“ROLE OF SALICYLIC ACID ON GROWTH, MEMBRANE LIPIDS AND THYLAKOID PROTEINS IN SALT STRESSED SORGHUM (SORGHUM BICOLOR L. MOENCH) PLANTS”

DISSERTATION SUBMITTED TO GOA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR

THE DEGREE OF MASTER OF SCIENCE IN BOTANY

BY

MR. VISHAL ARJUN JAMUNI

UNDER THE GUIDANCE OF

PROF. P. K. SHARMA

DEPARTMENT OF BOTANY

GOA UNIVERSITY

MAY 2022

CERTIFICATE

This is to certify that this dissertation is a bonafide and an authentic record of this research entitled **"Role of Salicylic acid on growth, membrane lipids and thylakoid proteins in salt stressed sorghum (*Sorghum bicolor* L. Moench) plants"**, carried out by **Jamuni Vishal Arjun**, student of Department of Botany, Goa University. This work is carried out under my supervision and guidance at the Plant Physiology laboratory, Department of Botany, Goa University, Taleigao Plateau, Goa, in partial fulfillment of the requirement for the award of 'MASTER OF SCIENCE IN BOTANY' degree of the University and that no part therefore has been presented before in any other degree or diploma of any University.

Prof. Prabhat Kumar Sharma

Dissertation Guide

Department of Botany

Goa University

DECLARATION

I hereby declare that the project entitled **"Role of Salicylic acid on growth, membrane lipids and thylakoid proteins in salt stressed sorghum (*Sorghum bicolor* L. Moench) plants"** submitted for the Master of Science in Botany to Goa University, is carried out by me under the supervision of Dr. P. K. Sharma, Senior Professor, Department of Botany, Goa University. The work is original and had not been submitted in any part or full by me or any other degree or diploma to this or any other University.

Mr. Vishal Arjun Jamuni

Department of Botany

Goa University

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Mr. Vishal Arjun Jamuni

Department of Botany

Goa University

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ABSTRACT

Salicylic acid (SA) is a naturally occurring plant hormone that regulates various physiological and biochemical functions in plants. It also plays an important role in tolerance to biotic and abiotic stresses as signalling molecule. The present study was conducted to assess whether exogenously applied SA as a foliar spray could ameliorate the adverse effects of salt stress on *Sorghum bicolor* L. Moench plants. The plants were grown under normal (Hoagland nutrient solution) and saline (100 and 150 mM NaCl in Hoagland solution) conditions. Different levels of salicylic acid (0, 0.1, 0.25 and 0.5 mM SA) were applied as a foliar spray at day 6, 7 and 8 after sowing (DAS) and all the measurements were taken at 12^{th} day. It was observed that salt stress reduced the growth of plants in both saline conditions as compared to control, however, foliar application of SA resulted in mitigating the salt stress to some extent in concentration dependent manner. We found that 0.1 mM SA treatment was effective in improving photosynthetic rate in sorghum seedlings. Our chromatographic data showed that SA could affect the membrane lipids as qualitative and quantitative changes in glycolipids and phospholipids in salt stressed sorghum plants. No qualitative changes in the thylakoid proteins were observed, however, slight quantitative change in protein profile of thylakoid membranes was observed. Study suggests that SA in a concentration dependent manner can influence Photosynthesis and lipid profile which may have role in mitigating the salt stress in sorghum plants. Further studies are to be carried out to elucidate the detail mechanism.

Keywords: *Sorghum, Salinity, Salicylic acid, Membrane lipids, Thylakoid proteins, Chlorophyll fluorescence, SDS-PAGE*

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INTRODUCTION

1.1. Salinity stress

All land plants experience environmental stress like high temperature, cold, UV, salinity, drought, alkalinity, pathogen infection, etc. Salinity is one of the most serious environmental factors limiting crop productivity because most crop plants are sensitive to salinity. Salinity negatively affects soil fertility and limit growth and productivity. The effects of salinity on plants are mainly due to: (1) decrease in osmotic potential of soil solution and (2) hindering mineral nutrients uptake (Ahmad *et al*., 2012). Saline areas occur where water tables are shallow and salts present in the alluvial clays are mobilized and brought to the surface.

According to reports, approximately 19.5% of all irrigated land and 2.1% of dry land is affected by salt stress (FAO, 2005). The occurrence of salinization in arid and semi-arid regions is due to high evaporation and inadequate amounts of precipitation for considerable leaching. Population explosion also creates an urgent need for the establishment of shelter, recreation centers, industrialization, urbanization, etc. and this affects land availability for mechanized farming. To sustain the nutritional need of the growing human population, agricultural practices must be intensified, irrespective of short supply of rain water, environmental effects of the use of inorganic chemicals (fertilizers), and salinity issues (Dai *et al*., 2011).

1.1.1. Effect of salinity on plant growth

Salt stress directly or indirectly influence plant physiological status by disturbing their metabolism, growth and productivity. Higher salinity levels caused significant reduction in growth parameters like leaf area, leaf length and root and shoot dry weight (Ashrafuzzaman *et al*., 2002). Waterlogging and seawater treatments decrease carotenoids in *Zea mays*

seedlings and induced reduction in chlorophyll and photosynthetic activity (Hill reaction and $^{14}CO_2$-light fixation) (El-Shihaby *et al*., 2002).

Salt stress can increase the accumulation of Na^+ and Cl^- in chloroplasts of crop plants, which leads to decreased growth rate that is often associated with decreased photosynthetic electron transport activity in photosynthesis (Boyer, 1976; Kirst, 1989). Salinity can also change the photosynthetic carbon metabolism, leaf chlorophyll concentration as well as photosynthetic efficiency (Seemann & Critchley, 1985).

1.1.2. Salt induced oxidative stress on lipids and proteins

Prolonged salinity stress leads to oxidative stress which produces reactive oxygen species (ROS) and the quenching activity of antioxidants is disturbed resulting into oxidative damage to biomolecules especially lipid membranes (Ahmad *et al*., 2010; Ahmad & Umar, 2011; Fadzilla *et al*., 1997). ROS are generally generated in mitochondria, chloroplasts and apoplastic space. In chloroplasts, ROS are produced by direct transfer of excitation energy from chlorophyll to produce singlet oxygen or by univalent oxygen reduction of photosystem I in Mehler reaction (Asada, 1999). ROS are highly reactive and in the absence of any protective mechanism they can alter normal metabolism through oxidative damage to lipids, proteins and nucleic acids. This oxidative stress causes ion leakage, membrane peroxidation and damage to nucleic acids, cell membranes and cellular structures (Noreen *et al*., 2009) and ultimately reduces the quality and total yield of the affected crop. The deleterious effects of ROS are limited or scavenged by antioxidant compounds (salicylate, ascorbate, glutathione, tocopherol, etc.) and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) (Foyer & Noctor, 2003). According to Horva´th *et. al*. (2007), it was found that inhibition of catalase, a H_2O_2 scavenging enzyme

by SA plays a major role in the generation of ROS. By increasing H_2O_2 concentration of the tissues, moderate doses of SA may activate the antioxidative mechanisms.

Salinity results in the reduction of photosynthesis, respiration, lipids and protein synthesis in plants (Ahmad & Prasad, 2012). Lipids are main constituent in biological membranes and are heterogenous in chain length, chain saturation and headgroup structure. They play an essential role in selective permeability of the plasma membranes of plant cells and regulating the activity of membrane enzymes, e.g., cation-stimulated ATPases. Lipids also have a direct role in physiological activities such as respiration, energy transport and photosynthesis (Radwan & Mangold, 1976). Recent studies have provided insight into the role of lipids in plant tolerance against environmental stress. The alteration in fatty acid composition is related to mechanism of biotic and abiotic factors in the plants (Upchurch, 2008).

Plants produce proteins in response to biotic and abiotic stresses and many of these proteins are induced by phytohormones such as ABA and salicylic acid (Hoyos & Zhang 2000). Higher salinity levels affect protein solubility, binding, stability and crystallization; stripping off the essential water layer from protein surface and increase hydrophobic interactions causing protein aggregation and precipitation (Sinha & Khare, 2014).

1.1.3. Effect of salinity on water relations

Activity of nutrients in soil solution is affected by high concentrations of salt ions resulting in nutritional disorders in plants (Grattan & Grieve, 1999). Soil salinity affects osmotic stress, decreasing water availability, soil structure, ionic toxicity, changes in the cellular ionic balance (Kirst, 1989). Higher salinity levels usually cause both hypertonic and hyperosmotic stresses ultimately leading to plant death. Accumulation of these ions in leaves through the transpiration flow is a general and long-term process occurring in salt-stress plants (Munns & Termaat, 1986). Nutrient uptake and accumulation are reduced under saline

conditions; however, this depends on the type of nutrients and composition of soil solution (Grattan & Grieve, 1999; Maas & Grattan, 1999; Homaee *et al*., 2002). Although plants selectively absorb potassium over sodium, Na^+-induced K^+ deficiency can develop on crops under salinity stress (Maas & Grattan, 1999).

1.1.4. Adaptation of plant to salt stress

Halophytes can respond and adapt to stress at individua cell level or synergistically as the organism level. Biochemical studies have proved that plants under salinity stress accumulate a number of metabolites, termed as compatible solutes or osmolytes for osmoprotection because they do not interfere with the plant metabolism and the accumulation of these solutes contribute to turgor maintenance in plants. Organic osmolytes can be four major chemical categories: small carbohydrates including sugars, polyols and their derivatives; amino acids such as glycine, proline, taurine and derivatives; methylamines such as glycine betain, methylsulphonium solutes including dimethylsulphonopropionate and Urea, these osmolytes are upregulated to prevent osmotic shrinkage in plants (Yancey, 2005). Tolerance of tissue to accumulated Na^+ or Cl^- ions by ion exclusion (Munns & Tester, 2008); Ion Homeostasis and compartmentalization; Antioxidants-ROS detoxification; programmed cell death are some of the plant strategies to salinity stress.

Salt Overly Sensitive (SOS) stress signaling pathway demonstrates role in ion homeostasis and salt tolerance, consisting of three major proteins SOS1, SOS2 and SOS3. SOS1 gene encodes plasma membrane Na^+/H^+ antiporter and is essential in regulating Na^+ from root to shoot (Gupta & Huang, 2014). SOS2 gene encodes serine/threonine kinase that is activates by salt stress elicited Ca^{2+} signals. The protein consists of a well-developed N-terminal catalytic domain and a C-terminal regulatory domain. SOS3 gene encodes myristoylated Ca^{2+} binding protein consisting of myristoylation site at its N-terminus that plays important

role in conferring salt tolerance (Ishitani *et al*. 2000). C-terminal regulatory domain of SOS2 protein containing a FISL motif (also known as NAF domain), serve as site of interaction for Ca^{2+} binding SOS3 protein. The resulting interaction between SOS2 and SOS3 protein results in activation of kinase, that then phosphorylates SOS1 protein thereby increasing its transport activity.

Increasing salinity is associated with a decrease in auxin, cytokinin, gibberellins and salicylic acid content in plant tissues and an increase in abscisic acid (ABA) content. Changes in hormone levels in plant tissues are thought to be an initial process controlling growth reduction due to salinity (Ghorbani *et al*. 2011). Crop stress tolerance can be improved by a number of means including selection and breeding, genetic modifications and use of osmoprotectants and growth regulating substances (Parida *et al*., 2004). In this regard, attention has come to be focused on the use of plant growth regulators, such as salicylic acid to control a number of stress-induced genes.

1.2. Salicylic acid

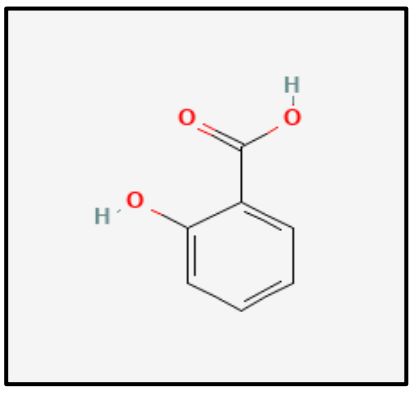


Fig 1.1. Structure of Salicylic acid (SA)

IUPAC Name: 2-hydroxybenzoic acid

Molecular Formula: $C_7H_6O_3$

Molecular weight: 138.12

Salicylic acid (SA) is a plant phenolic hormone-like endogenous growth regulator and considered to be a signaling molecule under stress situation. The word salicylic acid is derived from *Salix* sp., the scientific name for willow tree. SA belongs to a diverse group of plant phenolics usually defined as substances that possess an aromatic ring bearing a hydroxyl group or its functional derivative.

1.2.1. Chemical and physical properties of SA

Free SA is a crystalline powder that melts at 157-l59°C. It is moderately soluble in water and very soluble in polar organic solvents. The pH of a saturated aqueous solution of SA is 2.4 and fluoresces at 412 nm when excited at 301 nm, and this property can be used to detect this compound in a number of plant systems (Raskin *et al*., 1987). The physical properties of SA [pKa = 2.98, log K_{ow} (octanol/water partitioning coefficient) = 2.26] are nearly ideal for long-distance transport in the phloem. Therefore, unless free SA is actively transported, metabolized, or conjugated, it should translocate rapidly from the point of synthesis to distant tissues (Minnick & Kilpatrick, 1939).

1.2.2. SA biosynthesis and transportation

The shikimic acid pathway takes part in biosynthesis of most plant phenolic compounds; it converts simple carbohydrate precursors derived from glycolysis and pentose phosphate pathway to the aromatic amino acids including SA precursor phenylalanine. SA biosynthesis starts from two distinct and compartmentalized pathways that employ different precursor: the phenylamine Ammonia-Lyase (PAL) pathway in the cytoplasm and the Isochorismate synthase (ICS) pathway in the chloroplast (Khan *et al*., 2015). In PAL pathway. Trans-cinnamic acid is produced in the first step, which is subsequently converted to benzoic acid and then to SA by benzoic-acid-2-hydroxylase (BA2H). Benzoic acid is synthesized by

cinnamic acid either via β-oxidation of fatty acids or non-oxidative pathway. Cinnamate 4-hydroxylase (C4H) converts cinnamic acid to coumaric acid. Cinnamic acid produced from phenylalanine by the action of phenylalanine ammonialyase (PAL) is hydroxylated to form coumaric acid followed by oxidation of side chain and further hydroxylated to form SA (Khan, *et al*., 2015). In ICS pathway, chorismate is produced in the first step subsequently producing Isochorismate and then SA by isochorismate synthase (ICS) and isochorismate pyruvate lyase (IPL) respectively (Jayakannan & Bose, 2015).

In long distance transport, only methylated SA form travels in plant tissue both systematically and locally. This transport occurs in phloem within minutes of SA application. Most of the SA synthesized in plants is glucosylated and/or methylated as shown in Fig. 2. Glucose conjugation at the hydroxyl group of SA results in formation of the SA glucoside (SA 2-*O*-β-D-glucoside) as a major conjugate, whereas glucose conjugation at the SA carboxyl group produces the SA glucose ester in minor amounts. These conjugation reactions are catalyzed by the cytosolic SA glucosyltransferases that are induced by SA application or pathogen attack (Lee & Raskin, 1999). Interestingly, SA is also converted to methyl salicylate (MeSA) by an SA carboxyl methyltransferase and this volatile derivate is an important long-distance signal in systemic acquired resistance (Shulaev *et al*., 1997). MeSA can pass through cuticle by diffusion regardless of cuticular pH. After biosynthesis, SA can be transported freely in and out of the cells, tissues and organs. SA movement mediating transporters or SA conjugates between cell organelles remain obscure. SA is further glucosylated to produce MeSA 2-O-b-D-glucose, but this SA-conjugated form is not stored in the vacuole (Dean *et al*., 2005).

