^+ in the cytosol may be exported to the external environment (Shi et al., 2002b).

To increase physical and chemical fertility of the soil, an adequate and balanced supply of mineral nutrients is

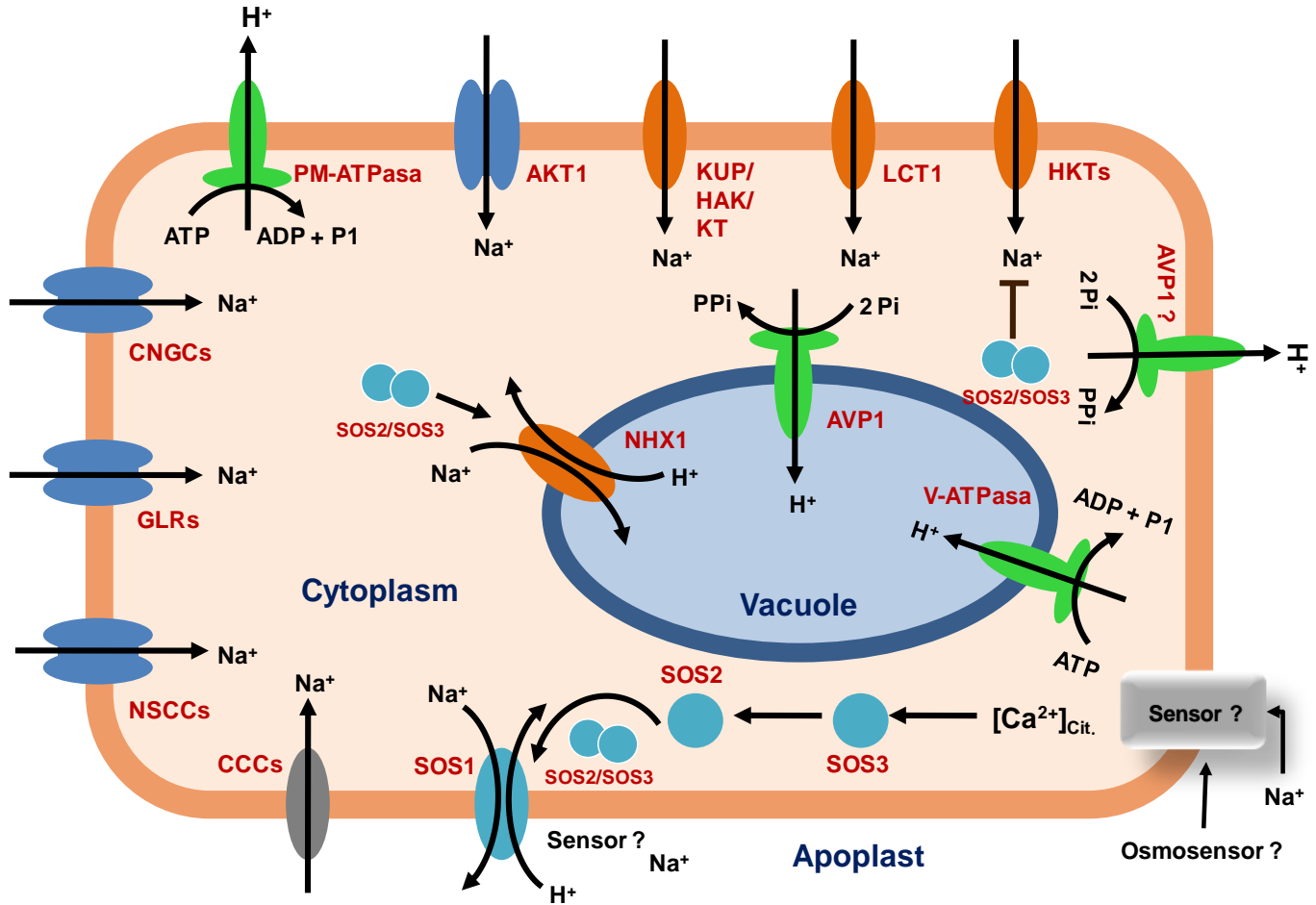


Figure 2. Main channels and transporters involved in Na⁺ transport in plants. NSCCs, which include glutamate like receptors (GLRs) and cyclic nucleotide gated channels (CNGCs,) are involved in the influx of Na⁺ into the cell. It has also been proposed that members of the AKT1 family (channels permeable to K⁺) may mediate the uptake of Na⁺ when external concentrations are increased. The KUP/HAK/KT family mediates the uptake of Na⁺ with high affinity; HKTs and LCT1 have high and low affinity, respectively, as the the CCCs are possible candidates for the uptake Na⁺ in high levels of salinity. The tranport of Na⁺ in plants involves a system of compartmentalization that is mediated by NHX1, which can be coupled to ATPases and pyrophosphatases. Na⁺ efflux systems can also involve signal transduction; this system is known as SOS and includes three components: SOS1, SOS2 and SOS3. The existence of sensor osmosensors for Na⁺ has also been proposed. Modified from Zhang et al. (2010)

important. There is growing evidence suggesting that the nutritional status of plants plays a critical role in increasing the resistance or tolerance of plants to environmental stresses (Marschner, 1995).

The absorption of K⁺ plays an important role in the growth and development of plants (Mengel and Kirkby, 1982; Ashley et al., 2006) because of the various functions performed by K⁺ in cells. Thus, salt-tolerant plants must maintain a high level of K⁺ in the cells (Göl, 2006).

Under salt stress, K⁺ deficiency occurs because the ionic radius and hydration energy of K⁺ and Na⁺ are similar, which prevents discrimination between these two ions (Zhang et al., 2010). Because of the competition between K⁺ and Na⁺, the selectivity and operation of transport systems for K⁺ and Na⁺ through various cellular membranes is essential for maintaining a proper

