Chapter 5
Changes in Photosystem II in Response to Salt Stress

Anjana Jajoo

5.1 Introduction

The population of world is expected to increase from about 6 billion people in year 2000 to more than 10 billion in 2050. To feed this increasing population, the average world cereal yield will need to reach 5 t ha^{-1} from its present 3 t ha^{-1}. At the same time, global climatic changes are posing more stressed conditions for the crops to grow and environmental stresses represent the most limiting factors for agricultural productivity. In general, abiotic stress often causes a series of changes at whole plant as well as molecular level that unfavorably affect growth, development and productivity of the plant. Abiotic stresses, singly or in combination, result in both general and specific detrimental effects on plant growth and development. A variety of distinct abiotic stresses exist, such as water (drought, flooding), high and low light, extreme temperature (chilling, freezing, heat), salinity, heavy metals (Allakhverdiev et al. 2008).

Looking to the severity of the decrease in crop yield caused by salinity (high salt stress) it is essential to understand physiology of the salt stressed plants. Knowledge of the type of damage that occurs to the plant during salt stress will direct research to design genetic modifications which may provide the plants with more salt tolerant machinery. This chapter particularly deals with the effects of salt stress on the process of photosynthesis the efficiency of which is responsible for the overall crop yield. If we can improve photosynthesis under salt stress conditions, definitely it will have a positive impact on the crop yield and production. Since in the process of photosynthesis, photosystem II is one of the most stress-susceptible components, we have focused more on it. We have also focused on the utility of chlorophyll a fluorescence induction kinetics to get quick information about the efficiency of photochemical reactions taking place in the leaf in the field conditions. This minireview presents a brief depiction

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and recent advances related to the effects of salt stress on photosynthesis, particularly, Photosystem II and shall be helpful to gain better understanding of the phenomenon of salinity stress and hence designing strategies to cope up with it. At the same time, Understanding the adaptive mechanisms exercised by the plants in natural stress conditions will help to improve salt tolerance in crop plants.

5.1.1 Environmental Stresses and Their Impact on Plant Growth

Plants are able to "sense" environmental changes and subsequently "respond" to stress. Most plants grow in environmental conditions that are, to a considerable degree, unfavorable to their growth.

Each environmental factor usually has a minimum and maximum level, beyond which plants cannot survive. Due to their stationary status, plants can only make metabolic and structural adjustment to cope with biological and non-biological trauma (Allahverdiyev et al. 2011).

Drought, salinity, extreme temperatures (cold and heat) and oxidative stress are often interrelated; these conditions singularly or in combination induce cellular damage. Due to the complex nature of stresses, not a single sensor, but multiple sensors may be responsible for perception of stress stimuli.

5.1.2 High Salt Stress

More than 800 million ha of land throughout the world are salt-affected (http://www.fao.org/ag/agl/agll/spush/). Growing concentration of salts in the rhizosphere have been because of natural causes such as salty raining waters near around the coasts, contamination from the parental rocks and oceanic salts and cultivation practices (Mahajan and Tuteja 2005). Increased salinisation of arable lands accompanied with water stress can result in 50% loss of arable lands by the year 2050. Sustainable salinization of arable land is getting more widespread and thus decreasing the yield from formerly productive soil everywhere in the world. Therefore, it requires increased water-use efficiency and salt tolerance for agricultural production in an ever-decreasing area of arable land (Tiburcio et al. 2012). Basically the term salinity implies high concentration of salts in soil, it is NaCl that constitutes the most part in soil salinity and that is why all plants have evolved several mechanisms to regulate NaCl accumulation or exclusion. High salinity causes both hyperionic and hyperosmotic stresses and finally affects the growth of the plant.

There is widespread occurrence of salt-affected soils and some plants are adapted (halophytes) to grow on such soils. However, most of our crops are salt-sensitive. As a consequence, salinity is a major threat to agriculture, especially in areas where secondary salinisation has developed through irrigation or deforestation. Attempts to improve the salt tolerance of crops have met with very limited success, due to the complexity of the genetic and physiological traits (Flowers and Flowers 2005).

5.2 General Effects of Salt Stress on Plants

Plant salt stress is a condition where excessive salts in soil solution cause inhibition of plant growth or plant death. No toxic substance has been found to restrict plant growth more as compared to salt, so salt stress presents an increasing threat to plant agriculture (Zhu 2007). Saline soil is characterized by toxic levels of chlorides and sulfates of sodium. The electrical conductivity of saturation extracts of saline soil is more than 4.0 dS/m (40 mM NaCl). The problem of soil salinity is increasing because of several reasons including the use of sea water for irrigation, improper drainage, salt accumulation in the root zone in arid and semi-arid regions due to high evaporative demand and insufficient leaching of ions as the rainfall is inadequate (Chinnusamy and Zhu 2003).

5.2.1 Effects of High Salt Stress on Plant Growth

Plants which have capacity to grow on highly saline environments are traditionally classified as glycophytes or halophytes (Flowers et al. 1977). Being the natural inhabitants of highly saline soils, halophytes efficiently excludes salts from their roots and leaves and some can tolerate salts that are more than twice the concentration of seawater. Salinity includes ionic stress (mainly due to Na^+, Cl^-, and SO_4^{2-}), osmotic stress, and secondary stresses such as nutritional imbalances and oxidative stress for glycophytes (Zhu 2002). Besides Na^+, some plant species are also sensitive to chloride, the major anion found in saline soils. High concentrations of Na^+ disturb osmotic balance and results in "physiological drought", preventing plant water uptake. Halophytic plants that are tolerant of sodium toxicity are probably inhibited by osmotic stress.