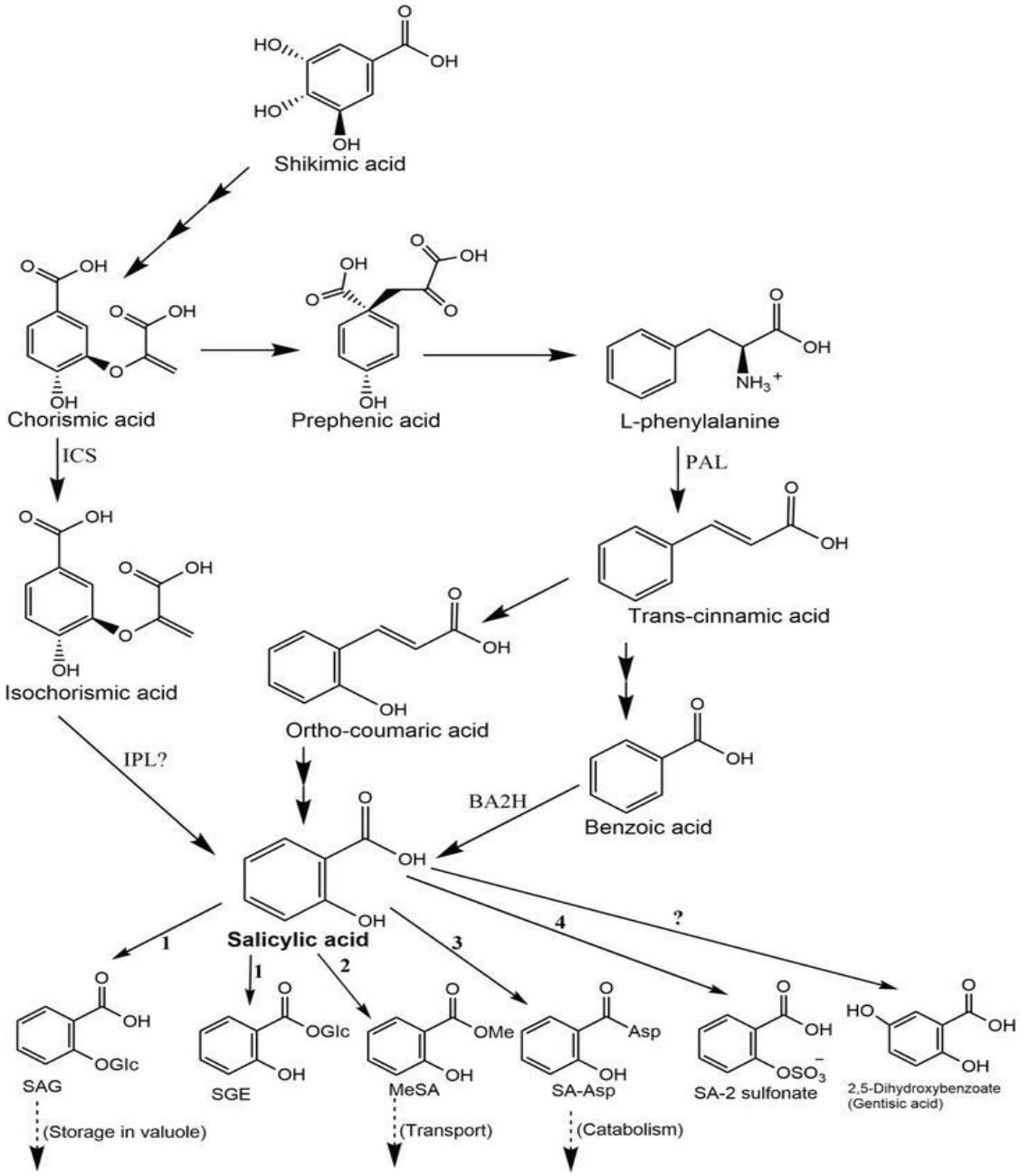


Fig 1.2. Salicylic acid (SA) biosynthesis pathway starting from Shikimic acid and accomplished by two different pathways.

Source: Hasanuzzaman *et al*. 2017

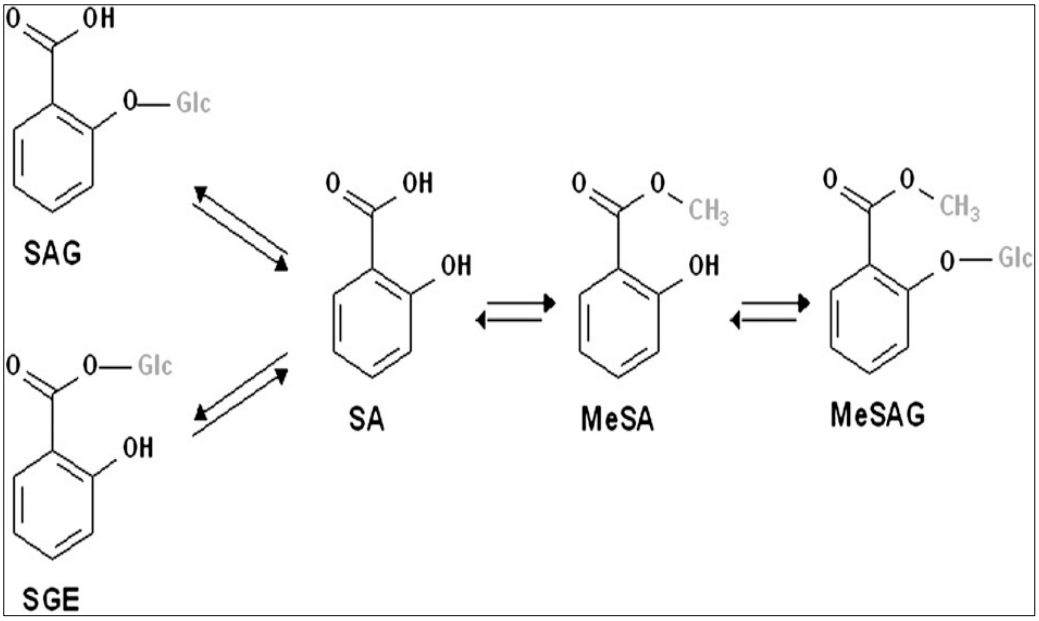


Fig 1.3. Structure of Salicylic acid and its derivatives. SA, salicylic acid; SAG, SA 2-O-β-D-glucoside; SGE, SA glucose ester; MeSA, methyl salicylate; MeSAG, methyl salicylate 2-O- β -D-glucose.

Source: Vicente & Plasencia, 2011

1.2.3. Role of SA against biotic stress

SA is known to play a major role in activation of defense mechanism against pathogen attack (Yalpani *et. al*, 1994; Szalai *et. al*, 2000). SA mediated oxidative burst that leads to cell death in hypersensitive response and in signal transduction pathway leading to gene expression. SA induces local and systemic acquired resistance (SAR) against the microbial pathogens (Shirasu *et al*., 1997) as well as considered to be plant secondary signaling molecule under stress conditions (Cameron, 2000). As a result of hypersensitive response (HR), necrotic lesions are developed in infected tissue accompanying SA induction (Malamy *et al*., 1990; Metraux *et al*., 1990) and subsequent activation of pathogenesis-related (PR) protein genes (Bol *et al*., 1990; Ohshima *et al*., 1990).

Pathogen attack induce SA synthesis by upregulating the Isochorismate Synthase 1 (ICS1) expression which encodes a key enzyme during SA production. Master regulatory protein of SA dependent defense response is the NPR1 (non-expressor of PR protein 1) which transcriptionally co-activates the PR gene expression. When SA concentration is low, the oligomeric NPR1 is in oxidized form localized in the cytoplasm (Jayakannan & Bose, 2015). Accumulation of SA leads to alteration in the cellular redox status that activates NPR1 by converting it into active monomer (Fu *et al*., 2012). SA when bound to NPR3 and NPR4 triggers this process of conversion of inactive oligomeric NPR1 to active monomeric NPR1, which are then transported into the nucleus, interacting with specific transcription factors and activating SA responsive PR genes (Dong, 2004). Prevention of SA accumulation by inhibiting ICS1 is done in presence of NPR1 in the nucleus by regulating the defense genes downstream playing a crucial step in the SA termination signaling followed by successful induction of defense response (Zhang *et al*., 2010).

1.2.4. Role of SA against abiotic stress

Interestingly, SA is does not only regulate the resistance to biotic stresses, but also provide tolerance to various abiotic stresses. The underlying mechanisms of SA-induced abiotic stress tolerance include (1) accumulation of osmolytes, such as glycinebetaine, proline, soluble sugars and amines, which can help maintain osmotic homeostasis, (2) regulation of mineral nutrition uptake, (3) enhanced reactive oxygen species scavenging activity, (4) enhanced secondary metabolite production, such as terpenes, phenolics, and compounds with nitrogen (alkaloids) and sulfur (glutathione, phytoalexins) and (5) regulation of other hormone pathways (Horváth *et al*. 2007; Khan *et al*. 2015). According to Hong and Hwang (2005), transgenic overexpression of some PR genes also enhanced the tolerance to different abiotic stresses in pepper and *Arabidopsis* plants.

SA could act as cell stress protector and also considered as plant growth regulator which has important role in regulating various plant physiological processes including photosynthesis (Fariduddin *et. al*, 2003; Waseem *et. al*, 2006; Arfan *et. al*, 2007). SA-induced increase in growth could be related to SA-induced enhancement in net photosynthetic rate under salt stress, particularly at 200mg/L SA level (Noreen & Ashraf, 2008).

Earlier reports suggest that SA could overcome the damaging effects of heavy metals in rice (Mishra & Choudhuri, 1999), drought and salt stress in wheat (Waseem, *et. al*, 2006; Arfan, *et.al*, 2007). Therefore, SA being an oxidant could be linked to oxidative stress. However, SA cannot induce abiotic stress tolerance in all types of plants or in other words the effectiveness of SA in inducing stress tolerance depends upon type of species or concentration of SA applied (Waseem *et. al*, 2006; Arfan *et. al*, 2007; Borsani *et. al*, 2001). Low concentration of SA (50 µM) exhibited growth-promoting and high concentration of SA (250 µM) exhibited growth-inhibiting properties in *Matricaria chamomilla* plants (Kova´cˇik *et. al*, 2009).

Enhancement of growth after SA supplementation has been reported in many plants, such as wheat (Shakirova *et. al*, 2003), soybean (Gutierrez-Coronado *et.al*, 1998) and maize (Gunes *et.al*, 2007).

1.2.5. Potential mechanism underlying SA mediated plant stress tolerance

SA plays an important role in plant growth and development as there is evidence that this hormone regulates physiological processes such as seed germination, vegetative growth, photosynthesis, respiration, thermogenesis, flower formation, seed production, senescence, and a type of cell death that is not associated with the hypersensitive response. In addition, SA also contribute to maintaining cellular redox homeostasis through the regulation of antioxidant enzymes activity (Durner and Klessig, 1995) and induction of the alternative

respiratory pathway (Moore *et al*., 2002), and regulating gene expression by inducing an RNA dependent RNA polymerase that is important for posttranscriptional gene silencing (Xie *et al*., 2001).

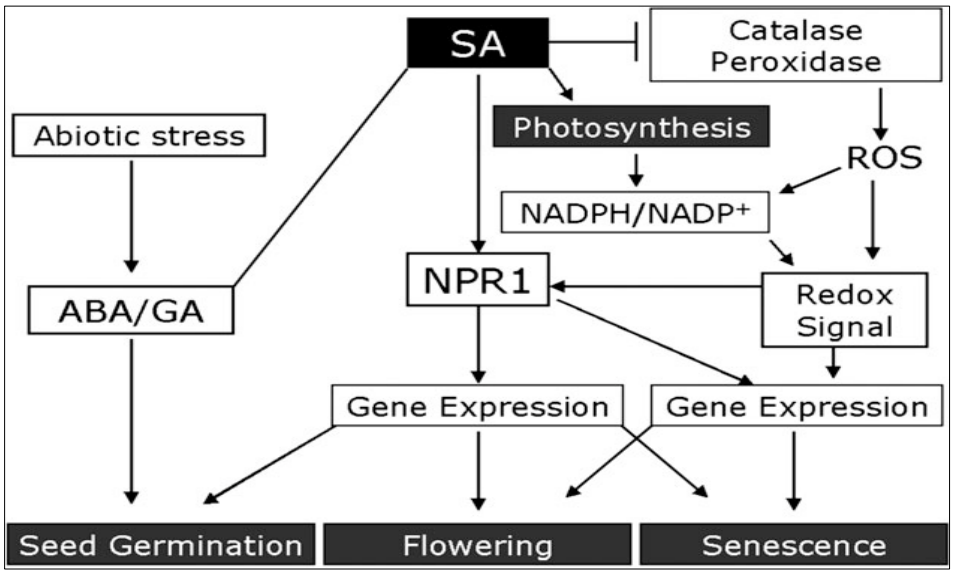


Fig 1.4. Model of salicylic acid (SA) function in plant growth and development.

Source: Vicente & Plasencia, 2011

SA induces several genes responsible for encoding chaperone, heat shock proteins (HSPs), antioxidants, and secondary metabolites [sinapyl alcohol dehydrogenase (SAD), cinnamyl alcohol dehydrogenase (CAD), and cytochrome P450] (Jumali *et al*., 2011). Also, involvement of SA in mitogen-activated protein kinase (MAPK) regulation, and in the expression and activation of *non-expressor of pathogenesis-related genes 1* (*NPR1*) has been reported (Chai *et al*., 2014). Nevertheless, the transcriptional reprograming that occurs during the plant defense response against biotic and abiotic stress was reported to be modulated by SA, where the transcription of different sets of defense genes can be controlled in a spatio-temporal manner via SA-mediated mechanisms (Herrera-Vásquez *et al*., 2015).

1.2.6. Exogenous application of SA

Several other studies also support a major role of SA in modulating the plant response to several abiotic stresses (Senaratna *et al*., 2000). The exogenous application of SA results in plant tolerance to many biotic and abiotic stresses, including fungi, bacteria, viruses (Delany *et al*., 1994), chilling, drought and heat (Senaratna *et al*., 2003). Bezrukova *et al*. (2001) reported that SA induces increased resistance to water deficit in tomatoes and to low and high temperature in bean plants. These functions may play a key role in plant tolerance to salt stress. Both salt and osmotic stress leads to oxidative stress and severe impairment of seedling survival.

The exogenous SA application to plants has diverse physiological effects, such as the inhibition of dry mass accumulation (Schettel & Balke, 1983), promotion of stomatal closure (Larque-Saavedra, 1979) and inhibition of ethylene synthesis (Leslie & Romani, 1986). SA is involved in the initiation of an alternative respiratory pathway (Elthon *et al*. 1989) through the regulation of a specific nuclear gene encoding the alternative oxidase protein in *Sauromatum guttatum* (Rhoads & McIntosh, 1991). Moreover, SA inhibited the synthesis of total soluble protein, the synthesis of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Pancheva & Popova, 1998) and Hill activity and minor changes were reported in the kinetics of O_2 evolution (Maslenkova & Toncheva, 1998) in association with the changes in net photosynthetic rate, chlorophyll and nitrate reductase activity (Fariduddin *et al*., 2003). The growth of barley seedlings improved when the grains were pre-treated with SA (Pancheva *et al*., 1996).

Previous studies have shown that exogenous SA application effectively regulates osmotic potential and plays a vital role in sustaining plant growth under osmotic stress. SA treatment resulted in retarding ethylene synthesis, interfering with membrane depolarization,

stimulating photosynthetic machinery, increasing chlorophyll content as well as blocking wound response in soybeans (Leslie & Romani, 1988; Zhao *et al*., 1995).

Exogenous application of SA has shown to affect a large variety of processes in plants, including stomatal closure, seed germination, ion uptake and transport, membrane permeability, fruit yield and glycolysis (Cult & Klessig, 1992).