cytoplasmic K⁺/Na⁺ relationship. High concentrations can inhibit Na⁺ transport systems in favor of K⁺ and K⁺/Na⁺-selective systems, which may influence the salt tolerance of plants (Alemán-Guillen, 2009).

In salinity, control of homeostasis depends on sodium efflux from the cytoplasm as the K⁺ concentration is maintained. Channels and K⁺ transporters may control the transport of Na⁺ (Jérémie-Diedhiou, 2006). In wheat species that are tolerant to salinity, a minor efflux of K⁺ from the roots in the presence of high concentrations of NaCl is correlated with tolerance to salt stress because this lower efflux of K⁺ from the roots maintains the K⁺/Na⁺ relationship in the plant (Cuin et al., 2008).

A high cytosolic K⁺/Na⁺ ratio is an essential requirement for plant growth at high salt concentrations (Zhu, 2003). In this regard, Volkov et al. (2004) found that in saline conditions, *Thellungiella halophila*, which is a close

relative of *A. thaliana*, was halotolerant but maintained a high ratio K^+/Na^+ in mesophyll cells, which are essential for photosynthesis. This higher ratio K^+/Na^+ could be explained by a higher selectivity for K^+ versus Na^+ that was attributed to transport systems (Volkov et al., 2004). Additionally, in *H. vulgare*, maintenance of the photosynthetic capacity under stress conditions resulted from a higher level of K^+ and a lower content of Na^+ in the cytoplasm of mesophyll cells (Garthwaite et al., 2005).

In summary, the study K^+ nutrition under saline conditions, particularly determining which carriers can block the influx of Na^+ (Zhu, 2001) and which transporters of K^+ and Na^+ affect the K^+/Na^+ relationship in the cytoplasm, is important for understanding the mechanisms underlying salt tolerance in plants (Attumi, 2007). Among the species of economic interest, pepper have a high sensitivity to salt stress compared with other vegetable crops, and we thus present a brief review of studies related to *Capsicum* and salinity stress.