Salt stress affects several important processes such as growth, photosynthesis, protein synthesis, and energy and lipid metabolism (Parida and Das 2005; Abbaspour et al. 2012). Some of the major effects of salt stress on plant growth and the mechanism of tolerance of salt stress by plants have been summarized in Fig. 5.1. Salinity causes increases in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter, and spongy cell diameter in leaves of bean, cotton, etc. Salt stress has various effects on plant physiological processes such as increased respiration rate and ion toxicity, changes in plant growth, mineral distribution, and membrane instability resulting from calcium displacement by sodium, membrane permeability, and decreased efficiency of photosynthesis (Sudhir and Murthy 2004). Salt stress can lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn result in generation of reactive oxygen species (ROSs) (Parida and Das 2005; Ahmad and Sharma 2008). To cope with the detrimental effects of salt stress, plants have evolved many biochemical and molecular mechanisms. Some of the biochemical strategies are (i) selective buildup or exclusion of salt ions, (ii) control of ion uptake by roots and transport into leaves, (iii) ion compartmentalization, (iv) synthesis

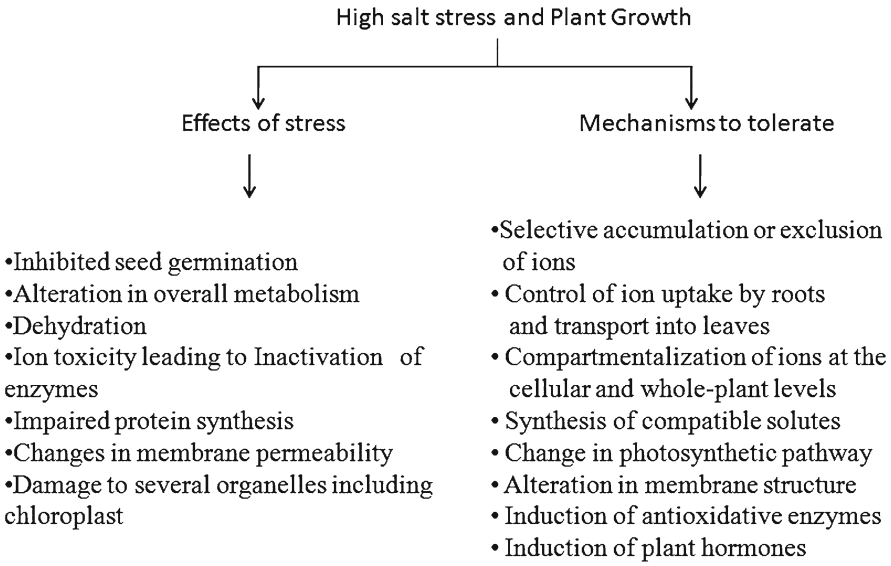

Fig. 5.1 Effects of salt stress on plant growth

of compatible osmolytes, (v) alteration in photosynthetic pathway, (vi) changes in membrane structure, (vii) induction of antioxidative enzymes and (viii) stimulation of phytohormones (Parida and Das 2005). Salinity is detrimental to plant growth as it causes nutritional constraints by decreasing uptake of phosphorus, potassium, nitrate and calcium, ion cytotoxicity and osmotic stress. Under salinity, ions like Na^+ and Cl^- penetrate the hydration shells of proteins and interfere with the function of these proteins. Ionic toxicity, osmotic stress, and nutritional defects under salinity lead to metabolic imbalances and oxidative stress (Chinnusamy and Zhu 2003).

Understanding the mechanisms of plant salt tolerance will lead to effective means to breed or genetically engineer salt-tolerant crops.

5.2.2 Effects of High Salt Stress on Photosynthesis

Several physiological processes contribute to limitation of plant growth by environmental factors. However, the dominant physiological process is photosynthesis. Plant growth as biomass production is a measure of net photosynthesis and, therefore, environmental stresses affecting photosynthesis also affect the growth and ultimately crop yield.

Photosynthesis is one of the most important metabolic processes in plants and its study provides information about the general "health" of plants. Photosynthesis serves as a global stress sensor in plants, algae and cyanobacteria. The modifications

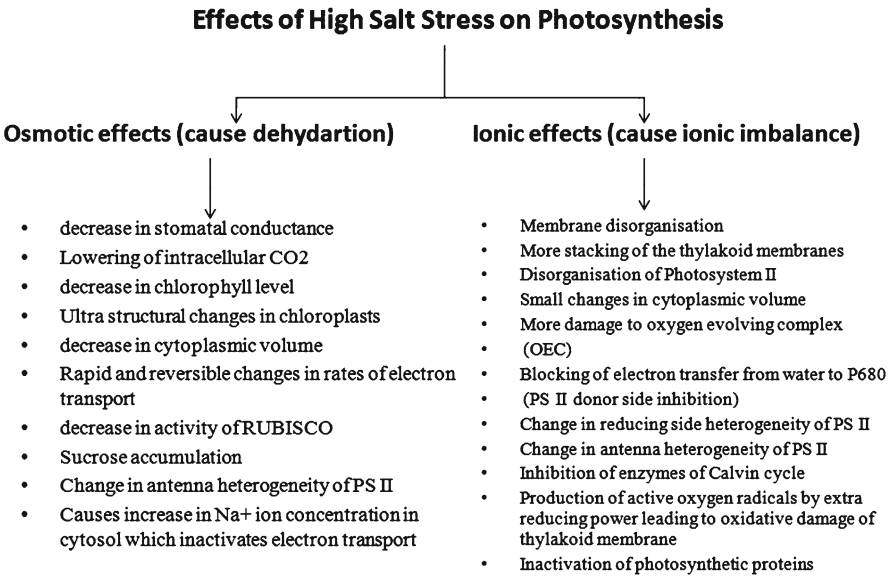

Fig. 5.2 Effects of salt stress including osmotic effects and ionic effects on the basic process of photosynthesis

of the chloroplast in response to various environmental stresses has been a topic of interest always. Photosynthesis occurs in two stages: the light reactions and the dark reactions. The chloroplast is the organelle where photosynthesis occurs in photosynthetic eukaryotes. Light reactions occur in the thylakoid stacks of the grana in the chloroplasts. Dark reactions occur in the stroma. Photosynthesis converts light energy into chemical energy via electron transport through pigment-protein complexes, Photosystem II (PSII) and Photosystem I (PSI). The light energy absorbed by pigments initiate the primary photochemical events followed by reactions that ultimately form the stable organic compounds. Since the two photosystems regulate photosynthetic efficiency and hence net productivity considerable attention has been paid to the effects of environmental stress on these photosystems.

Response of photosynthesis to drought and salinity stress is highly complex. It involves the interplay of limitations taking place at different sites of the cell/leaf and at different time scales in relation to plant development (Chaves et al. 2009). The stress is sensed at the levels of pigment composition, structural organization, primary photochemistry and the CO_2 fixation (Biswal et al. 2011; Shu et al. 2012; Mittal et al. 2012). Salt stress causes either short or long-term effects on photosynthesis. The short-term effect occurs after a few hours or within 1 or 2 days of the onset of exposure while long term effect arises after exposure to salt stress for several days.