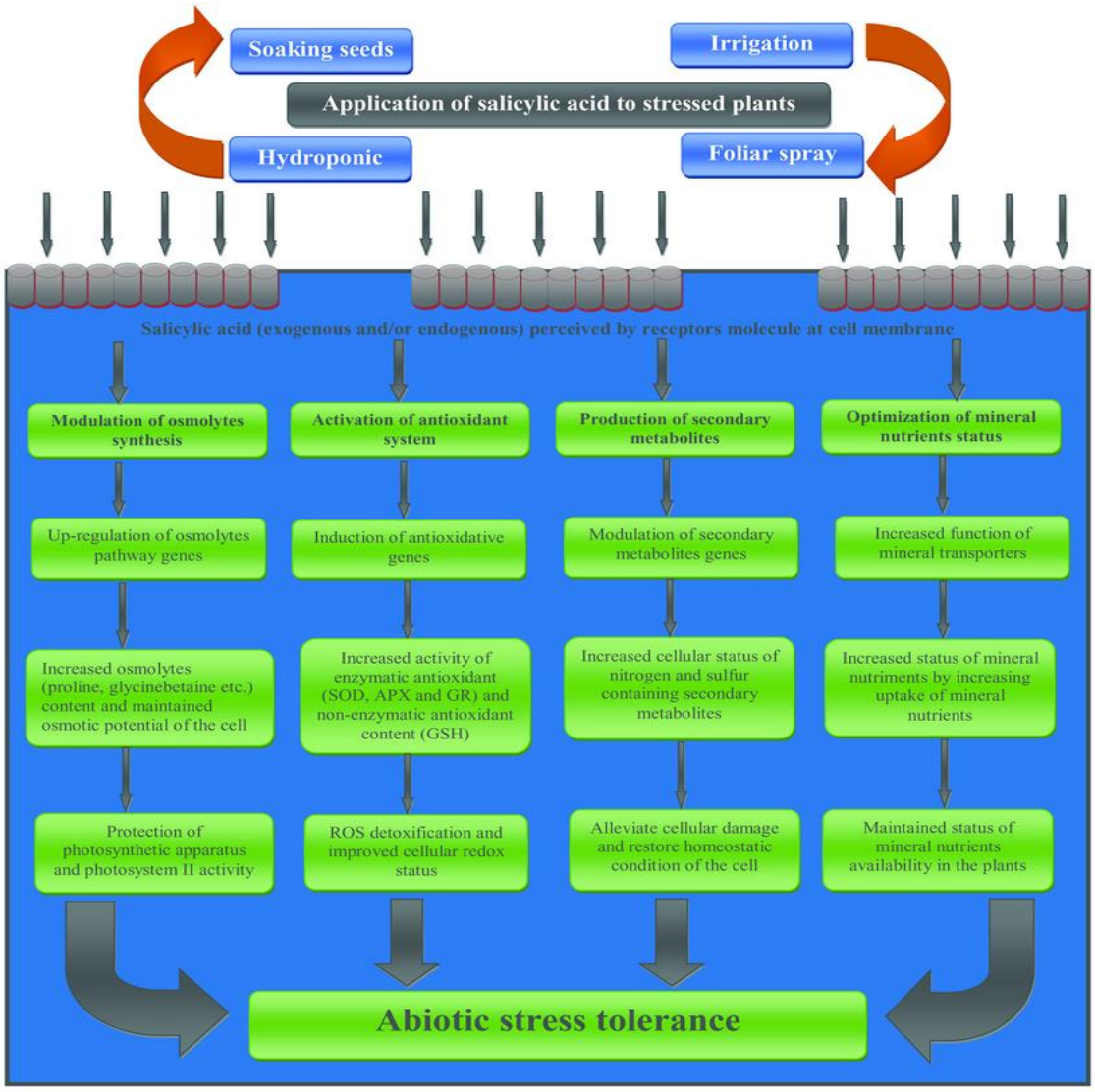


Fig 1.5. Descriptive model representing potential mechanisms underlying SA-mediated plant abiotic stress tolerance.

Source: Khan *et al*. 2015

1.3. Sorghum

Sorghum bicolor L. Moench. also called great millet, Indian millet, a C4 plant is the most widely grown summer crop because of its edible starchy seed. Sorghum is the fifth most important multipurpose cereal crop belonging to the grass family Poaceae (Ighbal, 2015). The plant likely originated in Africa and important food crop in Africa, Central America and South Asia. It is well adapted to arid and semi-arid regions because of its abiotic tolerance such as drought and salinity (Marsalis *et al*., 2010). Ulery and Ernst (1997) reported that sorghum is suitable for providing forage because of having high production amount per unit and the more efficiency of using water. The degree of salinity tolerance is also known to be moderately tolerant crop with the almost salinity tolerance of 6.8 dSm^{-1}, however, it varies in different varieties. Sorghum is sensitive to salt due to increased metabolic energy cost and reduced carbon gain.

It has economically important potential uses such as food (grain), feed (grain and biomass), fuel (ethanol production), fiber (paper), fermentation (methane production) and fertilizer (utilization of organic by products). *Sorghum* is rich in carbohydrates, with 10% proteins and 3.4% fat and contains calcium and small amounts of iron, vitamin B1, and niacin (Carvalho *et al*., 2002).

Sorghum, being a moderately salt tolerant plant may result into considerable reduction in its growth at higher salinity levels, therefore, improvement of its salinity tolerance by any means is a great challenge for plant scientists. However, no much work has been done on effect of salinity on membranes with respect to lipids composition and chloroplast membrane proteins. Although a variety of strategies are currently ongoing to counteract the salinity problems, application of SA can be considered as one of the convenient and cost-effective approaches of overcoming the salinity threat. Thus, in the present investigation, we have

assessed the ameliorative effect of exogenous application of SA on the adverse effects of salinity stress on sorghum.

GAPS IN KNOWLEDGE

The role of salicylic acid on lipids and thylakoid proteins of salt stressed plants is lacking. These are important parameters related to the photosynthetic efficiency of crop plants in terms of productivity. Therefore, the current study was conducted to reveal the effects of SA on lipids and thylakoid proteins and relate it to photosynthetic efficiency in sorghum, a C4 plant.

SIGNIFICANCE OF THE WORK

Our study determines the role of salicylic acid to alleviate the effects of salt stress on sorghum (*Sorghum bicolor* L. Moench) plants. The physiological and biochemical studies revealed the possible role of SA required to maintain the membrane integrity by altering lipids and thylakoid proteins composition of leaves to make the plants tolerant to salt stress.

In previous studies, the effects of SA were investigated on either salinity or temperature stress only and focused on changes in only one or two physiological parameters. In our study, we integrated the interactions between salinity stress and range of SA concentrations and reported the effects on a wide range of physiological and biochemical traits in sorghum crop at establishment stage.

OBJECTIVES

The aim of the current investigation was to study the effect of range of exogenously applied salicylic acid (SA) on salt stressed sorghum (*Sorghum bicolor* L. Moench) plants with 100 mM and 150 mM NaCl.

The following parameters were studied in detain in *Sorghum bicolor* L. Moench with reference to the effect of SA.

- Photosynthetic efficiency
- Qualitative and quantitative determination of membrane lipids and
- Qualitative and quantitative changes in thylakoid proteins.

MATERIALS AND METHODS

2.1. Plant material and chemicals

Sorghum (*Sorghum bicolor* L. Moench) was selected for this study, because it is categorized as a moderately salt tolerant plant. A homogenous lot of seeds of MHS-51 variety was purchased from Goa Bagayatdar Sahakari Kharedi Vikri Saunstha Maryadit, Goa and stored in desiccator. The free SA powder (Merk, tissue culture grade) was purchased from HiMedia, Mumbai.

2.2. Growth conditions and treatment

The experimental seeds were washed under running tap water and surface sterilized using 3% sodium hypochlorite ($NaHCO_3$) solution for 10 min. The seeds were washed thoroughly with tap water to remove traces of disinfectant and afterwards soaked in distilled water for 4 hours. The surface sterilized healthy seeds were sown in plastic pots filled with sterile vermiculite medium and irrigated with full strength Hoagland's nutrient solution (Table 1) containing 0, 100, and 150 mM NaCl. Salicylic acid (SA; 2-hydroxybenzoic acid) was dissolved in distilled water and different concentration solutions were prepared *viz*. 0, 0.1, 0.25 and 0.5 mM SA. A constant volume of about 60 sprays was sprayed as foliar application using manual automizer at day 6, 7 and 8 after sowing (DAS). The plant growth conditions were maintained at light intensity (PPFD) of 500±25µmol m^{-2} s^{-1}, 16 hours photoperiod, temperature at 25±2℃ and relative humidity (RH) at 70 ±5%. The pots were arranged in factorial design in Randomized Complete Block Design (RCBD) with three replicates. The plants were harvested at 12 days after sowing from all three different treatments and used for various analysis.

Table 2.1. The constituents of Hoagland nutrient solution (400X)

Components	Stock solution (g/L)
Macronutrients	
2M KNO_3	202 g/L
2M $Ca(NO_3)_2$ x $4H_2O$	236 g/0.5L
Iron (Spirit BQ iron chelate)	15 g/L
2M $MgSO_4$ x $7H_2O$	493 g/L
1M MH_4NO_3	80 g/L
Micronutrients	
H_3BO_3	2.86 g/L
$MnCl_2$ x $4H_2O$	1.81 g/L
$ZnSO_4$ x $7H_2O$	0.22 g/L
$CuSO_4$	0.051 g/L
H_3MoO_4 x H_2O Or Na_2MoO_4 x $2H_2O$	0.09 g/L 0.12 g/L
1M KH_2PO_4	136 g/L

pH 6.4 ±2 at room temperature 28±2 ℃

The experiments were repeated thrice with three replicates of each treatment.

2.3. Chlorophyll fluorescence measurements

The Chlorophyll fluorescence (Fv/Fm ratio), which is an indicator of photosynthetic efficiency, was determined using a pulse amplitude modulation chlorophyll fluorometer (PAM 101, Walz Germany) according to the method of Sharma *et al.* (1997). Measurements were made on upper (adaxial) surface of $1cm^{-2}$ leaf disk, which was dark adapted for 10 min prior to the measurements at room temperature to inhibit light dependent reactions by oxidizing PSII electron acceptor molecules. The dark-adapted leaf was exposed to very low irradiance with a light intensity of 3-4 μmol m^{-2} s^{-1} (modulated light) to measure initial fluorescence (F_0). Maximum fluorescence (Fm) was determined by exposure to a saturating pulse of white light of ≈ 4000 μmol m^{-2} s^{-1} for 0.06 s. The coefficient of Variable fluorescence (Fv) was calculated by subtracting F_0 from Fm (**Fv = Fm – F_0**) along with maximum quantum yield (**Fv/Fm**) ratio. After this, the leaf was exposed to actinic light of ≈ 600 μmol m^{-2} s^{-1} and allowed to reach a steady state terminal fluorescence yield (Fs). Another short burst of saturating light at Fs was used to measure secondary maximum chlorophyll fluorescence (Fm'). After reaching to a steady state again, the leaf was exposed to infra-red radiation (15 μmol $m^{-2}s^{-1}$ for 5 s) to measure minimum fluorescence F_0'. The following photochemical activities were calculated as given below. The PSII efficiency (ΦPSII) was calculated as **(Fm' - Ft / Fm')**

$$\boldsymbol{PSII\ efficiency\ (\Phi PSII)} = \frac{\boldsymbol{Fm' - Ft}}{\boldsymbol{Fm'}}$$

2.4. Extraction of total lipids

The total lipids were extracted according to the method described by Turnham and Northcote (1984). The freshly harvested 2g of leaf tissue was chopped into fine pieces and soaked overnight in 70% ethanol to remove pigments. Ethanol was drained and the tissue was then boiled in ample amount of isopropanol solvent on flame using spirit lamp for 5-10 minutes to inhibit lipase activity, it was then cooled and drained. The tissue was ground into fine powder using liquid nitrogen and homogenized in chloroform/methanol (1:2 v/v) thoroughly containing a few crystals of butylated hydroxytoluene (BHT; 2.6 di-tert-butyl-p-cresol) as an antioxidant agent and the final volume was made to 15mL. The extract was transferred into a separating funnel and left overnight at room temperature for separation. The upper layer was collected using micropipette and the residue was washed with chloroform/methanol (1:1 v/v). The lipid extract was centrifuged for 5 minutes at 2000×g, 4℃ to get rid of cell debris. The supernatant (~15 mL) was taken in a separating funnel and to it 4 mL distilled water and 5 mL chloroform was added and mixed followed by adding 5 mL of 0.88% potassium chloride (KCl). The mixture was shaken vigorously for 5 min with constant removing of cap to release pressure and allowed to separate for 30 min. The solvent phase (lower layer) of chloroform contains appreciable amounts of extracted lipids was collected in a glass screw capped tubes by releasing the knob and used for further quantitative and qualitative analysis.

2.4.1. Total lipids determination

The total lipids extracts were used for estimation of glycolipids and phospholipids.

***Estimation of glycolipids*:** The total sugars present in the total lipids extract were determined by phenol-sulphuric method as described by Kushawa and Kates (1981). 100µL of total

lipids extract was taken in a test tube and the final volume was made to 2mL using distilled water. 1mL of 5% phenol solution was added and the contents were mixed well. Later, 5mL of conc. H_2SO_4 acid was added and again the contents were mixed properly. The mixture was incubated in a hot water bath at 100℃ for about 5 min. The tube was allowed to cool down at room temperature for 15 minutes. The development of orange colour was read at 490 nm against a reagent blank. The amount of sugars present in the extracted total lipids sample was calculated using a standard curve established with 1mM glucose solution.

***Estimation of phospholipids*:** The phosphorous level in phospholipids present in the lipids extract were determined by Bartlette method according to Christie (1982). Lipid extract of 100µL was taken in a test tube and the final volume was made to 4.3mL using distilled water. To the tubes, 0.5mL of ammonium molybdate solution was added. The mixture was incubated at room temperature for about 10 minutes. Later, 0.2mL of freshly prepared ANSA (1-amino-2-naphthol-4-sulphonic acid) solution was added to the mixture and the tube was placed in hot water bath at 100℃ for 20 min, cooled and the absorbance of the contents was read at 660nm against a reagent blank. 1mM Potassium dihydrogen phosphate solution was used as standard and using the calibration curve the amount was calculated.

2.4.2. Thin Layer Chromatography (TLC)

Thin layer chromatography (TLC) was carried out for the extracted total lipids extract according to the method as described by Liljenberg and Von Arnold (1987). The extracted lipids sample was completely dried under nitrogen gas. The dried lipids extract was redissolved in 50µL of chloroform and the separation of lipids was done using sheet of aluminium foil coated TLC Silica gel 60 (Merck, Germany). 2µL of redissolved lipid sample was loaded onto a plate while drying under fan. The two different eluting solvents were of

the following --- chloroform: methanol: glacial acetic acid: water in the ratio of 85:15:10:3.5 v/v for phosphoglycolipids and diethyl ether: water in ratio of 90:1 v/v for neutral lipids. The loaded sheets were placed in the solvent systems for about 10 minutes to allow the components to separate based on their affinity towards mobile and stationary phases. Once the solvent had run up to a certain level on stationary phase and separation of mixture were attained, the plates were removed and kept in iodine vapour chamber for detection. The developed spots of dark brown colour were observed and based on the distance the Rf value was calculated using the formula given below. The respective lipids were identified by the Rf values relative to authentic standards.2

$$\boldsymbol{Retention\ factor\ (Rf)value} = \frac{Distance\ travelled\ by\ the\ sample\ (in\ cm)}{Distance\ travelled\ by\ the\ solvent\ (in\ cm)}$$

2.5. Thylakoid protein profile

2.5.1. Chloroplast isolation

The intact chloroplasts were mechanically isolated according to the method given by Sharma & Singhal (1992). The freshly harvested 2g of leaves tissue were finely chopped and homogenized in 50mL grinding media (GM) containing sorbitol, Tricine, Na_2 EDTA, $MgCl_2$, $MnCl_2$ (pH 7.2). The homogenate was filtered using 8 layers of muslin cloth. The filtered extract was centrifuged for 5 minutes at 5000×g, 4℃ and the supernatant was discarded and pellet was washed and resuspended using resuspending media (RM) containing sorbitol, Tricine, Na_2 EDTA, $MgCl_2$, $MnCl_2$, NaCl (pH 7.8) making final volume to 1mL in an Eppendorf tube. All the steps were performed at 4℃. Chlorophyll concentration was determined according to Arnon (1949) taking OD at 652nm.

2.5.2. Preparation of protein samples

500µL of resuspended chloroplast was mixed in 200µL of 4X SDS proteins solubilizing buffer (1M Tris-HCl at pH 6.8, SDS, 1% Bromophenol blue, 100% Glycerol and β-mercaptoethanol) for 30 minutes in ice and stores overnight for complete solubilization of protein samples at 4℃. The solubilized samples were incubated in dry bath at 90℃ for 5 min and transferred to ice immediately. The samples were then centrifuged at 10,000×g, 4℃ for about 30 seconds to get rid of debris. The supernatant was collected in an eppendorf tube and used it for protein estimation and SDS-PAGE. The protein concentration was determined according to the method of Bradford (1976) using bovine serum albumin (BSA) as a standard.