SALINITY AND GENUS *CAPSICUM*: AN UPDATE

Peppers are members of the family Solanaceae. The genus *Capsicum* includes approximately 25 wild species and five domesticated species (Bosland and Votava, 2000). Among the domesticated species, *Capsicum annuum* is the most economically important species for agriculture (Morales-Garcia, 2008). Peppers are an important agricultural crop, not only for their economic importance but also for the nutritional value of their fruit, mainly because they are an excellent source of natural pigments and antioxidant compounds in addition to their excellent flavor and pungency (McMahon et al., 2002; Navarro et al., 2006).

For optimum production, peppers require environmental conditions (temperature and light) typical of tropical regions and semi-arid climate, where irrigation water is usually limited or has a high concentration of salt. Salinity decreases the performance of peppers and imposes stress conditions on plants (Navarro et al., 2006). The shortage of high-quality water where peppers are grown has necessitated the use of saline water for irrigation.

According to the U.S. Department of Agriculture (USDA), pepper (*C. annuum*) and tomato (*S. lycopersicum*) are among the major crops that are moderately sensitive to salinity (de la Peña and Hughes, 2007). Kanber et al. (1992) and Kadir et al. (2004) mentioned that peppers as moderately sensitive to this type of stress. Other authors consider peppers to be susceptible or very susceptible to salinity (Navarro et al., 2002, 2006; Aktas et al., 2006), but little information exists about the effect of salinity on the *Capsicum* genus in general.

In *C. annuum*, salinity affects the growth of the plants, where germination, plant height, the length of the root and general biomass are significantly reduced.

Productivity is also reduced, with a yield of fruits that are smaller and of lower weight. The number of fruits per plant is also reduced (Chartzoulakis and Klapaki, 2000; Rubio et al., 2009). The membrane permeability, activity of water channels (aquaporins), stomatal conductance, photosynthetic rate and the balance of ions are also affected (Carvajal et al., 1999; Navarro et al., 2003; Cabanero et al., 2004; Aktas et al., 2006).

Throughout their entire ontogeny, pepper plants are susceptible to salt stress, but damage at seedling is more serious and results in growth retardation and even death of the seedling. In adult plants, symptoms of leaf necrosis, wilting and defoliation have been observed, and darkening in the roots or burns the tips have also been observed. Long-term salt stress reduces vegetative growth, mainly shoot growth (De Pascale et al., 2003). In general, plant species are more tolerant during germination and become more sensitive during emergence and early stages of plant growth.

In *C. annuum*, the reduction in performance begins when the electrical conductivity is higher than 1.5 dSm^{-1} in the saturated soil extract and 1 dSm^{-1} in irrigation water (FAO, 2003); the yield was diminished by 50% to an EC of 8.6 and 5.8 dS m^{-1} , respectively, and it increased proportionally with increasing EC (Goldberg, 2009). In studies under greenhouse conditions, 50 mM NaCl prolonged the time of germination, but the percentage of germinating plants was not affected. Similarly, the growth of *C. annuum* is affected in a concentration range of 10 to 50 mM, depending on the conditions and time of exposure to stress (Palma et al., 1996; Chartzoulakis and Klapaki, 2000; Yilmaz et al., 2004; Morales-Garcia, 2008). The total yield of the fruit was significantly reduced at concentrations above 10 mM NaCl, with a reduction of 95% in the presence of 150 mM NaCl (Chartzoulakis and Klapaki, 2000). Furthermore, it has been found that some genotypes of *C. annuum* can tolerate 150 mM NaCl in nutrient solution for 10 days; to select salt tolerant genotypes a concentration of 100 mM NaCl was used, and the severity of salt stress was evaluated in the leaves (Aktas et al., 2006). The NaCl concentration that affects the growth of *C. annuum* thus varies between 0 to 150 mM NaCl.