Salt stress is exhibited in two ways: by causing a change in the osmoticum of the surroundings (osmotic stress) and by causing change in the ionic composition of the medium (ionic stress). As shown in Fig. 5.2, osmotic and ionic effects on photosynthesis are manifested in different ways. The accumulation of salt ions in plants can

cause osmotic stress, ionic toxicity and induce nutritional deficiencies (Munns 2002). When Na^+ and Cl^- ions reach high concentrations in leaves, they cause impairment in both biochemical and photochemical processes of photosynthesis (Munns and Tester 2008). The salt-induced ionic toxicity effects are capable to induce acute photosynthetic damages (photochemistry and gas exchange) due to stomatal and biochemical limitations (Silva et al. 2011).

As shown by Downton et al. (1985), there is development of a thicker leaf with less chlorophyll per unit area during salt treatment which permitted stomatal conductance and intercellular partial pressure of CO_2 to decline without restricting photosynthesis and had the benefit of greatly increasing water use efficiency. Chlorophyll content has been shown to decrease in salt sensitive/susceptible plants as compared to salt-tolerant plants. At the same time salt stress increases the efficiency of photophosphorylation by stimulating the cyclic photosynthetic electron flow around PS1 (Sudhir and Murthy 2004). The reduction in photosynthetic rate has also been shown to be due to the reduction in stomatal conductance which restricts availability of CO_2 for carboxylation. Stomatal closure minimizes loss of water by transpiration and this affects chloroplast light-harvesting and energy-conversion systems thus leading to alteration in chloroplast activity.

Photosynthetic rate is lower in salt-treated plants, but the photosynthetic potential is not greatly affected when rates are expressed with regard to chlorophyll or leaf area. Decreases in photosynthetic rate are due to several factors: (1) dehydration of cell membranes which reduce their permeability to CO_2, (2) salt toxicity, (3) reduction of CO_2 supply because of hydroactive closure of stomata, (4) enhanced senescence induced by salinity, (5) changes of enzyme activity induced by changes in cytoplasmic structure (Parida and Das 2005). Electron microscopy has shown that the thylakoidal structure of the chloroplasts becomes disorganized, the number and size of plastoglobuli increases, and their starch content decreases in plants treated with NaCl (Hernandez et al. 1999). In the mesophyll of sweet potato leaves, thylakoid membranes of chloroplast are swollen and most are lost under severe salt stress (Mitsuya et al. 2000).

5.3 Effects of Salt Stress on Photosystem II

5.3.1 General Effects of High Salt Stress on PS II

The four major protein components of the photosynthetic electron transport chain are Photosystem II (PSII), Photosystem I (PSI), the cytochrome ($Cytb_6f$) complex, and ATP synthase. The majority of PS II reaction centers (RC) with their main light harvesting complex (LHC) II are located in the grana while Photosystem I (PSI) is localized in stroma-exposed thylakoid membranes. PS II is a multi-subunit complex whose function is to organize the chlorophylls for light harvesting and harbor the electron transport cofactors needed for the oxidation of water.

Effects of salt stress in cyanobacterium *Spirulina platensis* (Sudhir et al. 2005) showed a decrease in PS II mediated activity and an increase in PS I activity. It was ascribed to changes in the thylakoid membrane protein profile which led to the decreased energy transfer from light harvesting antenna to PS II. Salt adapted cells can maintain a high conversion efficiency of excitation energy through the down regulation of PS II RCs (Lu and Vonshak 2002). In the cyanobacterium, salt stress inhibits the apparent quantum efficiency of photosynthesis and photosystem II (PSII) activity while stimulating photosystem I (PSI) activity and dark respiration significantly. Salt stress also results in a decrease in overall activity of the electron transport chain (Lu and Vonshak 1999). Experimental evidence shows that at low salinity (100 mM) PSII mediated electron transport activity increases while a decrease in PS II activity is observed at high salinity in *B. parviflora* (Parida et al. 2003). High salt stress has a negative influence on PS II activity and the effect carried with the duration of stress application and on the cultivar used. In cynobacteria, under salt stress there is loss in chlorophyll protein (47 kDa) and a core membrane linker protein 94 kDa that can attach phycobilisome to thylakoid (Garnier et al. 1994). The 23 kDa protein which is extrinsically bound to PS II is also dissociated under salt stress (Sudhir et al. 2005). The short term stress leads to acclamatory changes in the functional aspects of PS II.

5.3.2 *Effects of Salt Stress on Chl a Fluorescence Induction Kinetics*

Chl *a* fluorescence kinetics is an informative tool for studying effects of different environmental stresses on photosynthesis (Kalaji et al. 2011). Due to its intricate connection with several processes taking place during the conversion of light due to its intricate connection with the numerous processes taking place during the energy conversion of light into a stable chemical form, Chl *a* fluorescence has proven to be an open window in the heart of the photosynthesis process (Papageorgiou and Govindjee 2004; Stirbet and Govindjee 2011). Chl *a* fluorescence is becoming a popular tool for plant management especially photosynthesis research. Chl *a* fluorescence originates mainly from PS II. Most interesting aspect of this technique is that chlorophyll fluorescence parameters start to fluctuate before other visual symptoms due to stress appear. Various parameters arising from fluorescence measurements can be exploited to gain information about status and efficiency of different components of PSII in Early stages of development and stress. When a dark-adapted photosynthetic sample is illuminated, Chl *a* fluorescence emission exhibits a fast rise to a maximum followed by a decline to a steady state over some minutes (Stirbet and Govindjee 2011). Analysis of the intermediate data points of the fast fluorescence rise forms the basis of the so-called "OJIP curve", whose shape is universal for all photosystems containing Chl *a*. The OJIP transient has the potential to be used for the characterization of the photochemical quantum yield of PS II photochemistry, and the electron transport activity. A representative OJIP curve has