2.5.3. SDS-PAGE

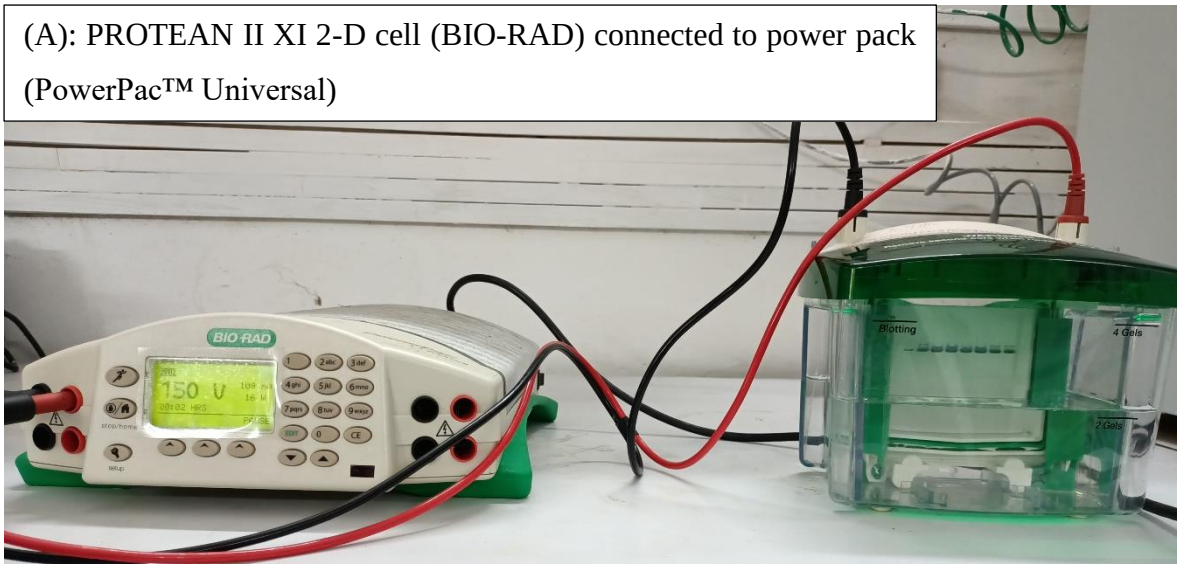
Based on the protein concentration of samples, 15µg equivalent of chloroplast protein was electrophoresed on 15% resolving and 4% stacking polyacrylamide gels and the buffer system of Laemmli (1970) was used. The composition of resolving and stacking gels is mentioned in Table 2.2. The solubilized proteins centrifuged at 1,000 rpm for about a min prior to loading on the gel. The protein samples were run at 150V for 30 min with a PROTEAN II XI 2-D cell (BIO-RAD) connected to power pack (PowerPac™ Universal). After separation of proteins based on their molecular density, the gels were subsequently washed with distilled water and then poured water out and stained with staining solution containing 0.2% Coomassie Brilliant Blue R-250 (Sigma), 10% GAA and 30% ethanol for overnight on Gel Rocker (NeoLab) for complete staining of protein bands. This was followed by removal of excess stain using destainer solution containing 10% GAA and 30% ethanol for 4 hours and finally the gels were washed in distilled water. The gels were scanned

using Bio-Rad Gel Densitometer (GS 800) and the separated protein bands were analysed using Quantity One software from Bio-Rad. The molecular weights of different protein bands were determined with the help of standard protein markers (Himedia, pre-stained 10-245 kDa).

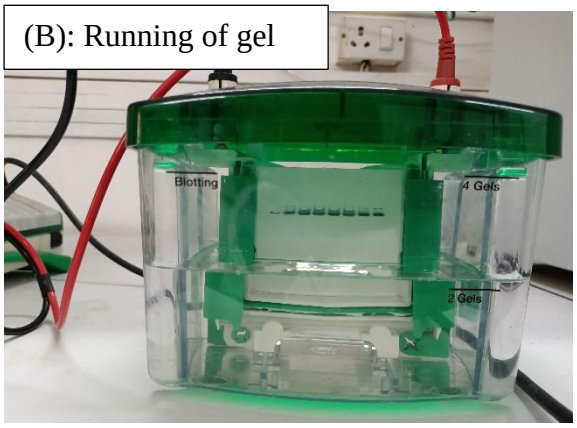
Table 2.2. Composition of SDS-PAGE gels (Mini-Protean II) according to Laemmli (1970).

Composition	**Resolving Gel (15%)** **2 Gels**	**Stacking Gel (4%)** **2 Gels**
40% Acrylamide Stock	3.0 mL	500 µL
20% SDS	200 µL	100 µL
1.5 M Tris-HCl, pH 8.8 (RG Buffer)	2.6 mL	----
1.0 M Tris-HCl, pH 6.8 (SG Buffer)	-----	500 µL
Milli-Q water	2.2 mL	3.9 mL
10% Ammonium Persulfate	20 µL	48 µL
TEMED	8 µL	8 µL

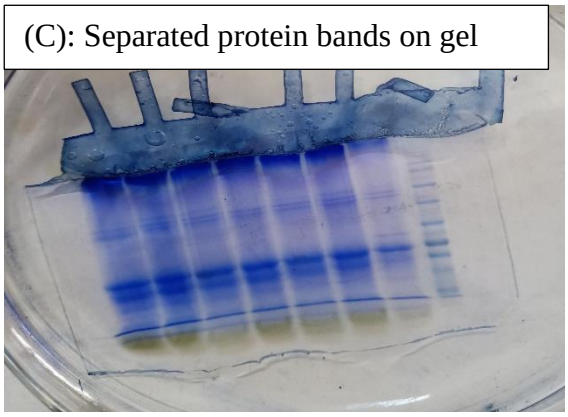
(A): PROTEAN II XI 2-D cell (BIO-RAD) connected to power pack (PowerPac™ Universal)



(B): Running of gel



(C): Separated protein bands on gel



(D): Bio-Rad Gel Densitometer (GS 800)

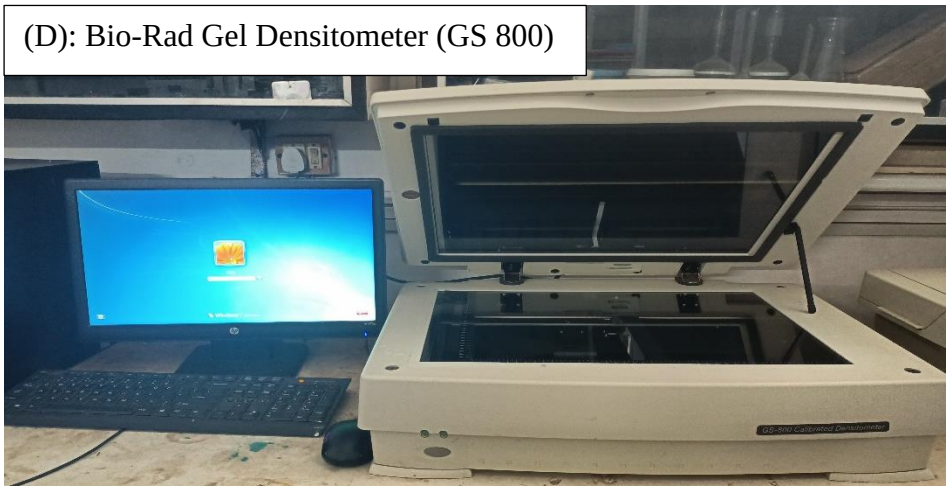


Fig 2.1. SDS-PAGE set up. A-D shows series of events from running of gel till visualization of bands.

RESULTS

3.1 Morphological study

Fig. 3.1. Effect of SA concentrations on plant growth in NaCl treated Sorghum plants.

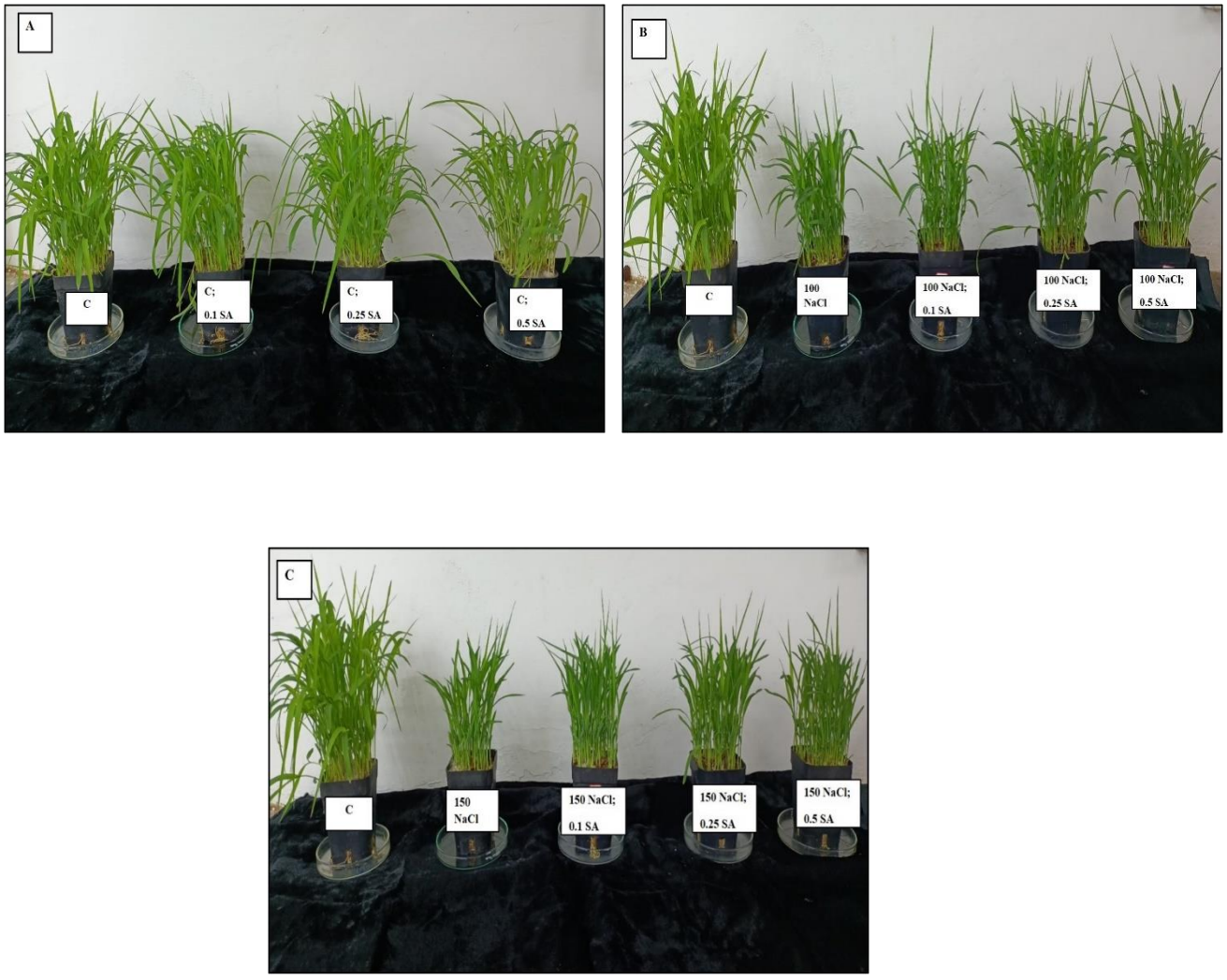


Fig. 3.1. Photographic images of *Sorghum bicolor* L. Moench plants grown under salinity stress and sprayed with SA. A: Plants grown in Control (Hoagland nutrient solution); B: Plants grown with 100 mM NaCl treatment; C: Plants grown with 150 mM NaCl treatment. All the three treatments were sprayed with 0, 0.1 mM SA, 0.25 mM SA and 0.5 mM SA.

Phenotypic observations were done to study the effect of SA concentrations on the salt stressed sorghum plants. Control plants grown in Hoagland nutrient solution, spraying SA increased the foliage growth. Plants treated with 0.25 mM SA showed better growth as compared to other SA treatments **(Fig. 3.1. A)**. With increase in salinity concentrations the growth of plants reduced drastically. However, spraying SA improved the plant growth in both saline conditions and the best results were obtained upon application of 0.25 mM SA **(Fig. 3.2. B-C)**. Overall, 0.25 mM SA promised to be a better dose in terms of increase in foliage growth.

3.2. Chlorophyll Fluorescence

Table 3.1. Effect of concentrations of SA on Fv/Fm ratio (Photosynthetic efficiency of PSII) and e'PSII (Quantum efficiency of PSII) in NaCl treated Sorghum plants; ± represents the standard deviation.

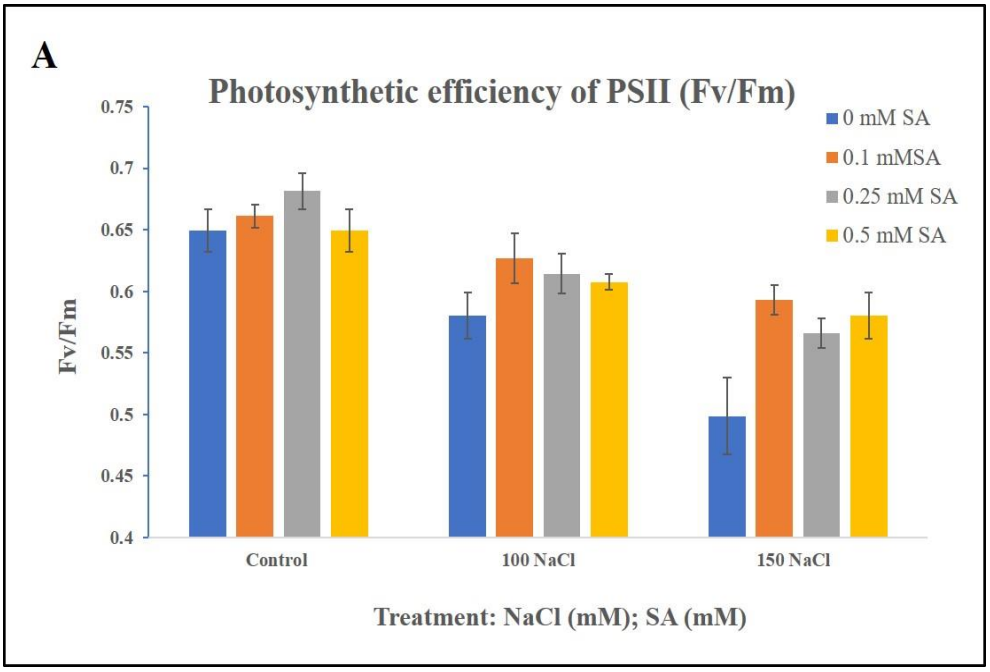
Treatment	Fv/Fm	% Change	e'PSII	% Change
Control	0.64±0.017	0	0.59±0.038	0
Control; 0.1 mM SA	0.66±0.009	1.80	0.60±0.047	1.70
Control; 0.25 mM SA	0.68±0.014	4.92	0.61±0.015	4.23
Control; 0.5 mM SA	0.64±0.017	0	0.55±0.024	-6.27
100 mM NaCl	0.58±0.018	-10.61	0.55±0.029	-5.75
100 mM NaCl; 0.1 mM SA	0.62±0.020	7.96	0.60±0.022	8.76
100 mM NaCl; 0.25 mM SA	0.61±0.016	5.81	0.57±0.030	3.34
100 mM NaCl; 0.5 mM SA	0.60±0.006	4.63	0.58±0.009	4.46
150 mM NaCl	0.49±0.031	-23.20	0.47±0.019	-19.26
150 mM NaCl; 0.1 mM SA	0.59±0.012	18.90	0.53±0.014	12.90
150 mM NaCl; 0.25 mM SA	0.56±0.012	13.43	0.52±0.027	10.85
150 mM NaCl; 0.5 mM SA	0.58±0.018	16.40	0.51±0.032	8.52

Chlorophyll fluorescence was taken to study various photosynthetic parameters such as Fv/Fm (Photosynthetic efficiency of PSII) and e'PSII (Quantum yield of PSII) (Table 3.1).