The varying degrees of toxicity to salt stress depend on the pepper cultivar, species and growing conditions (Chartzoulakis and Klapaki, 2000; Niu et al., 2010). Regarding the selection of tolerant genotypes, in the case of pepper, very little information is available in the literature regarding the genetic variability in the genus *Capsicum* (Aktas et al., 2006) and only a few genotypes have been evaluated for their genetic tolerance to salinity (Chartzoulakis and Klapaki, 2000). It is known that the root system of some genotypes of pepper is not affected by salt stress. Yildirim and Guvenc (2006) have reported pepper cultivars that could be useful as sources of genes to develop pepper varieties that will be tolerant to abiotic stress. Similarly, considering the severity of symptoms,

genotypes of pepper that are tolerant and sensitive to salinity have been selected through the analysis of growth of shoots and roots, as well as the concentration of K^+ , Na^+ and Ca^{2+} in the tissues of both organs (Aktas et al., 2006). Recently, in an evaluation of the response of genotypes of *C. annuum* and *C. chinense* to irrigation with salt water, the genotypes of *C. chinense* showed greater sensitivity (Niu et al., 2010a, b).

One of the main problems related to salinity in *C. annuum* manifests in the aerial region of the plant, particularly in the leaves. In sensitive genotypes, chlorosis and necrosis of the leaves was observed (Aktas et al., 2006), as well as a reduction in leaf area (Chartzoulakis and Klapaki, 2000). This effect was accompanied by a reduction in chlorophyll content, a relatively low rate of photosynthesis and net assimilation of CO_2 and a low conductance (Martinez-Ballesta et al., 2004; Lycoskoufis et al., 2005). At high concentrations of NaCl (100 to 150 mM), the photosynthetic rate was reduced to approximately 85% (Bethke and Drew, 1992), which led to decreased plant growth and to ultimately death of the plants.

High concentrations of salts in the soil are responsible for reduced productivity of economically important species cultivated worldwide. Peppers are a major vegetable crop and are not exempt from this effect of salinity. In *C. annuum*, the total fruit yield was affected at concentrations above 10 mM NaCl, and this effect was more severe with increasing concentrations. As previously mentioned, the number of fruits per plant and their weight is reduced by salinity (Chartzoulakis and Klapaki, 2000). Furthermore, the severity of salt stress on pepper fruits depends on the state of maturity of the fruit. At low maturity, salinity has a beneficial effect (Navarro et al., 2006); in contrast, at later stages of maturity, salinity affects the morphology and organoleptic properties of the fruit (Rubio et al., 2010). Additionally, the Ca^{2+} content of the fruits decreases under saline conditions. Recently, a high sensitivity of *C. chinense* fruits was observed when compared with other genotypes of *C. annuum* (Niu et al., 2010a).

Furthermore, pepper plants under conditions of salt stress are not able to adjust their osmotic potential to maintain proper water content in the leaves as mentioned by Navarro et al. (2002, 2003); in contrast, it has been reported that Cl^- and NO_3^- are used by pepper plants to perform osmotic adjustment. It also appears that sucrose is the main carbohydrate that accumulates to maintain turgor (Martinez-Ballesta et al., 2004). The effect of NaCl is mainly osmotic at low concentrations; however, at high concentrations of salt, toxicity of Na^+ and Cl^- is also involved. Rubio et al. (2010) mentioned that in pepper, the osmotic effect seems to be more important than the ionic effect, and the effect in pepper plants appears to be based on salinity; therefore, more research is required to determine the physiological basis of this response (Navarro et al., 2003).