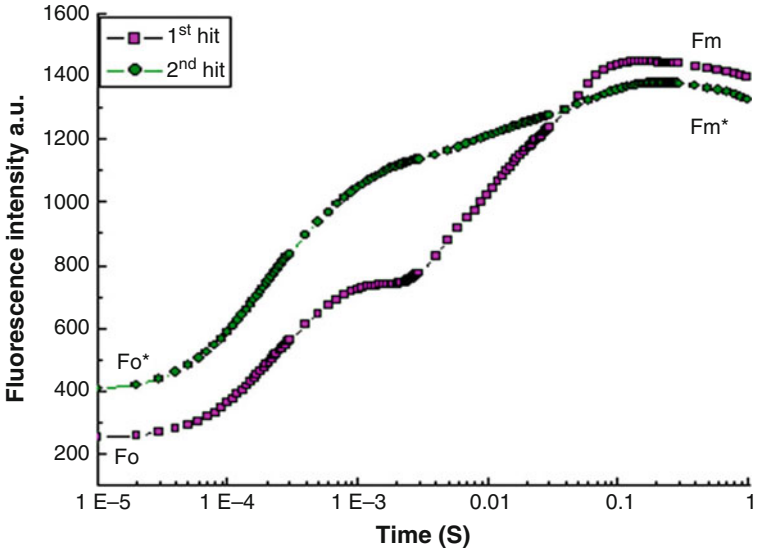

Fig. 5.3 A representative fluorescence induction curve (first hit) and a curve obtained after second hit. The graphs have time axes in logarithmic scale

been shown in Fig. 5.3 illustrating various intermediate phases. The OJIP transient is the fast Chl *a* fluorescence rise, measured at high light intensities [generally at 3000 μmol photons m-2 s-1]. The OJIP transient starts at O or Fo [minimum fluorescence, all Q_A is oxidized] and reaches a maximum called P or Fm (all Q_A is reduced) in ~200 ms. The intermediate steps are called J and I and they are situated at ~2 ms and ~30 ms. The O to J phase is due to the net photochemical reduction of Q_A to Q_A^-. The intermediate I step and the final P step have been proposed to be due to existence of fast and slow reducing plastoquinone (PQ) pool, as well as due to different redox states of the reaction centers (RC) of PS II which reduces the PQ pool (Govindjee 1995; Haldimann and Strasser 1999). The popular JIP-test is a tool to analyse the polyphasic rise of the Chl *a* fluorescence transient and has been developed to investigate in vivo the "vitality" of plants and the adaptive behavior of the photosynthetic apparatus to different stresses (Christen et al. 2007) like high temperature, salinity stress (Mehta et al. 2010b). By measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry can be obtained (Maxwell and Johnson 2000). At a given moment, the physiological state of the sample determines the shape of the fluorescence transient of any sample. Diagnosis and early detection of various stresses using this non-invasive method is highly useful even more because field investigation can be conducted with high laboratory precision.

Polyphasic chlorophyll *a* fluorescence transient was measured to evaluate the effects of high salt stress on the photochemical efficiency of PS II. The OJIP transient represents the successive reduction of electron transport pool of PS II (Govindjee 1995). As evident in Fig. 5.4, the intensity of fluorescence in the induction curve

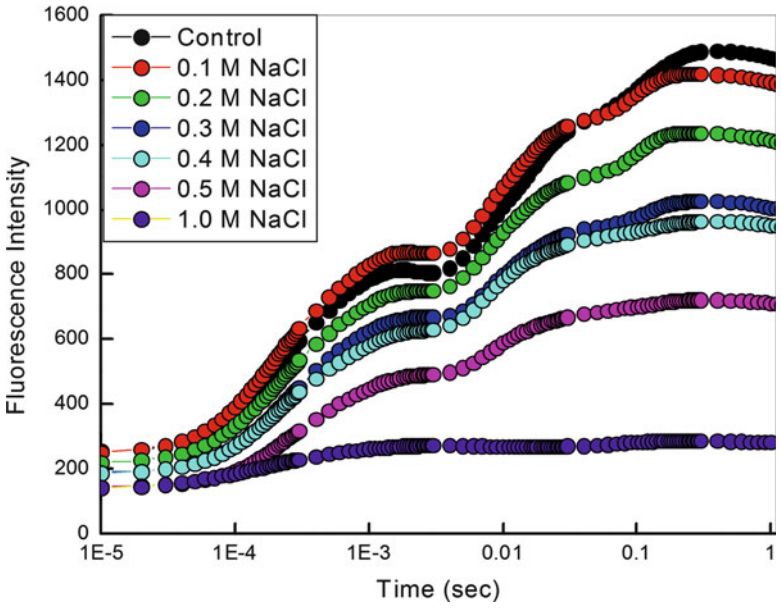

Fig. 5.4 The OJIP Chl *a* fluorescence transient curves (log time scale) in wheat leaves gradually exposed to various concentration of NaCl

decreased with increase in NaCl concentration. A significant decline in the minimal fluorescence (Fo), variable fluorescence (Fv) and maximal fluorescence (Fm) was observed with an increase in salt concentration. This decline in the fluorescence yield of leaves can be attributed to an inhibition of electron flow at oxidizing site of PS II (Lu and Vonshak 2002). The decrease in Fm and fluorescence at J, I, P has been suggested to be due to two reasons, first by inhibition of electron transport at the donor side of the PS II which results in the accumulation of P_{680}^{+} and second due to a decrease in the pool size of Q_A^{-}. Area over the fluorescence induction curve between Fo and Fm is proportional to the pool size of the electron acceptor Q_A on the reducing side of PS II. The area is dramatically reduced in case the electron transfer from reaction center to quinone pool is blocked. A decrease in area over the fluorescence curve with increase in NaCl concentration has been observed which suggests that high salt stress inhibits the electron transfer rates at the donor side of PS II. Fv/Fm ratio was not affected significantly in high salt treatment. ABS/RC i.e. effective antenna size of an active reaction centers, is influenced by ratio of active/inactive RCs and with increase in NaCl concentration the value of ABS/RC increased.