Photosynthetic efficiency (Fv/Fm) decreased with increase in salt stress. Sorghum seedlings grown with 100 mM NaCl and 150 mM NaCl showed **decrease** in Fv/Fm by **10.61%** and **23.21%** respectively as compared to the non-saline plants. In control plants grown, application of 0.1 mM SA and 0.25 mM SA showed **increase** in the Fv/Fm ratio by **1.80%** and **4.92%** respectively as compared to absolute control. However, plants sprayed with 0.5 mM SA showed no change in Fv/Fm as compared to the absolute control plants. Plants grown in 100 mM NaCl, spray with 0.1 mM SA **elevated** the Fv/Fm value by **7.96%** compared to plants without SA application. However, application of 0.25 mM SA and 0.5 mM SA **increased** the value by **5.81%** and **4.64%** respectively as compared to saline control. Likewise, in plants grown with 150 mM NaCl, the Fv/Fm ratio **increased** with 0.1 mM SA treatment by **18.91%** in comparison to plants without SA. However, 0.25 mM and 0.5 mM SA application **increased** the value by **13.43%** and **16.40%** respectively. Our data presented in **Fig 3.2. A** shows plants sprayed with **0.1 mM SA** had **maximum** photosynthetic efficiency for 100 mM NaCl as well as 150 mM NaCl stress, while concentration of SA of 0.25 mM and 0.5 mM showed minimum effect to mitigate salt stress.

The **Quantum efficiency of PSII (e'PSII)** decrease with increase in salinity stress. Plants grown in 100 mM and 150 mM NaCl, the e'PSII was seen to **decrease** by **5.75%** and **19.26%** respectively as compared to control. Application of 0.1 mM SA and 0.25 mM SA **improved** the quantum efficiency by **1.70%** and **4.24%** respectively in non-saline plants as compared to unsprayed plants. However, application of 0.5 mM SA **reduced** the efficiency by **6.28%** in comparison to non-saline control. In 100 mM NaCl treated plants, 0.1 mM SA spray **improved** the quantum efficiency by **8.76%** compared to respective saline control. The plants sprayed with 0.25 mM SA and 0.5 mM SA also showed **increased** efficiency by

3.34% and **4.46%** respectively in comparison to saline control. Similar trend was observed in plants grown with 150 mM NaCl. Plants sprayed with 0.1 mM SA **increased** the quantum efficiency of PSII by **12.90%** in comparison to saline control. Also, spraying the plants with 0.25 mM SA and 0.5 mM SA **improved** the efficiency upto **10.85%** and **8.53%** respectively in comparison to saline control. Our data (**Fig. 3.2. B)** portrays that **0.1 mM** SA **increased** the quantum efficiency of PSII as compared to other SA concentrations in both 100 mM NaCl and 150 mM NaCl treated plants.



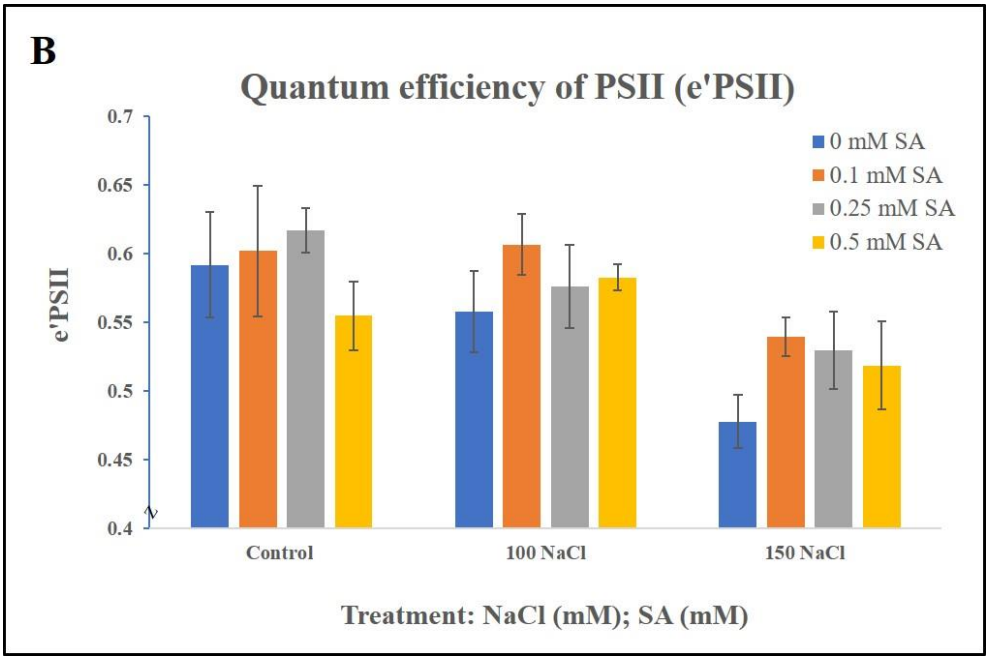


Fig. 3.2. Effect of SA on chlorophyll fluorescence of salt-stressed sorghum plants. A: Photosynthetic efficiency of PSII (Fv/Fm); B: Quantum efficiency of PSII (e'PSII). The vertical bars represent the standard deviation (*n=3*).

3.3. Total lipids determination

Table 3.2. Effect of SA concentrations on membrane lipids (glycolipids and phospholipids) in NaCl treated Sorghum plants; ± represents the standard deviation.

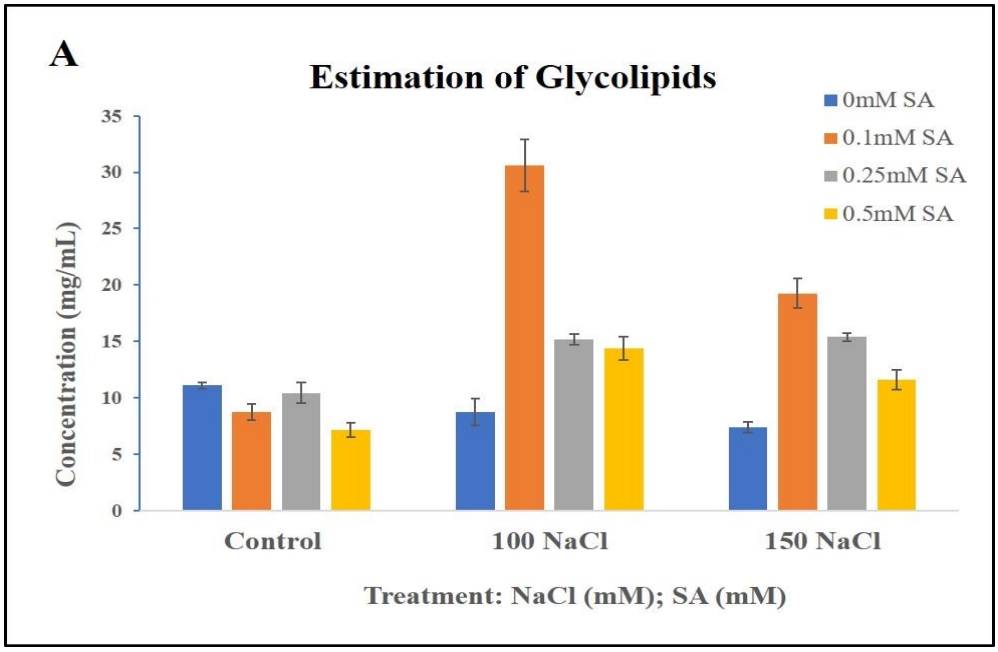
Treatment	Glycolipids (mg/mL)	% Change	Phospholipids (mg/mL)	% Change
Control	11.07±0.308	0	20.50±0.959	0
Control; 0.1 mM SA	8.75±0.703	-20.95	29.22±0.829	42.5
Control; 0.25 mM SA	10.42±0.922	-5.85	39.61±0.959	93.21
Control; 0.5 mM SA	7.15±0.625	-35.43	74.18±3.743	261.78
100 mM NaCl	8.72±1.201	-27.00	54.15±5.458	164.10
100 mM NaCl; 0.1 mM SA	30.61±2.30	251.07	118.12±1.495	118.12
100 mM NaCl; 0.25 mM SA	15.17±0.447	73.97	62.50±0.829	15.41
100 mM NaCl; 0.5 mM SA	14.36±1.01	64.77	75.94±6.598	40.22
150 mM NaCl	7.38±0.461	-33.28	17.61±0.167	-14.10
150 mM NaCl; 0.1 mM SA	19.23±1.307	160.27	97.61±7.991	451.97
150 mM NaCl; 0.25 mM SA	15.39±0.359	108.31	42.43±5.318	140.95
150 mM NaCl; 0.5 mM SA	11.60±0.872	57.04	64.07±11.568	263.82

Total membrane lipids were determined by estimating the glycolipids and phospholipids content in sorghum leaves grown under salinity stress and studying the possible role of SA (Table 3.2).

Glycolipids was observed to **decrease** greatly in salt stressed plants. Plants grown with 100 mM NaCl and 150 mM NaCl showed decrease in glycolipids content by **27%** and **33.28%** respectively in comparison to the control plants. The control plants grown in Hoagland nutrient solution did not show any change with application of SA. In 100 mM NaCl grown plants, spray of 0.1 mM SA **amplified** the glycolipids concentration by **251.07%** however there was **increase** of only **73.97%** and **64.77%** in glycolipids content in plants sprayed with 0.25 mM SA and 0.5 mM SA respectively as compared to plants grown in saline control. Analogous trend was observed in plants grown in 150 mM NaCl. Plants grown with 150 mM NaCl spray of 0.1 mM SA **improved** the glycolipids content by **160.28%** as compared to saline control. On application of 0.25 mM SA and 0.5 mM SA **increased** the amount of glycolipids concentration by **108.31%** and **57.04%** respectively as compared to plants with no SA spray. Our data in **Fig. 3.3.A** displays that **0.1 mM SA** spray was most effective under 100 mM and 150 mM NaCl stress to mitigate salt stress by **increasing** glycolipids content, while higher concentrations had minimum effect on development of tolerance against salt stress in sorghum plants. The results also indicate that spray of different concentrations of SA increased the glycolipids content in salt stressed plants as compared to plants without salinity stress.

Phospholipids concentration was observed to be increased in 100 mM NaCl plants while it decreased in 150 mM NaCl grown plants. The phospholipids content **elevated** in 100 mM NaCl stressed plants by **164.11%** while the content **declined** in 150 mM NaCl grown plants by **14.11%** as compared to control plants. The sorghum plants grown in control Hoagland

nutrient solution showed **increase** in phospholipids content with increase in concentration of SA of 0.1 mM SA, 0.25 mM SA and 0.5 mM SA by **42.5%**, **93.21%** and **261.79%** respectively as compared to the plants without SA application. In 100 mM NaCl grown sorghum plants, application of 0.1 mM SA **increased** the phospholipids content by **118.12%** however there was only **15.41%** and **40.23% increase** in phospholipids concentration in plants sprayed with 0.25 mM SA and 0.5 mM SA respectively as compared to plants without SA spray. Similar trend was observed in plants grown with 150 mM NaCl. When applied with spray of 0.1 mM SA, there was tremendous **increase** in phospholipids concentration in plants by **451.97%** whereas only **140.96%** and **263.82%** was increased in plants sprayed with 0.25 mM SA and 0.5 mM SA respectively as compared to plants without SA application. Our data in **Fig. 3.3.B** displays that spray of **0.1 mM SA** was most effective to mitigate salt stress by **increase** in phospholipids concentration, while increase in SA concentration had minimum effect on development of tolerance against salt stress in sorghum plants. The results also show that spraying plants with different concentrations of SA grown in salt stress increased phospholipids concentration as compared to plants grown in control nutrient medium.



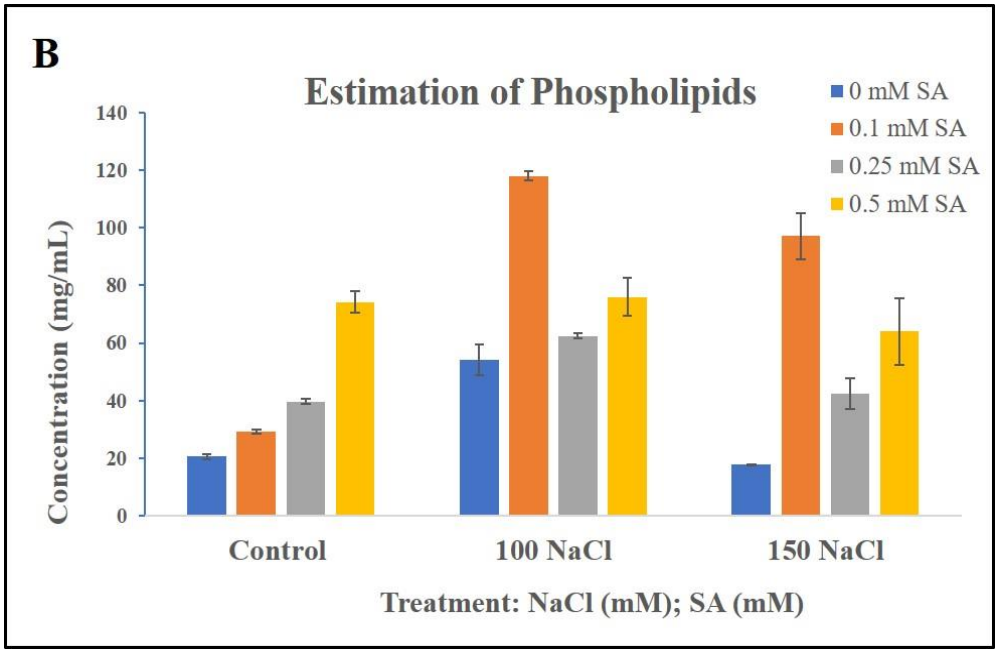
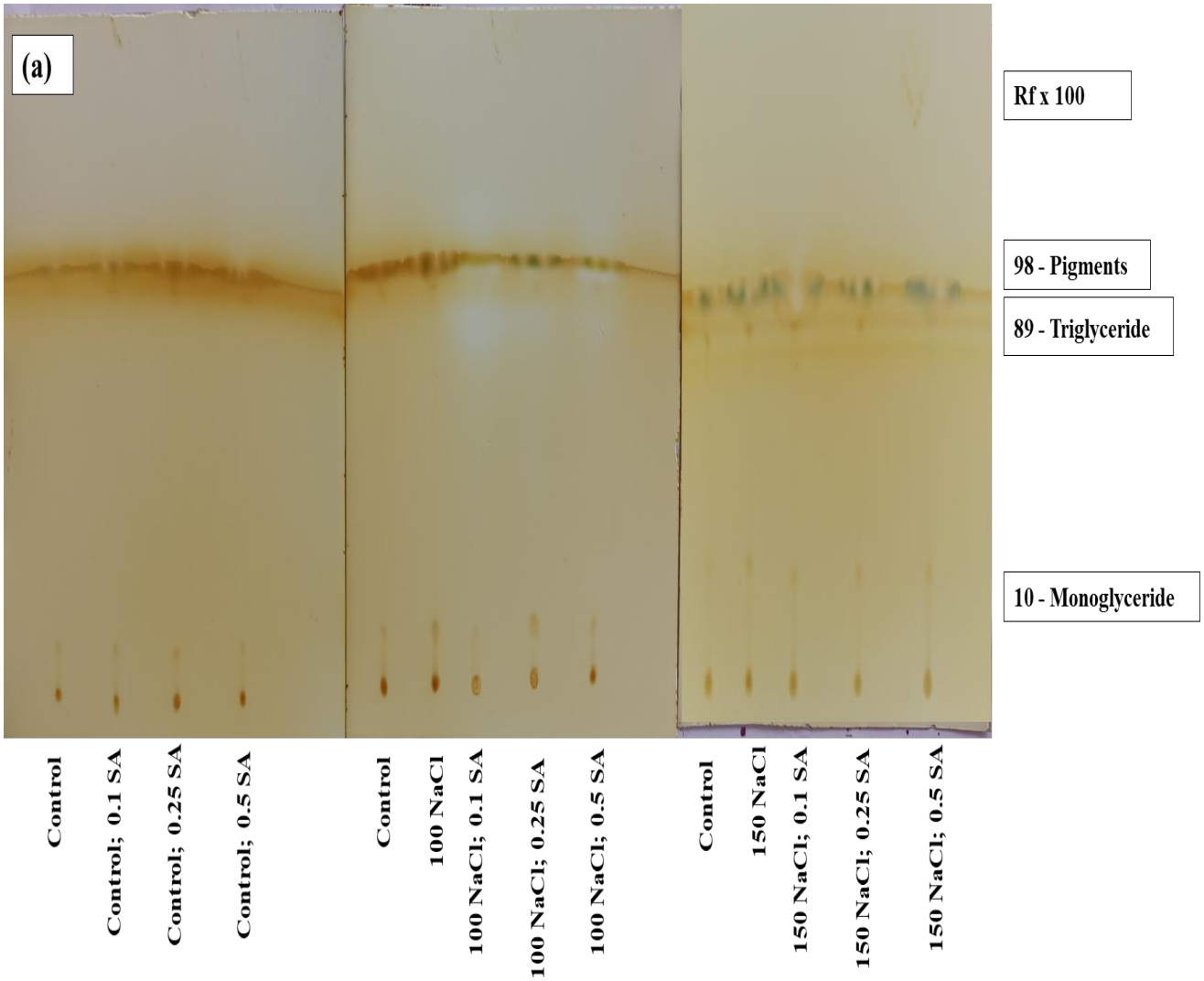


Fig. 3.3. Effect of SA on membrane lipids content of salt stressed plants. A: Concentration of Glycolipids (mg/mL); B: Concentration of Phospholipids (mg/mL). The vertical bars represent the standard deviation (*n=3*).