Salinity also has a significant effect on nutrient concentration in plant tissue, which decreases with increasing concentrations of Na^+ (Martinez and Carvajal, 2008). In *C. annuum*, salinity significantly reduces the content of K^+ , Ca^+ and Mg^+ ; additionally, the concentration of Na^+ and Cl^- in the tissues increases as salinity increases. Na^+ and Cl^- mainly accumulate in the roots (Lycoskoufis et al., 2005); therefore, it has been suggested that low accumulation of Na^+ in the leaves may occur because pepper plants are capable of Na^+ recirculation control, thereby preventing damage to photosynthetic tissue (Blom-Zandstra et al., 1998). Other studies have also suggested that pepper plants have the ability to limit the uptake and transport of Na^+ and Cl^- in the root zone to aerial parts (Chartzoulakis and Klapaki, 2000). It has also been reported that Na^+ accumulates mainly in basal cells of the pich root and stem (Blom-Zandstra et al., 1998).

In seedlings of pepper, the content of K^+ and the K^+/Na^+ relationship in the leaves is negatively affected by increased concentrations of NaCl. A tolerant variety had a lower content of Na^+ and increased accumulation and K^+/Na^+ ratio compared with the corresponding sensitive varieties (Yilmaz et al., 2004). These findings indicate that tolerance of pepper varieties to salt stress is related to mechanisms of Na^+ absorption that are under the control of the levels of K^+ . Previously the K^+/Na^+ ratio was considered to be an important marker of salinity tolerance, but this marker has not been used to select salt tolerant genotypes (Aktas et al., 2006). Additionally, we have observed that under conditions of salinity stress, K^+ deficiency may occur in pepper (*C. annuum*), and consequently, Na^+ enters the cell, which decreases the K^+/Na^+ ratio in the cytoplasm and affects metabolic processes that are dependent on K^+ (Kaya and Higgs, 2003).

To identify the possible mechanisms involved in the influx of Na^+ , a mutant *C. annuum* that has been studied that has the ability to hyperaccumulate Na^+ in all plant tissues and has a lower content of K^+ in the roots. The results in this mutant suggest that there is an alteration in the mechanisms of transport of Na^+ that causes the accumulation of this ion. Evidence also suggests that at least four different processes of ion transport are affected by mutation, possibly because of effects on four transport proteins: NSCCs or HKTs (influx of Na^+), Na^+/H^+ exchanger (efflux of Na^+), K^+/H^+ symporter (influx of K^+ with high affinity) and outward-rectifying K^+ channels (upstream K^+) (Murthy and Tester, 2006).

Additionally, the role of aquaporins (water channels) and their relationship to water uptake and nutrients in *C. annuum* have been studied (Carvajal et al., 1999; Martinez-Ballesta et al., 2003). It has been suggested that one of the first steps to increase the tolerance to salinity is to control the flow of water through aquaporins (Bohnert and Jensen, 1996). In contrast, it has been shown that stress caused by an excess or deficiency of

nutrients (K^+ , Ca^{2+} , Mg^{2+} and Na^+) affects water uptake and transport, which supports the idea that aquaporins play an important role in pepper plants under salt stress (Cabanero and Carvajal, 2007). Similarly, the type of nutrient supplied and how it is perceived by the roots has been linked to the functionality of aquaporins and ATPase activity in certain circumstances (Martinez-Ballesta et al., 2003; Cabanero and Carvajal, 2007). In contrast, Martinez-Ballesta et al. (2003) indicated that there is no relationship between these two membrane proteins in pepper plants in saline conditions.

Salinity induced Ca^{2+} deficiency in different species and affects water relationships on a large scale. Previous studies in pepper plants showed that salinity reduced the concentration of Ca^{2+} in the roots, which was restored when Ca^{2+} was added to the treatments (Cabanero et al., 2004). Moreover, the reduction in the putative function of aquaporin in pepper plants treated with salinity appears to not be caused by an osmotic effect (Carvajal et al., 1999). Recent evidence has suggested that the availability of Ca^{2+} in cells and on the plasma membrane of pepper plant roots (*C. annuum*) under salt stress conditions participates in the functionality and regulation of aquaporins in the plasma membrane (Cabanero et al., 2006). Martinez-Ballesta et al. (2008) mentioned two different effects of Ca^{2+} on aquaporins of pepper plants under salt stress: an increase in the concentration of cytosolic Ca^{2+} associated with the perception of salt stress may allow closure of aquaporins, and when the requirement for Ca^{2+} is reduced by salinity, extracellular calcium upregulates aquaporins, which indicates a positive role of Ca^{2+} in the regulation of water transport in pepper roots under salt stress (Martinez-Ballesta et al., 2008). Other cations may also have specific responses or interact with other nutrients to regulate the metabolism of plants under salt stress.