The kinetics of relative variable fluorescence (Vj) can give information about effects of high salt stress in electron transport chain on acceptor side of PS II. Vj is equivalent to (Fj – Fo/Fm – Fo) where Fj is the fluorescence at J step i.e. at 2 ms. Efficiency with which a trapped exciton can move an electron in to the electron transport chain further than Q_A^{-} (Ψo, which is calculated as ETo/TRo) was also measured. Increase in the value of Vj by 29% and a decrease in the value of Ψo by

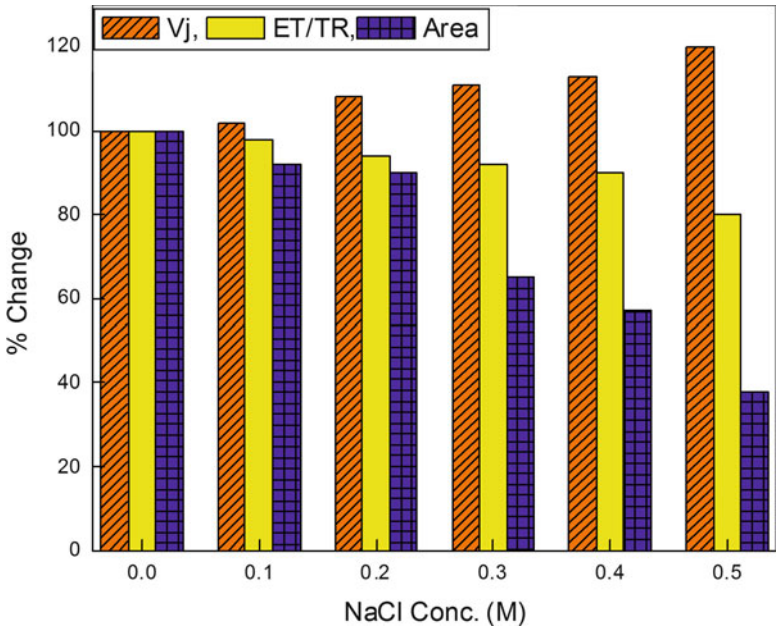

Fig. 5.5 Change (in %) variable fluorescence (Vj), efficiency of forward electron transfer (ETo/TRo) beyond Q_A and area over the curve in response to salt stress in wheat leaves

26% (Fig. 5.5) in 0.5 M NaCl treatment suggested a loss in reoxidation capacity of Q_A^- and an inhibition of electron transport at the acceptor side of PS II (Lu and Vonshak 1999) and also beyond Q_A^-. In recovery studies it was observed that the damage at acceptor side of PS II was recovered completely while damage at the donor side of PS II was recovered more than 80%. The rapid decline in photosynthesis under NaCl stress is reversible and specific to osmotic stress, where as the slow decline is irreversible and specific to ionic stress (Zhang and Xing 2008).

Another important parameter of JIP test is the performance index (PI) which is an indicator of sample vitality. It is the combined measurement of three functions: amount of photosynthetic reaction centers (RC/ABS), the maximal energy flux which reaches to the PS II reaction centers and the rate of electron transport at the onset of illumination. PI can be calculated as

$$PI_{ABS} = RC / ABS.\Phi_{Po} / (1-\Phi_{Po}).\Psi_{Eo} / (1-\Psi_{Eo})$$

Where Φ_{Po}, is the exciton trapped per photon absorbed and Ψ_{Eo}, is the probability that an electron can move further than Q_A^-. With increase in NaCl concentration a significant decrease in the value of performance index was observed and its value became half of the control in 0.5 M NaCl treatment (Mehta et al. 2010a). Since Fv/Fm ratio was not decreased significantly with high salt stress it can be said that high salt stress did not influence the number of quanta absorbed per unit time. The ratio $\Psi_{Eo}/(1-\Psi_{Eo})$ decreased with increase in NaCl concentration and became 57% of the

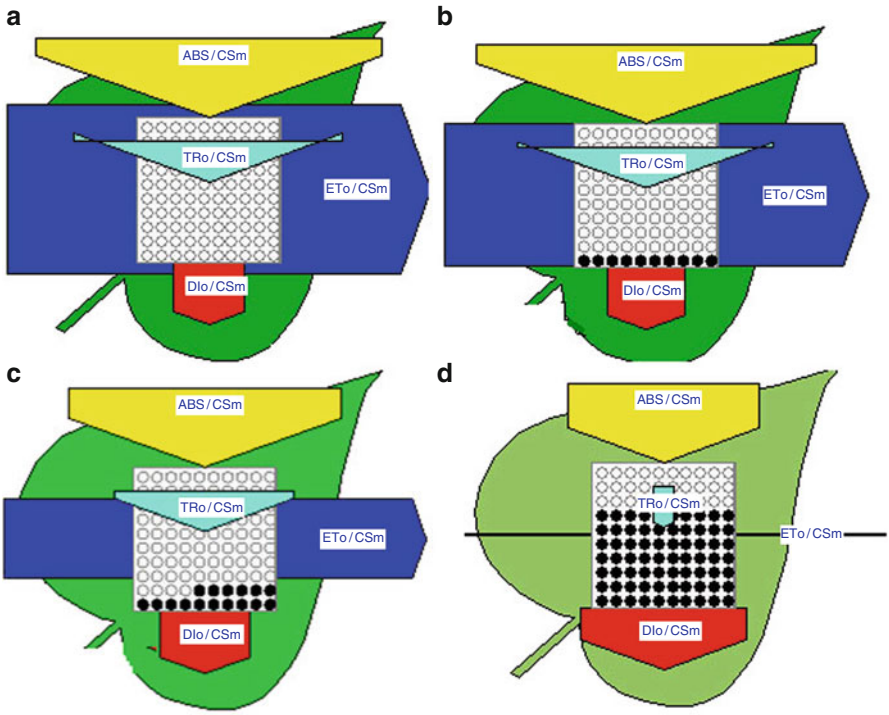

Fig. 5.6 Energy pipeline leaf model of phenomenological fluxes (per cross section, CS) in gradually stressed wheat leaves exposed to various concentrations of NaCl gradually. (**a**) Control (**b**) 0.3 M NaCl (**c**) 0.5 M NaCl and (**d**) 1 M NaCl. The value of each parameter can be seen in relative changes in width of each *arrow*. Active RCs are shown as *open circles* and inactive RCs are *closed circles*

control, suggesting that the efficiency of the forward electron transport rates were decreased. These results are in accordance with the data of ET/TR (Fig. 5.5).

It is possible to visualize the derived parameters by means of dynamic energy pipeline model of the photosynthetic apparatus (Krüger et al. 1997). The leaf model deals with the phenomenon-logical energy fluxes (per cross-section). Electron transport in a PS II cross section (ETo/CS) deals with the reoxidation of reduced Q_A via electron transport over a cross section of active and inactive RCs (Force et al. 2003). At various NaCl concentrations, a decrease in the electron transport per excited cross section (ETo/CS) due to inactivation of reaction center complex was observed. Density of the active reaction centers (RC/CS) reflects the number of active RCs in PS II cross section (indicated as open circles) which decreased with increase in salt concentration (Fig. 5.6). A decrease in RC/CS ratio suggests that the active RCs are converted into inactive RCs. ABS/CS is the number of photons absorbed by an excited PS II cross section (Force et al. 2003). At high salt concentration, a decrease in the energy absorbed per excited cross section (ABS/CS) was observed indicating that the energy absorption efficiency of PS II was decreased with increase in salt concentration.