3.4. Thin Layer Chromatography (TLC)

Fig.3.4. Effect of SA concentrations on membrane lipids (Neutral lipids and Phosphoglycolipids) in NaCl treated Sorghum plants.



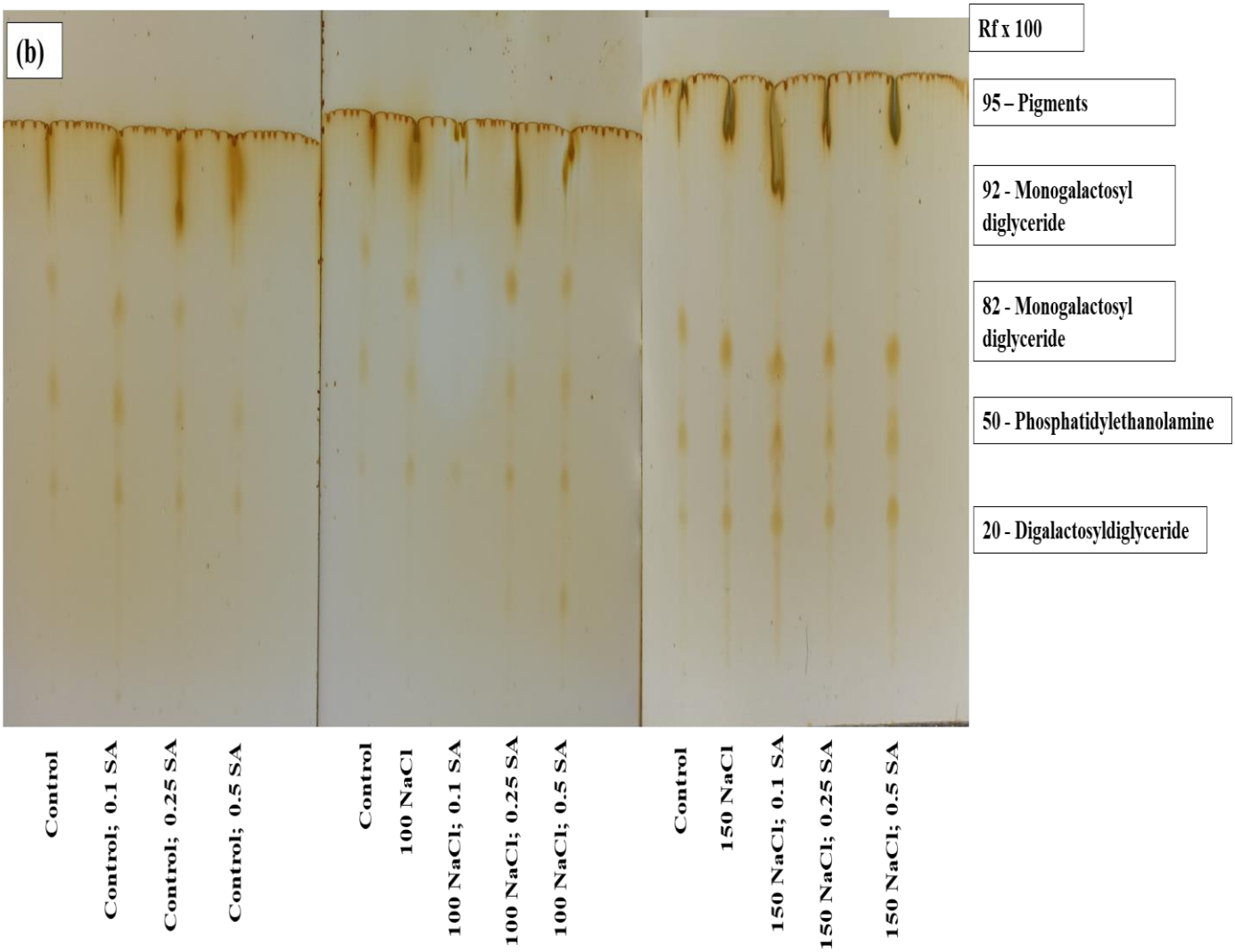


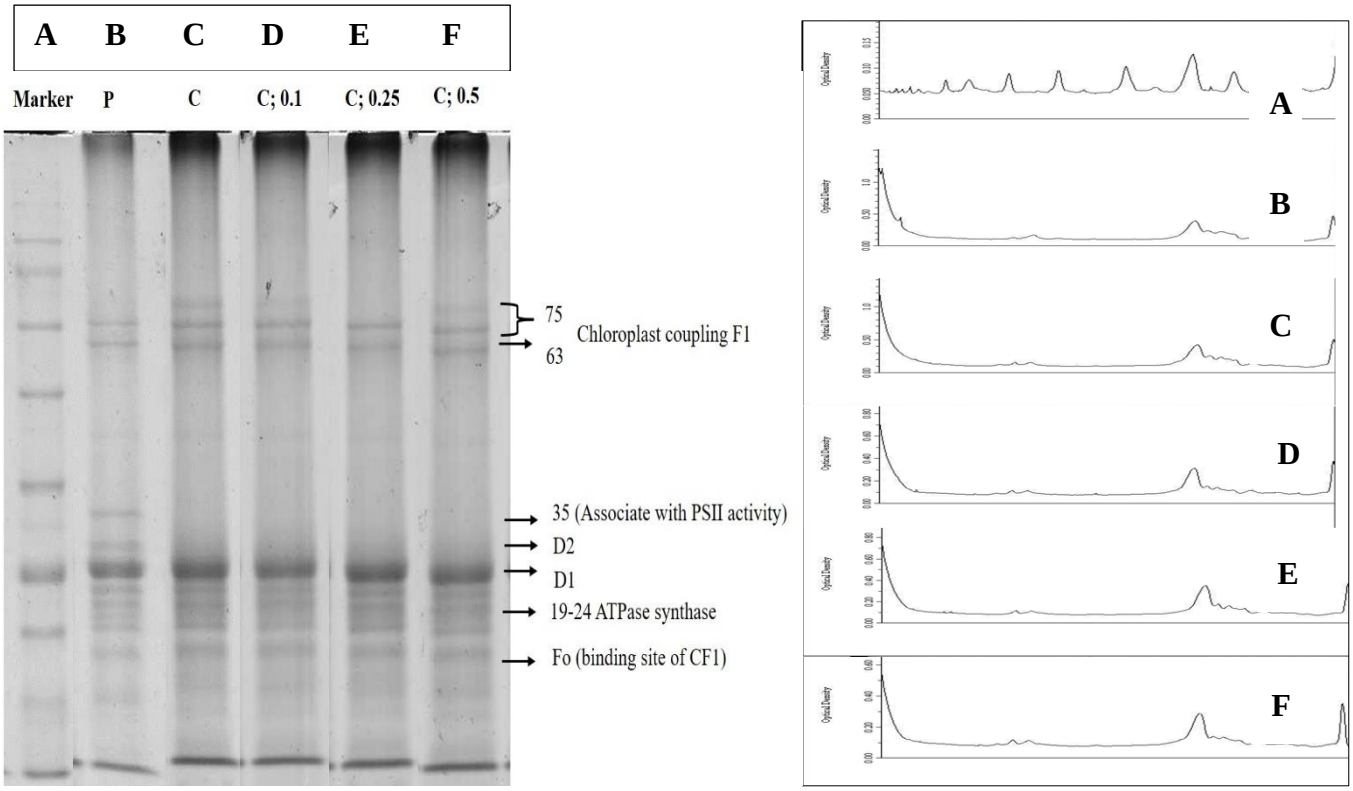
Fig. 3.4. TLC profile of salt stressed sorghum plants supplemented SA on membrane lipids (a) Neutral lipids and (b) Phosphoglycolipids in *Sorghum bicolor* L. Moench. Treatments: NaCl (mM); SA (mM).

SA-induced changes were observed in profile of qualitative analysis of membrane lipids (Neutral lipids and Phosphoglycolipids) of *S. bicolor* gown under salinity stress as shown in **Fig.3.4. a, b**. The visual determination was done based on the appearance on brown spot on the plates and corresponding lipids were identified based on their Rf values. TLC plate **(a)** shows neutral lipids qualitative results. Spraying with SA on plants grown in Hoagland nutrient solution did show any visual differentiation as compared to control plants with no SA. Only Monoglycerides and Pigments were observed on the plate. Similar results were observed in plants grown under 100 mM NaCl stress. However, the spots were darker in SA sprayed treatments as compared to unsprayed one. Plants grown in 150 mM NaCl conditions, depicted presence of Triglycerides along with Monoglycerides and Pigments spots. The lanes with corresponding to sprayed SA showed slightly longer spots in comparison to control and unsprayed 150 mM treated plants.

Phosphoglycolipids qualitative results are displayed in TLC plate **(b)**. Plants grown in control medium, spraying 0.1 mM SA showed darker phospholipid spots as compared to plants sprayed with higher concentration of 0.25 mM SA, 0.5 mM SA and unsprayed plants. However, plants grown in 100 mM NaCl showed darker and bigger spots when sprayed with 0.25 mM SA and 0.5 mM SA as compared to plants sprayed with 0.1 mM SA, control and with no SA spray. In 150 mM NaCl stressed plants, the visualized spots were dense as compared to plants grown in control and 100 mM NaCl conditions. The plants sprayed with 0.1 mM SA and 0.5 mM SA showed bigger and darker spots as compared to 0.25 mM SA sprayed, control and unsprayed plants.

3.5. Protein profiling (SDS-PAGE)

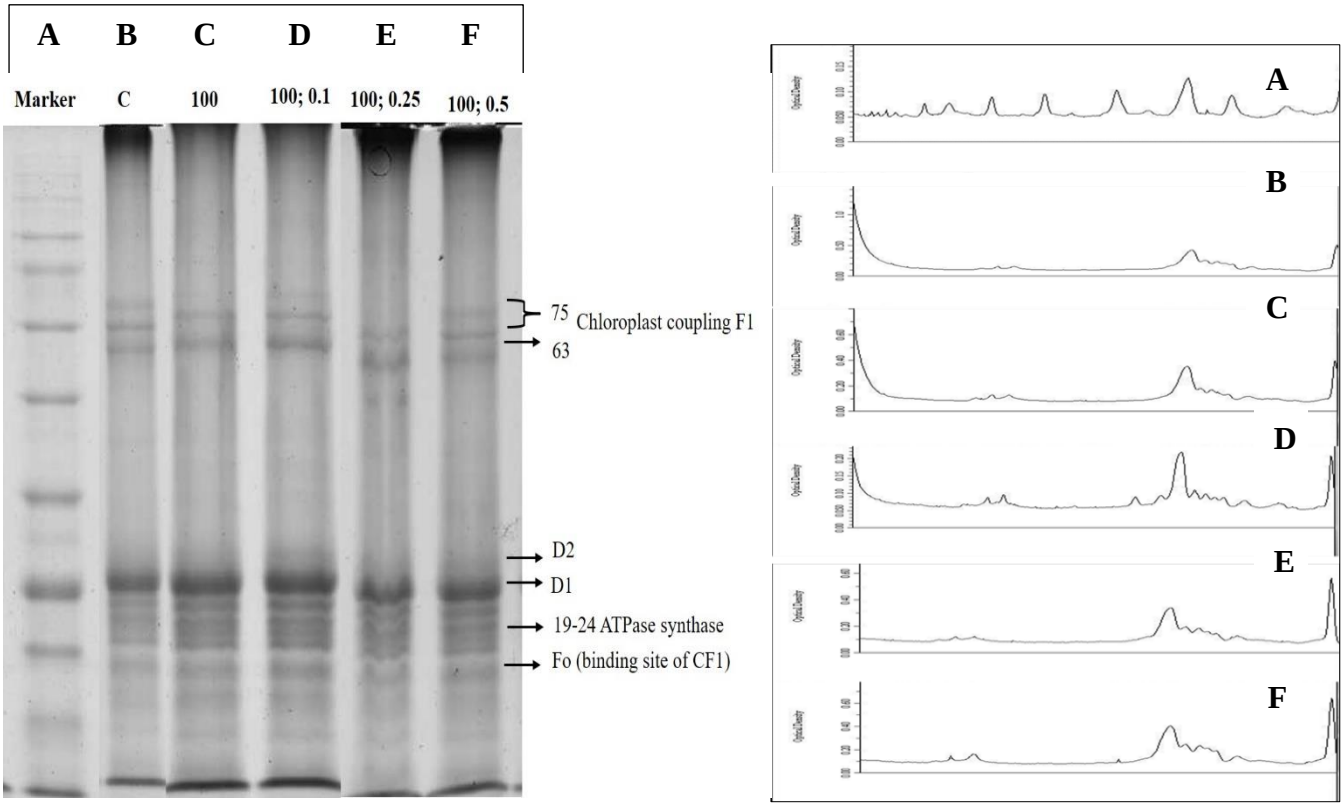
Fig.3.5-3.7. Effect of SA concentrations on polypeptides composition of thylakoid proteins in NaCl treated Sorghum plants.



(A): Effect of SA on polypeptide composition of thylakoid proteins

(B): Optical density profile of thylakoid membrane polypeptides

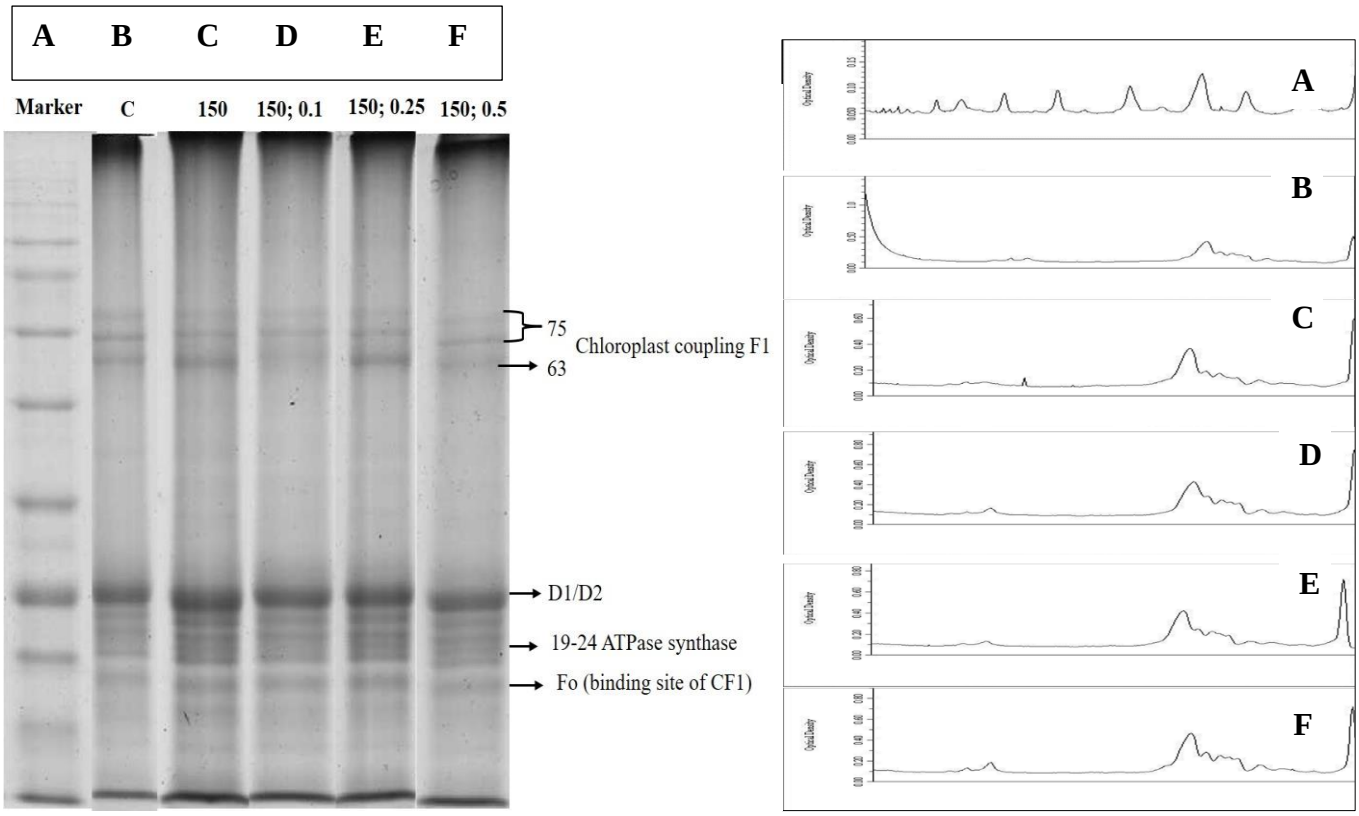
Fig. 3.5. Qualitative and quantitative determination of thylakoid membrane polypeptides from leaves of sorghum plants grown in Hoagland solution (Control) and treated with different concentration of SA. (A) Marker, (B) Polyhouse, (C) Control, (D) Control+0.1 mM SA, (E) Control+0.25 mM SA and (F) Control+0.5 mM SA.