At the molecular level, several genes of *C. annuum* have been reported to be expressed in response to biotic and abiotic stress. Under conditions of osmotic stress, a gene encoding an embryogenesis abundant (LEA) protein was identified from *C. annuum*. This gene *cadhn* encodes a dehydrin, which is a LEA protein that is involved in the resistance to osmotic stress. It has been shown that this gene is induced by osmotic stress and abscisic acid in the tissues of the leaves; salinity and (to a lesser extent) cold stress also induced *Cadhn* (Chung et al., 2003). The overexpression of the transcription factor ERF/AP2 of *C. annuum* (CaPF1) increases the ability of transgenic *Pinus virginiana* plants to tolerate various stresses during vegetative growth. The transgene *CaPF1* increases the tolerance to salt stress and oxidative tissue damage and counteracts the inhibitory effect of salt stress in the callus, shoots and plants of *P. virginiana* (Tang et al., 2006). Previous studies have shown that CaPF1 improves the tolerance to heavy metals and pathogens (Yi et al., 2004; Tang et al., 2005).

An extracellular peroxidase of *C. annuum* (CaPO2)

participates in the defense response of pepper plants to abiotic stress and fungal pathogens. CaPO2 is strongly induced by drought, cold and salinity. The loss function of CaPO2 by silencing increases the susceptibility of pepper plants to salt stress and osmotic stress. Overexpression of CaPO2 in *A. thaliana* increased the tolerance to salinity, drought, oxidative stress and pathogen stress (Choi and Hwang, 2011). Similarly, the gene *CaXTH3* that encodes xyloglucan endo-trans-glucosylase/hydrolase in *C. annuum* has been reported to increase the tolerance to drought stress and high salinity in Arabidopsis (Choi et al., 2010). Furthermore, cloning and gene expression of the osmotin of *Nicotiana tabacum* in pepper increased salt tolerance and prevented the accumulation of ROS as well as the effects of ROS in cells. Osmotin plays an essential role in the adaptive response of plants to environmental factors, particularly salinity stress (Subramanyam et al., 2010). Also, total deoxyribonucleic acid (DNA) of *Rhizophora apiculata*, a salt resistant seashore plant, was introduced into *Capsicum annuum* via pollen tubes formed after self-pollination. The transformed progenies showed obvious stronger salt tolerance compared with control. About 55% of the transgenes can survive, flower and fruit when grown under salt stress. By contrast, no control survived under the same condition (Lin et al., 2009). These results indicate that it is feasible to introduce exogenous DNA through pollen tubes, and improve salt tolerance in *Capsicum*.

Within the genus *Capsicum*, the five domesticated species include *C. chinense*. The habanero pepper is a crop of great economic importance to vegetable growers in the state of Yucatán, México, only second to the tomato. In contrast to *C. annuum*, there have been few studies on the effect of salinity in *C. chinense*. Two recent papers that evaluated the response of pepper genotypes to saline conditions revealed that habanero pepper plants had greater sensitivity to growth and productivity in salt stress conditions, with a higher mortality rate than other genotypes (Niu et al., 2010a, b).

CONCLUSION

Because peppers are important vegetables and are valued for their aroma, flavor, nutraceutical potential and pungency, their sensitivity to salinity is of agricultural concern. Therefore, improvement in the tolerance to salinity stress should be a priority in areas where different pepper species are grown.

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