Salt stress involves osmotic as well as ionic components. Sorbitol can be used to give osmotic stress while high salt concentration (NaCl) provides osmotic as well as ionic stress. A comparison of the effects observed in these two cases will help us to differentiate between the effects caused by osmotic and ionic components of the high salt stress. Osmotically, 0.5 M NaCl should behave as 1 M sucrose. Hyperosmotic conditions cause an efflux of water through water channel thereby decreasing cytoplasmic volume and reversibly inactivating the photosynthetic machinery (Allakhverdiev and Murata 2008). It was observed that the treatment of wheat leaves with 1 M sucrose caused a decrease in the efficiency of light reaction $\Phi_{Po}/(1-\Phi_{Po})$, rate of biochemical reaction ($\Psi_{Eo}/(1-\Psi_{Eo})$) and performance index (PI) by 11%, 20% and 30% respectively (Mehta et al. 2010a). The effects due to 1 M sucrose were recovered totally when the leaves were immersed in distilled water. In comparison, the effects observed in these parameters in 0.5 M NaCl were much higher and the effects were not totally reversible. It suggests that the effects observed in the samples treated with 0.5 M NaCl exhibit both the osmotic and ionic components of NaCl. The initial reversible effects may be ascribed to the osmotic aspects while the later, irreversible effects may be because of the ionic aspects of NaCl (Allakhverdiev and Murata 2008). These results are in contention with earlier studies in *Arabidopsis thaliana* where measurement of delayed fluorescence in high salt stressed seeds demonstrated that the rapid decline in photosynthesis under NaCl stress is reversible and specific to osmotic effects, where the slow decline is irreversible and specific to ionic stress (Zhang and Xing 2008).

Thus according to Mehta et al. (2010a, b) high salt stress inhibits the electron transport rates by ~75% at the donor and by ~25% at the acceptor side of PS II. As compared to acceptor side, the donor side of PS II is significantly affected by high salt stress. Inactive PS II centers increased with increasing salt concentration. Complete recovery of the damage caused at the acceptor side was observed, while damage to donor side could be recovered by more than 80%.

5.3.3 Effects of High Salt Stress on Heterogeneity of PS II

It is well established that PSII of higher plants is not homogenous in nature (Lavergne and Briantais 1996). The PSII varies in its structure and function both and this diverse nature of PSII is known as Photosystem heterogeneity. The concept of PSII heterogeneity originated in order to explain the biphasic nature of the kinetics of primary PSII activity. Two main types of PSII heterogeneity have been studied widely i.e., PSII antenna heterogeneity and PSII reducing side heterogeneity. On the basis of the differences in the antenna size the concept of α, β and γ centers has been introduced while on the basis of acceptor/reducing side function, Q_B-reducing and Q_B-non-reducing centers have been defined. Extent and nature of PSII heterogeneity may vary under different physiological conditions (Tongra et al. 2011; Lavergne and Briantais 1996) i.e. salinity stress, temperature stress (Mathur et al. 2010a, b), etc.

On the basis of the biphasic data obtained from fluorescence kinetics, presence of two distinct populations of PSII centers (termed as PSIIα and $PSII_{\beta}$) in the chloroplast was suggested (Melis and Homann 1976; Melis and Duysens 1979; Black et al. 1986). In a step-wise process in the development of PSII units, the addition of about 80 Chl to LHC II-inner portion of the antenna of $PSII_{\gamma}$ (contains ~ 50 Chl) yields $PSII_{\beta}$ (~ 130 Chl). In a second step, the addition of another 80chl to LHC II-peripheral part increases the antenna size to yield $PSII_{\alpha}$ [20]. The dominant form, PSII α, is localized in the grana partition regions [Andersson and Melis 1983] and is responsible for the majority of the water oxidation activity and plastoquinone reduction. These centers possess a Chl a core complex, an accessory Chl *a-b* light harvesting inner antenna (LHC II-inner), and a peripheral antenna (LHC II-peripheral) containing a combined total of about 210–250 Chl a and Chl b molecules (Morrissey et al. 1989). These have a higher absorption cross-section area due to association with the peripheral Chla/b LHCs. $PSII_{\alpha}$ are characterized by a large light harvesting antenna and excited states transfer between PSII units is possible in them as exhibited by the sigmoidal fluorescence rise when measured with DCMU. In contrast, $PSII_{\beta}$ are mainly located in stromal region of thylakoid membranes and are characterized by smaller light harvesting antenna of $PSII_{\alpha}$ and there is no possibility of the excited states transfer between PS IIs as reflected in an exponential fluorescence rise in the presence of DCMU. Smaller antenna size has been ascribed to the absence of peripheral LHC II in PSII. The α and β centers are similar in terms of their intrinsic trapping and fluorescence properties (Melis 1991). However they show difference in the recombination rate (reopening) in the α and β centers (Melis and Homann 1976). In addition to differences in their lateral location, the two types of PSII differ in terms of their kinetic properties, apparent mid-point potential of their primary electron acceptors, connectivity to the plastoquinone pool and their DCMU sensitivity as well (Sundby et al. 1986). The $PSII_{\gamma}$ is localized in stroma lamellae region, has the smallest antenna size among the three components and has the longest lifetime. $PSII_{\alpha}$ is believed to be the major 'normal' PSII centers whereas $PSII_{\beta}$ and $PSII_{\gamma}$ represent the two minor groups of 'abnormal' PSII centers with low quantum efficiencies due to their slow electron donation systems. The slow rate of $PSII_{\beta}$ and PSII γ as compared to the α center might be due to slow electron donation to their reaction center, which might undergo many turnovers via back reaction under continuous excitation, until their reduced primary acceptors were stabilized by the electron donation into the system. Some important characteristics of PS IIα, β and γ centres are shown in Fig. 5.7.