(A): Effect of SA on polypeptide composition of thylakoid proteins

(B): Optical density profile of thylakoid membrane polypeptides

Fig. 3.6. Qualitative and quantitative determination of thylakoid membrane polypeptides from leaves of sorghum plants grown in 100 mM NaCl and treated with different concentration of SA. (A) Marker, (B) Control, (C) 100 mM NaCl, (D) 100 mM NaCl+0.1 mM SA, (E) 100 mM NaCl+0.25 mM SA and (F) 100mM NaCl+0.5 mM SA.



(A): Effect of SA on polypeptide composition of thylakoid proteins

(B): Optical density profile of thylakoid membrane polypeptides

Fig. 3.7. Qualitative and quantitative determination of thylakoid membrane polypeptides from leaves of sorghum plants grown in 150 mM NaCl and treated with different concentration of SA. (A) Marker, (B) Control, (C) 150 mM NaCl, (D) 150 mM NaCl+0.1 mM SA, (E) 150 mM NaCl+0.25 mM SA and (F) 150 mM NaCl+0.5 mM SA.

Protein profiling was carried out for sorghum plants to study the effect of SA on NaCl stressed plants using SDS-PAGE. The results showed that spraying plants with SA induced significant protein pattern changes. The optical density of protein bands elevated with 100 mM NaCl treatment while there was slight decrease in 150 mM NaCl treated plants as compared to 100 mM NaCl. In our study we observed that spraying SA did not show much difference in qualitative analysis based on visualization of protein bands in all the three treatments (Fig. 3.5 – 3.7). However, our quantitative results based on optical density of bands showed that plants sprayed with SA showed higher peaks.

The different proteins were identified based on the molecular weights and comparing with protein ladder. D1 protein was found to be abundant in both qualitative and quantitative study. Other proteins such as chloroplast coupling F1, ATPase synthase proteins and Fo proteins were also found to be elevated upon SA application.

DISCUSSION

From the results of the present study, it is suggested that salt stress reduced growth of *Sorghum bicolor* L. Moench plants and showed that it is not tolerant enough to high levels of salinity. The reduction in plant growth by salinity stress might be related to adverse effects of excess salt anion homeostasis, water balance, mineral nutrition and photosynthetic carbon metabolism (Manivannan *et al.* 2015). However, exogenous application of SA improved the plant growth in salt stressed plants (Fig. 3.1). These results are in agreement with those of El-Tayeb (2005) and Arfan *et al*. (2007) who reported that exogenous foliar application of SA ameliorated the adverse effects of salt stress on growth of barley and wheat, respectively. Similarly, reports suggest foliar application of SA caused increase in yield of wheat under water stress (Singh & Usha, 2003). El-Tayeb (2005) reported that SA-induced increase in growth could be related to enhanced activity of antioxidants that protect the plants from oxidative damage. Therefore, photosynthesis which is a major controlling factor for plant growth and yield might have been increased due to SA application.

Increased F_0 under salt stress may be related to the initial damage occurring in PSII, likely due to low water availability. This increase in F_0 under salt stress may be dependent on structural conditions affecting the probability of the energy transfer within the pigments of the light harvesting complex to the PSII reaction center (Krause & Weis, 1984). Salt stress may have several effects on PSII activity, one of which may be increased number of inactive reaction centers where electrons cannot be transferred out of reduced Q_A and thus higher measured F_0. Another possibility is low energy transfer from LHCII to PSII reaction center and thus higher fluorescence from LHCII which may have been caused by the dissociation of LHCII from the PSII core (Havaux, 1993). The donor side of PSII is reported to be affected more by salt stress, as compared to the acceptor side of PSII (Mehta *et al*. 2010).

The Fv/Fm ratio is frequently used as an indicator of the photo-inhibition or injuries caused to the PSII complexes (Roháček, 2002). The low Fv/Fm values in sorghum under saline conditions (Table 3.1) could have resulted from the inactivity of the reaction centers, which may favor greater energy dissipation in the form of heat and fluorescence, as deduced from the high Fv/Fm values. This may be associated with increased heat sinks which may absorb light in a similar manner as that of active reaction centers, but are unable to store the excitation energy as redox energy and dissipate their total energy as heat (Hermans *et al*. 2003). Netondo *et al*. (2004) found that Fv/Fm decreased by about 9 and 10% and electron transport rate decreased by 20 and 25% in two sorghum varieties grown under 250 mM NaCl. Thus, the changes observed in photochemical activity of PSII can contribute to the limitations of photosynthesis activity under salt stress. In our study, foliar application of range of SA, 0.1 mM to a greater extent improved chlorophyll fluorescence by increasing Fv/Fm especially under saline conditions (Fig. 3.2 A). SA treatment relieved the decreasing degree of Fv/Fm under salinity stress, suggesting that, to a certain extent SA can regulate the chloroplast energy state. SA is known to prevent the excessive accumulation of ROS and increase the repair rate of PSII, thus maintain the PSII activity (Tang *et al.* 2017).

In the present study, significant decrease of photosynthetic quantum yield (e'PSII) was observed in sorghum at high levels of salinity (Table 3.1), which underlines the drop of photosynthetic net CO_2 uptake. According to Everard *et al*. 1994, reduced quantum yield may result from a structural impact on PSII. Our results indicated that the quantum yield was improved in the salt stressed leaves by SA addition (Fig. 3.2.B) and caused considerable enhancement in net photosynthetic rate under salt stress, particularly at 0.1 mM SA level. This increase in photosynthetic rate due to exogenously applied SA was in agreement with earlier studies which reported that exogenously applied SA increased the photosynthetic rate in different crops, e.g., barley (Pancheva *et al*., 1996), soybean (Khan *et al*. 2003), wheat

(Singh & Usha, 2003), and maize (Khan *et al*. 2003; Khodary, 2004). Furthermore, a positive relationship between photosynthetic rate and growth has been observed, which suggests that SA-induced changes in photosynthetic rate might have contributed in growth enhancement under saline conditions. Similar relationship between growth and photosynthetic capacity was found in different crops e.g., *Brassica* spp. (Nazir *et al*., 2001), wheat (Arfan *et al*., 2007).

Glycolipids, especially galactolipids, are important for photosynthesis, as they are the building blocks for the thylakoid membrane (Dörmann & Benning, 2002). They are rich in unsaturated fatty acids and alterations in fatty acid profiles of glycolipids has been shown to significantly affect membrane structural and functional properties of chloroplast (Sui & Han, 2014). In our study, there is a significant decrease in sorghum leaves plasma membrane glycolipid content with increasing NaCl concentration (Table 3.2). Salinity-induced reduction in glycolipids has been observed in barley in response to NaCl (Brown & DuPont, 1989). This may due to peroxidation of lipids due to ROS production resulting in electrolyte leakage of membranes under saline conditions. The change in lipid profile of membranes may also be due to highest rate of Cl^- accumulation and leading to chloride toxic effects. Silva *et al*. (2003) found that increase in Cl^- content of cowpea plants, especially in roots has effect on fatty acid profile of glycolipids. However, Silva *et al*. (2007) pointed out that in some cases the increase in Cl^- content did not appear to cause damage to cells, which correlates with our studies in case of phospholipids content in 100mM NaCl grown plants (Fig. 3.3.B). Borsani *et al*. (2001) reported that excess salt affected the functional and structural integrity of cell membranes, reduced the activity of various enzymes and inhibited nutrient uptake from roots. This is also thought to be a consequence of increased galactolipase and lipoxygenase activities, which are normally induced by environmental stress or cell senescence (Hasanuzzaman *et al*. 2014) and have a preference for the

breakdown of monogalactosyldiacylglycerol (MGDG) (Skórzyńska *et al*. 1991). A decreased digalactosyldiacylglycerol (DGDG) content with increasing NaCl concentration is argued to be responsible for membrane structural disorder (Wang *et al*. 2014), instability of the membrane (Williams, 1998) and an alteration in membrane protein activity (Schuler *et al*. 1991).

Observed increase in glycolipid content in leaves upon SA treatments (Fig. 3.3 A) in our study is probably due to a greater contribution of galactolipids, such as MGDG and DGDG, demonstrating the importance of this group of lipids to membranes in photosynthetically active tissues (Dörmann and Benning, 2002). Therefore, sufficient MGDG lipids are required for self-regulation of membranes in regards to its lipid (MGDG) to protein (LHCII) ratio to maintain a stable bilayer structure (Garab *et al*. 2000). The increase in levels of MGDG and DGDG when sprayed with SA in our study are the main constituents of plastid membranes, which are rich in unsaturated fatty acids such as linoleic and α-linolenic (Somerville *et al*. 2000) and may assist in mitigating the salt effect by better maintaining structural and functional integrity of cell membrane.

Membrane phospholipids serve as structural and signaling molecules in plant cells (Furt *et al*. 2011) and reports have shown alterations in amount in plants subjected to salinity stress. Sorghum being moderately tolerant to NaCl, treating with 100 mM salt increased the phospholipids content (Table 3.2). Kerkeb *et al*. (2001) reported that increased phospholipids level induced tolerance in tomato under saline environment. The phospholipids membranes are impermeable to charged O_2 molecules; therefore, SOD is present for the removal of O_2 in the compartments where O_2 radicals are formed (Takahashi & Asada, 1983).

We observed that the phospholipids content was upregulated upon SA application (Fig. 3.3. B). Studies in maize have shown that the proportion of phospholipids in the root plasma membrane of a salt-tolerant genotype was approximately 1.7-fold higher when directly compared to a sensitive genotype (Salama *et al*., 2007) after spraying with SA, suggesting a positive correlation between SA-induced increase in phospholipid content and plant salt tolerance. According to Rodas-Junco (2020), phospholipids signaling is a component of SA response to stress. Krinke *et al*. (2009) demonstrated that SA stimulation led to rapid activation of Phospholipase D (PLD) and modification of PLD affected transcriptomic changes stimulated by SA. Interestingly, it was also shown that PI–PLC (Phosphatidylinositol−Phospholipase C) substrates and products participate in SA-triggered transcriptomic remodeling (Bejaoui *et al*. 2016). However, major components in the signaling pathway mediated by phospholipids in response to SA with a focus on plant membrane stability is not much known.

Neutral lipids mainly monoacylglycerols (MG) and triacylglycerols (TG) accumulation in vegetative tissues can serve as an energy reservoir under times of stress and are primarily stored in lipid droplets (LD) or plastoglobules (chloroplast localized LD) (Légeret *et al*. 2016). In our present study, high TG levels were observed in 150 mM stressed plants and it increased upon spraying SA (Fig. 3.4 A). Recent evidence has shed light on the role of triacylglycerols (TG) in plant stress tolerance. In leaves of the glycophyte *S. carnosa*, SA regulated increase in plastoglobule size and number under salt stress was linked to increased accumulation of TG lipids as a result of thylakoid galactolipid mobilization is suggested by Bejaoui *et al*. (2016). It was hypothesized that this could be an intermediate step in the conversion of thylakoid fatty acids to phloem-mobile sucrose, recruiting membrane carbon for the normal growth of plants under salt stress. Upregulation of TG by SA is proposed to

be an adaptive feature of salt-tolerant plants and may provide precursor lipid molecules such as DAG and FA for phospholipid synthesis to increase membranes stability.

Among all the major phosphoglycolipids, Phosphatidylcholines (PC), Phosphatidylglycerol (PG) and Phosphatidylethanolamine (PE) are the predominant components (together accounting for 15%–80%) in membranes. In our study, increased PE levels upon SA application was detected (Fig. 3.4 B). Upregulation of choline kinase (CK) mRNA levels and enzyme activity was observed in leaf rosettes of *A. thaliana* upon SA application in response to salt stress, which may contribute to the increased PC biosynthesis (Tasseva *et al*. 2004). However, Interrelationship between choline and SA response in plants and mechanism behind regulation need to be further investigated.

The salt-stressed sorghum's pigment-protein complexes showed a gradual decrease in the polypeptides contents upon increasing the NaCl concentrations (Fig. 3.7 and 3.8). A decrease in the polypeptide contents in SDS-PAGE of sorghum suggests salinity induced proteolysis of thylakoid membrane proteins. The decrease in the abundance of thylakoid membrane proteins can be related to decrease in Fv/Fm and quantum yield stress (Fig. 3.2 A and B) suggest that NaCl may have affected the number of functional reaction centers of PSII and combination state of chlorophyll molecules in light-harvesting two complexes (LHC2) (Dalal & Tripathy, 2018). Higher expression levels of proteins in the moderately salt-tolerant sorghum depicted the stabilizing capacity during salt stress conditions. A similar increase in protein level has been observed under drought (Ghabooli *et al*., 2013) and salt stress (Wu *et al*., 2014). Disorganization of the chloroplast membrane system, due to damages and swelling of thylakoid membranes (both stromal and granal) has been reported on maize and wheat under stress condition (Tian *et al*. 2013; Shao *et al*. 2016). A number of studies have demonstrated that exogenously applied SA maintained the integrity of chloroplast and thylakoid structures under stress conditions (Aldesuquy *et al*. 2018). Similarly, exogenous

SA application mitigated the inhibitory effect of salt stress on the photosynthetic capability of sorghum. Our results indicates that exogenous SA application improved thylakoid lipid membrane remodeling and structurual stability in leaves of sorghum under salt stress conditions which is confirmed by obtaining high optical density of polypeptides in the SA-treated plants (Fig. 3.7. and 3.8.). This can indicate that SA maintained an environment suitable for the function of critical integral proteins during stress via modulation of the ROS signal and adjusting chloroplasts and thylakoid membrane fluidity (Aldesuquy *et al*. 2018). However, our qualitative results did not show much variations in all treatments in sprayed and unsprayed plants.

D1 protein is the most sensitive member in PSII and provides binding sites for various cofactors and maintains conformation stability of PSII reaction center. Under stress, damage of D1 protein will lead to change of PSII reaction center conformation, hindrance of electron transfer and destruction of PSII reaction center (Yamamoto *et al*. 2008). Previous studies shown that one of the ways of signal transduction through salicylic acid was to induce reversible phosphorylation of proteins. It has been known that there is a kind of salicylic acid-induced protein kinase (SIPK) and a variety of defensive reaction to stresses that can be induced by enhancing SIPK activity (Zhang & Liu, 2001). Hence, it can be concluded that salicylic acid may accelerate the repair and turnover of proteins (especially D1 protein) and thus protect photosynthetic system by inducing protein kinase activity and reversible phosphorylation of proteins. The results also indicate that SA play important role in regulating the thylakoid membrane proteins under stress. However, the detailed mechanism needs to be further investigated.

CONCLUSION

Although sorghum is considered a crop moderately tolerant to salinity stress, the seedling stage is more sensitive to salinity and it is crucial for establishment. The results of this study support the hypothesis that Salicylic acid treatment might play an important role in modulating the physiological processes which eventually lead to protect plants under salt stress conditions. SA is of great potential to improve and maintained the integrity of the cell membrane and enhanced thylakoid membrane stability by modulating the expression or intensity of certain lipids and polypeptides and effectiveness of SA in alleviating the adverse effects of salt stress was dose dependent.