In addition to heterogeneity on the antenna size, PSII centers also display heterogeneity related to the reducing side of Q_A^- in relation to electron flow to the plastoquinone pool. It has been shown that a number of PS II centers, though photochemically competent, are unable to transfer electrons efficiently from electron acceptor Q_A^- to secondary electron acceptor Q_B (Lavergne 1982; Graan and Ort 1986; Guenther et al. 1988). These centers are termed as PS II Q_B-non-reducing using Lavergne's nomenclature (Lavergne 1982). In such centers Q_A^- can be reoxidized only by a back reaction with the donor side of PSII (Schanker and Strasser 2005). Q_B-non-reducing differs from Q_B reducing center in being incapable of

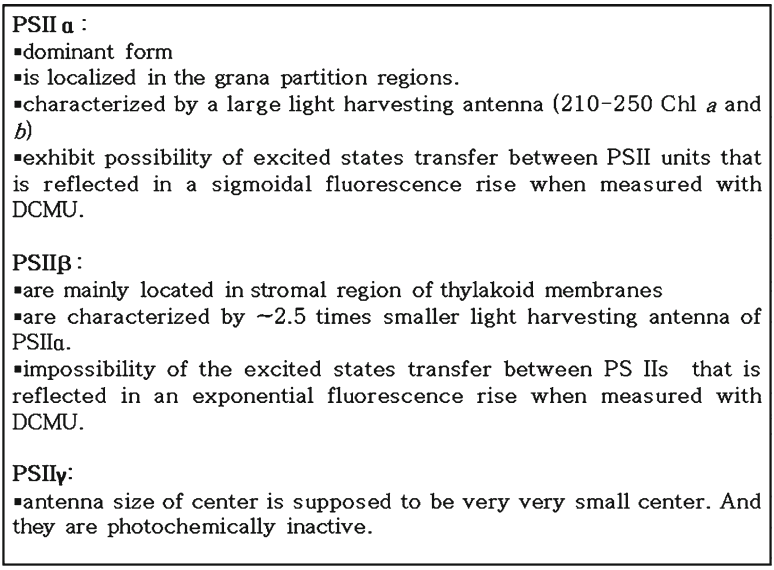

Fig. 5.7 Important characteristics of PS II alpha (α) and beta (β) and gamma (γ) centers

reducing the PQ pool. Q_B-non-reducing is normally either equal to $PSII_\beta$ or a subset of it (Guenther et al. 1988). It is found that the electron transfer from the primary acceptor Q_A to PQ is more than 1,000 times slower in the inactive centers as compared to the active centers (Hsu 1992).

The biological processes involved in adaptation to stress in plants are complex. Changes in several biological macromolecules including proteins and lipids are involved in these adaptive mechanisms. Rearrangement in PS II seems to be an adaptive mechanism of plants to tolerate stress conditions.

To evaluate the effect of high salt stress on reducing side and antenna size heterogeneity of Photosystem II, the chlorophyll *a* fluorescence transient curves were measured in the absence and presence of DCMU.

5.3.3.1 Effect of High Salt Stress on Reducing Side Heterogeneity

Reducing side heterogeneity of PSII, relative amount of Q_B reducing and Q_B-non-reducing centers was measured by the double-hit method as described in Strasser and Tsimilli 1998. According to this method, fluorescence measurement is induced by two subsequent pulses (each of 1 s). The first pulse (denoted as first hit) is given after a dark period which is long enough to ensure the reopening of all reaction centers. It is followed by a second pulse (denoted as second hit) and the duration of the dark interval between two hits was 500 ms. As shown in Table 5.1, the amount of Q_B non-reducing centers increase in salt stressed leaves. In control leaves, the Q_B non-reducing centers were found to be 13% which became 31% in 0.5 M salt treatment.

Table 5.1 Amounts of Q_B non reducing and Q_B reducing centers in response to high salt stress in wheat leaves

NaCl Concentration (M)	% of Q_B non-reducing centers	% of Q_B reducing centers
Control	13 ± 1	87 ± 2
0.1	14 ± 1	86 ± 2
0.2	20 ± 1	80 ± 2
0.3	22 ± 1	78 ± 1
0.4	24 ± 1	76 ± 2
0.5	31 ± 1	69 ± 3
Recovery	31 ± 1	69 ± 2
1.0	34 ± 1	66 ± 3
Recovery	33 ± 1	67 ± 3

In leaves treated with 0.5 M NaCl no recovery was observed and the number of non Q_B reducing centers were almost the same as in the 0.5 M NaCl treated leaves. This result suggests that the damage at the reducing side of PS II was permanent.

5.3.3.2 Effects of High Salt Stress on Antenna Heterogeneity

The kinetics of complementary area of DCMU treated fluorescence induction curve was calculated by the equation [B = ∫(Fm − Ft)dt], where B is the double normalized (between 0 and 1) kinetics of complementary area (Strasser et al. 2000). The B kinetics of the first light pulse was fitted with three exponentials which correspond to three different types of PS II centres (PS II α, β and γ centers) differentiated on the basis of their lifetimes. As shown in Fig. 5.8, in wheat leaves grown in control conditions, the lifetime of the fastest α component was found to be 0.41 ms, contributing to 71% of the total amplitude. The β component was about 3.8 fold slower (life time ~1.34 ms) and contributed to about 27% of the total amplitude. The γ component had longest lifetime (8.79 ms) and contributed only to 2% of the total amplitude in control leaves. With increase in salt concentration the percentage of α centers decrease while that of β and γ centers increase. The relative ratio of α: β: γ centers in control leaves was 71:27:2 while it becomes 33:40:25 in salt stressed leaves (1 M NaCl). It was observed in recovery studies that changes caused by 0.5 M NaCl was almost recoverable but those caused by 1 M NaCl were partially recoverable. These results indicate that the damage caused due to high salt stress in antenna size heterogeneity were not permanent but temporary and largely reversible, suggesting that the α, β and γ centers were interconvertible to most extent.

It is known that the relative variable fluorescence [(Ft − Fo)/(Fm − Fo)] is directly proportional to the number of closed RCs as well as linearly related to the rate at which centers close. This has been explained by the connectivity of PS II units. $PSII_\alpha$ showed a non-exponential (sigmoid) rise while $PSII_\beta$ were characterized by an exponential rise of the time course of complementary area (CA) whereas (Melis and Homann 1976). The non-exponential fluorescence rise of $PSII_\alpha$ is generally reflects energetic connectivity between these PSIIs while the exponential rise for

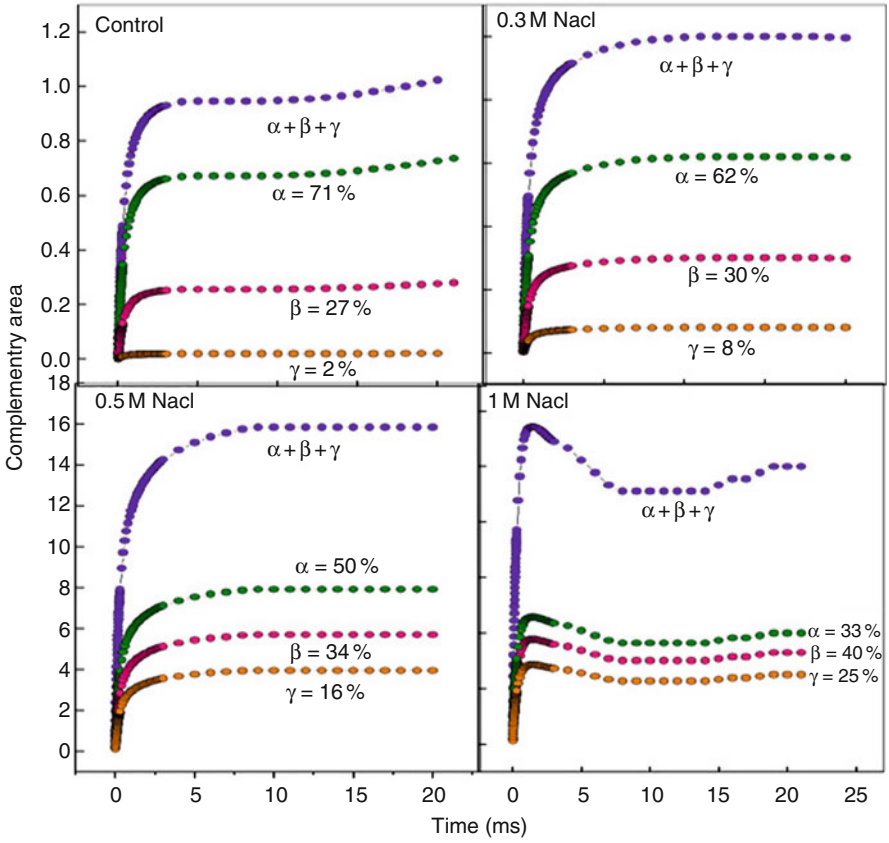

Fig. 5.8 Complementary area curves (linear time scale) showing percentage of alpha (α) and beta (β) and gamma (γ) centers in control and salt-treated wheat leaves

$PSII_\beta$ reflected the mutual energetic separation of these PSII. In salt stressed leaves, the α component (sigmoidal phase) of chlorophyll *a* fluorescence induction curve decreased while the β component (exponential phase) increased (Mehta et al. 2010b). A loss in connectivity also reflects that the fraction of closed RCs i.e. Q_B-non-reducing centers has increased (Strasser and Tsimilli 1998).

The energy pipeline models of the photosynthetic apparatus also help to study the antenna and reducing side heterogeneity of PS II (Krüger et al. 1997; Strasser 1987; Strasser et al. 2000) which help to calculate specific energy fluxes. The parameter ABS/RC demonstrates average antenna size and expresses the total absorption of PSII antenna chlorophylls divided by the number of active (in the sense of Q_A reducing) reaction centers. The parameter TRo/RC refers only to the active (Q_A to Q_A^-) centers (Force et al. 2003). Under high salt stress, the flux ratios ABS/RC, TRo/RC and DIo/RC increased (Mehta et al. 2010b). The ratio of ABS/RC seems to have increased due to inactivation of some active RCs. TRo/RC which represents the

maximal rate by which an exciton is trapped by the RC resulting in the reduction of Q_A, increased indicating that all the Q_A has been reduced but it is not able to oxidize back due to stress. It also means that under high salt stress, the reoxidation of Q_A^- is inhibited so that Q_A cannot transfer electrons efficiently to Q_B. The parameter DIo/RC reflects the ratio of the dissipation of untrapped excitation energy from all RCs with respect to the number of active RCs. Dissipation may occur in various ways such as heat, fluorescence and energy transfer to other systems. It is also influenced by the ratios of active/inactive RCs. The ratio of total dissipation to the amount of active RCs (DIo/RC) increased due to the high dissipation of energy from the active RCs. All these energy flux ratios conclusively suggest that the number of inactive centers have increased due to high salt stress in wheat leaves.

Thus it is concluded that increase in salt concentration caused an alteration in antenna and reducing side heterogeneity of PS II. An increase in the relative amounts of Q_B non-reducing centers and a change in the relative amounts of α, β and γ centers were caused by an increase in the salt concentration. Salt stress led to the conversion of the active α or center into inactive β and γ centers. Recovery studies suggested that the change in antenna size was recovered while the changes in reducing side heterogeneity could not be recovered.

5.4 Conclusion and Future Perspectives

Light and dark reaction of photosynthesis are inhibited by salt stress. High salt stress inhibited the electron transport rates at the donor side of PS II by ~75% while the acceptor side was inhibited by ~25%. Thus under high salt stress, the donor side of PS II is more significantly affected as compared to acceptor side. Inactive PS II centers increase with increasing salt concentration. Most of the damage caused by high salt stress is recovered when the normal conditions were restored. Increase in salt concentration cause an alteration in PS II heterogeneity as well. Increase in salt concentration cause a change in the relative amounts of α, β and γ centers and an increase in the relative amounts of Q_B non-reducing centers. High salt stress leads to the conversion of the active α centers into inactive β and γ centers. The changes in antenna heterogeneity are recovered while changes in reducing side heterogeneity are not recovered while that of. Alteration in heterogeneity of PS II seems to be an adaptive mechanism of plants to face harsh environmental conditions. The structure and function of PS II is manipulated temporarily under high salt stress in the form of change in heterogeneity. Following are the unresolved areas which need to be addressed in the future:

1. Most of the stresses are inter-related and their relationships are complex. Synergistic effects of various stresses are known, for ex. salt stress inhibits the repair of PSII from light induced damage. Such relationships need to be examined in detail and with various stress combinations.
2. Normally, individual stress is monitored in lab conditions that obviously do not simulate the conditions in the field where plant experiences multiple stresses

simultaneously. However in field studies it is difficult to distinguish between effects of individual stress. Study of individual stress in lab and in field conditions should be carried out, interpreted and differentiated very cautiously.
3. Further research to differentiate between the osmotic and ionic components of salt stress is required.

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