We observed that 0.1 mM SA treatment as compared to 0.25 mM and 0.5 mM SA is to be must effective dose which successfully improved photosynthetic rate, membrane lipids composition and thylakoid protein content under salinity stress in sorghum seedlings. In general, SA seems to be a promising method that could be used to ameliorate the negative effects of salt stress on sorghum in areas where salinity is a major constraint. However, detail study is needed to optimize the dose dependent effectiveness of SA treatments to seeds and leaf to draw firm conclusion.

REFERENCES

1. Ahmad, P. and Prasad, M.N.V. (2012). Abiotic Stress Responses in Plants: Metabolism, Productivity and Sustainability. *Springer Science + Business Media*, New York.
2. Ahmad, P. and Umar, S. (2011). *Oxidative Stress: Role of Antioxidants in Plants*. Studium Press, New Delhi, 19-53.
3. Ahmad, P., Hakeem, K. R., Kumar, A., Ashraf, M., Akram, N. A. (2012). Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). *Afr. J. Biotech*, 11: 2694–2703.
4. Ahmad, P., Jaleel, C.A., Salem, M.A., Nabi, G., Sharma, S. (2010). Roles of Enzymatic and nonenzymatic antioxidants in plants during abiotic stress. *Crit. Rev. Biotech*., 30: 161–175.
5. Ahmad, P., Sharma, S. (2008). Salt stress and phyto-biochemical responses of plants. *Plant Soil Environ*, 54: 89–99.
6. Aldesuquy, H.S., Ibraheem, F.L. and Ghanem, H.E. (2018). Exogenously supplied salicylic acid and trehalose protect growth vigor, chlorophylls and thylakoid membranes of wheat flag leaf from drought-induced damage. *J. Agri. Forest Meteorol. Res*. 1(1):13–20.
7. Arfan, M., Athar, H.R. and Ashraf, M. (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physiol*. 6: 685–694.
8. Arfan, M., H.R. Athar and Ashraf, M. (2007). Does exogenous application of salicylic acid through the rooting medium modulate growth and photosynthetic capacity in two differently adapted spring wheat cultivars under salt stress? *J. Plant Physiol*. 6(4):685-694.
9. Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant physiology*. 24(1): 1.
10. Asada, K. (1999). The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol*., 50: 601-639.

11. Ashrafuzzaman, M., M.A.H. Khan and S.M. Shahidullah. (2002). Vegetative growth of maize (*Zea mays*) as affected by a range of salinity. *Crop Res. Hisar*, 24: 286–291
12. Bejaoui, F., Salas, J.J., Nouairi, I.; Smaoui, A., Abdelly, C., Force, E.M. and Ben Youssef, N. (2016). Changes in chloroplast lipid contents and chloroplast ultrastructure in *Sulla carnosa* and *Sulla coronaria* leaves under salt stress. *J. Plant Physiol*. 198, 32–38.
13. Bezrukova, M.V., Sakhabutdinova, R., Fatkhutdinova, R.A., Kyldiarov, I. and Shakirova, F. (2001). The role of hormonal changes in protective action of salicylic acid on growth of wheat seedlings under water deficit. *Agrochemiya*, 2: 51-54.
14. Bol, J.F., Linthorst, H.J.M. and Cornelissen, B.J.C. (1990). Plant pathogenesis- related proteins induced by virus infection. *Annu. Rev. Phytopathol*, 28: 113-138.
15. Borsani, O., Cuartero, J., Fernandez, J.A., Valpuesta, V. and Botella, M.A. (2001). Identification of two *loci* in tomato reveals distinct mechanisms for salt tolerance. *Plant Cell*. 3:873–887.
16. Borsani, O., Valpuesta, V. and Botella, M.A. (2001). Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. P*lant Physiol.* 126: 1024–1034.
17. Boyer, J.S. (1976). *Water deficits and photosynthesis*. In: Kozlowsky, T.T. (ed.): Water deficit and plant growth. 153–190. Academic press, New York, San Francisco, London.
18. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*. 72(1-2): 248-254.
19. Brown, D.J. and DuPont, F.M. (1989). Lipid composition of plasma membranes and endomembranes prepared from roots of barley (*Hordeum vulgare* L.). *Plant Physiol*. 90:955 – 961.

20. Cameron, R.K. (2000). Salicylic acid and its role in plant defense responses: what do we really know? *Physiol. Mol. Plant Pathol*. 56: 91–93.
21. Carvalho, L.F., Medeiros, Filho S., Rossetti, A.G., Teófilo, E.M. (2002). Osmoconditioning in Sorghum seeds. *Rev Bras Sementes*, 22:185-92.
22. Chai, J., Liu, J., Zhou, J. and Xing, D. (2014). Mitogen-activated protein kinase 6 regulates NPR1 gene expression and activation during leaf senescence induced by salicylic acid. *J. Exp. Bot*. 65, 6513–6528. doi:10.1093/jxb/eru369
23. Chakraborti, N. and Mukherji, S. (2003). Effect of phytohormone pretreatment on nitrogen metabolism in *Vigna radiata* under salt stress. *Biol. Plant*. 46: 63-66.
24. Chen F, D'Auria JC, Tholl D, Ross JR, Gershenzon J, Noel JP, Pichersky E. (2003). An *Arabidopsis thaliana* gene for methylsalicylate biosynthesis, identified by a biochemical genomics approach, has a role in defense. *The Plant Journal*, 36, 577–588.
25. Christie, W. W. (1982). *Lipid analysis* (Vol. 207). Oxford: Pergamon press.
26. Cult, J.R. and Klessig, D.F. (1992). Salicylic acids in plants: a changing perspective, *J. Pharmaceut. Technol.* 16: 26–34.
27. Dai, X., Huo, Z., Wang, H. (2011). Simulation for response of crop yield to soil moisture and salinity with artificial neural network. *Field Crop Res*. 121: 441–449.
28. Dean, J.V., Mohammed L.A. and Fitzpatrick T. (2005). The formation, vacuolar localization, and tonoplast transport of salicylic acid glucose conjugates in tobacco cell suspension cultures. *Planta*, 221, 287–296.
29. Delany, T.P., Uknes, S., Vernooij, B., Friedrich, L., Weymann, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E. and Ryals, J. (1994). A central role of salicylic acid in plant disease resistance. *Science*, 266: 1247-1250.

30. Ding, C.K., Wang, C.Y., Gross, K.C. and Smith, D.L. (2002). Jasmonate and salicylate induce expression of pathogenesis-related protein genes and increase resistance to chilling injury in tomato fruit. *Planta*. 214: 895-901.
31. Dörmann, P. and Benning, C. (2002). Galactolipids rule in seed plants. *Trends Plant Sci*. 7: 112–118.
32. Durner, J. and Klessig, D.F. (1995). Inhibition of ascorbate peroxidase by salicylic acid and 2,6-dichloroisonicotinic acid, two inducers of plant defense responses. *Proceedings of the National Academy of Sciences*, USA 92, 11312–11316.
33. Durrant, W. E. and Dong, X. (2004). Systemic acquired resistance. *Annu. Rev. Phytopathol*. 42: 185-209.
34. El-Shihaby, O.A., M.M.N. Alla, M.E. Younis and Z.M. El-Bastawisy. (2002). Effect of kinetin on photosynthetic activity and carbohydrate content in waterlogged or sea-water treated *Vigna sinensis* and *Zea mays* plants. *Plant Biosyst*., 136: 277–290
35. El-Tayeb, M.A. (2005). Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul*. 45:215-224.
36. Elthon, T. E., Nickels, R. L. and McIntosh, L. (1989). Monoclonal antibodies to the alternative oxidase of higher plant mitochondria. *Plant Physiology*. 89(4): 1311-1317.
37. Everard, J.D., R. Gucci, S.C. Kann, J.A. Flore and W.H. Loeschner. (1994). Gas exchange and carbon partitioning in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity. *Plant Physiol*. 106:281–292.
38. Fadzilla, N.M., Finch, R.P., Burdon, R.H. (1997). Salinity, oxidative stress and antioxidant responses in shoot cultures of rice. *J. Exp. Bot*. 48: 325–331.
39. FAO. (2005). *Global Network on Integrated Soil Management for Sustainable Use of Salt Affected Soils*. FAO Land and Plant Nutrition Management Service: Rome, Italy.

40. Fariduddin, Q., Hayat, S. and Ahmad, A. (2003). Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. *Photosynthetica*. 41: 281–284.
41. Fariduddin, Q., Hayat, S. and Ahmad, A. (2003). Salicylic acid influences net photosynthetic rate, carboxylation efficiency, nitrate reductase activity and seed yield in *Brassica juncea*. *Photosynthetica*. 41(2): 281-284.
42. Foyer, C.H. and Noctor, G. (2003). Redox sensing and signaling associated with reactive oxygen in chloroplasts, peroxisomes and mitochondria. *Physiol. Plant.*, 119: 355-364.
43. Furt, F., Simon-Plas, F. and Mongrand, S. (2011). Lipids of the plant plasma membrane. In *The Plant Plasma Membrane*; Murphy, A.S., Peer, W., Schulz, B., Eds.; Springer: Berlin/Heidelberg, Germany.19: 3–30.
44. Garab, G., Lohner, K., Laggner, P. and Farkas, T. (2000). Self-regulation of the lipid content of membranes by non-bilayer lipids: A hypothesis. *Trends Plant Sci*. 5: 489–494.
45. Ghabooli, M., Khatabi, B., Ahmadi, F. S., Sepehri, M., Mirzaei, M., Amirkhani, A., . . . Salekdeh, G. H. (2013). Proteomics study reveals the molecular mechanisms underlying water stress tolerance induced by *Piriformospora indica* in barley. *J. Proteome*. 4: 289–301.
46. Grattan, S.R. and Grieve, C.M. (1999). Mineral nutrient acquisition and response by plants grown in saline environments. In: Pessarakli M. (ed.). *Handbook of Plant and Crop Stress*. Marcel Dekker, New York: 203–229.
47. Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E.G. and Cicek, N. (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Plant Physiol*. 164: 728–736.

48. Guo, R., Zhou, J., Hao, W., Gong, D. Z., Zhong, X. L., Gu, F. X., Liu, Q., Xia, X., Tian, J. N., Li, H. R. (2011). Germination, growth, photosynthesis and ionic balance in *Setaria viridis* seedlings subjected to saline and alkaline stress. *Can. J. Plant Sci*, 91: 1077–1088.

49. Gupta, B. and Huang, B. (2014). Mechanism of salinity tolerance in plants: physiological, biochemical, and *molecular characterization. International journal of genomics*.

50. Gutierrez-Coronado, M.A., Trejo-Lopez, C. and Saavedra, A.L. (1998). Effects of salicylic acid on growth of roots and shoots in soybean. *Plant Physiol. Biochem*. 36: 653–665.

51. Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K. and Fujita, M. (2014). Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) Varieties. *BioMed Res. Int.* 2014: 1–17.

52. Hasanuzzaman, M., Nahar, K., Anee, T. I. and Fujita, M. (2017). Glutathione in plants: biosynthesis and physiological role in environmental stress tolerance. *Physiology and Molecular Biology of Plants*. 23(2): 249-268.

53. Havaux, M. (1993). Rapid photosynthetic adaptation to heat stress triggered in potato leaves by moderately elevated temperatures. *Plant Cell Environ*. 16:461–467.

54. Hermans, C., Smeyers, M., Rodriguez, R.M., Eyletters, M., Strasser, R.J. and Delhaye, J.P. (2003). Quality assessment of urban trees: a comparative study of physiological characterization, airborne imaging and on-site fluorescence monitoring by the O-J-I-P-test. *J. Plant Physiol*. 160:81–90.

55. Herrera-Vásquez, A., Salinas, P. and Holuigue, L. (2015). Salicylic acid and reactive oxygen species interplay in the transcriptional control of defense genes expression. *Front. Plant Sci.* 6:171. doi:10.3389/fpls.2015.00171

56. Homaee, M., Feddes, R.A. and Dirksen, C. (2002). A macroscopic water extraction model for nonuniform transient salinity and water stress. *Soil Sci. Soc. Am. J*., 66: 1764–1772.

57. Hong, J. K. and Hwang, B. K. (2005). Induction of enhanced disease resistance and oxidative stress tolerance by overexpression of pepper basic PR-1 gene in *Arabidopsis*. *Physiol. Plant*. 124:267-277.

58. Horva´th, E., Szalai, G. and Janda, T. (2007). Induction of abiotic stress tolerance by salicylic acid signaling. *J. Plant Growth Regul*. 26: 290–300.

59. Horváth, E., Csiszár, J., Gallé, Á., Poór, P., Szepesi, Á. and Tari, I. (2015). Hardening with salicylic acid induces concentration-dependent changes in abscisic acid biosynthesis of tomato under salt stress. *J. Plant Physiol*. 183:54-63.

60. Horváth, E., Szalai, G. and Janda, T. (2007). Induction of abiotic stress tolerance by salicylic acid signaling. *Journal of Plant Growth Regulation*. 26(3): 290-300.

61. Hoyos, M. E. and Zhang, S.Q. (2000). Calcium-independent activation of salicylic acid-induced protein kinase and a 40-kilodalton protein kinase by hyperosmotic stress. *Plant Physiology*. 122: 1355-1363.

62. Ighbal, M.A. (2015). Agronomic management strategies elevate forage sorghum yield: A review. *J. Adv. Bot. Zool*. 3: 1–6.

63. Jayakannan, M., Bose, J., Babourina, O., Rengel, Z., and Shabala, S. (2015). Salicylic acid in plant salinity stress signaling and tolerance. *Plant Growth Regulation*. 76(1): 25-40.

64. Jumali, S. S., Said, I. M., Ismail, I. and Zainal, Z. (2011). Genes induced by high concentration of salicylic acid in '*Mitragyna speciosa*'. *Aust. J. Crop Sci*. 5, 296–303.

65. Kang, H.M. and Saltveit, M.E. (2002). Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. *Physiol. Plant* 115: 571–576.

66. Karakas, B., P. Ozias-Akins, C. Stushnoff, M. Suefferheld and M. Rieger. (1997). Salinity and drought tolerance of mannitol accumulating transgenic tobacco. *Plant Cell Environ*., 20: 609–616

67. Kerkeb, L., Donaire J.P. and Rodriguez-Rosales, M.P. (2001). Plasma membrane H^+-ATPase Activity is involved in adaptation of tomato to NaCl. *Physiol Plant*, 111:483–490.

68. Khan, M.I.R., Fatma, M, Per, T.S., Anjum, N.A. and Khan, N.A. (2015). Salicylic acid induced abiotic stress tolerance and underlying mechanisms in plants. *Front. Plant Sci*. 6:462. doi:10.3389/fpls.2015.00462

69. Khan, W., Prithiviraj, B. and Smith, D. (2003). Photosynthetic response of corn and soybeen to foliar application of salicylates. *J. Plant Physiol*. 160:485-492.

70. Khodary, S.E.A. (2004). Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Intl. J. Agri. Biol*. 6:5-8.

71. Kirst, G.O. (1989). Salinity tolerance of eukaryotic marine algae. *Ann. Rev. Plant Physiol*., *Plant Mol. Biol*. 40: 21-53.

72. Koocheki, A., Ghorbani, R., Mondani, F., Alizade, Y. and Moradi, R. (2011). Pulses production systems in term of energy use efficiency and economical analysis in Iran. *International Journal of Energy Economics and Policy*. 1(4): 95-106.

73. Kova´cˇik, J., Gru´z, J., Bacˇkor, M., Strnad, M. and Repcˇa´k, M. (2009). Salicylic acid-induced changes to growth and phenolic metabolism in *Matricaria chamomilla* plants. *Plant Cell Rep*. 28: 135–143.

74. Krause, G.H. and Weis, E. (1984). Chlorophyll fluorescence as a tool in plant physiology. II. Interpretation of fluorescence signals. *Photosynth. Res*. 5: 139–157.

75. Krinke, O., Flemr, M., Vergnolle, C., Collin, S., Renou, J.P., Taconnat, L., Yu, A., Burketova, L., Valentova, O., Zachowski, A. and Ruelland, E. (2009). Phospholipase D activation is an early component of the salicylic acid signaling pathway in *Arabidopsis* cell suspensions. *Plant Physiol*. 150:424–436.

76. Kushwaha, S., Kates, M., Sprott, G. D. and Smith, I. C. P. (1981). Novel polar lipids from the methanogen *Methanospirillum hungatei* GP1. *Biochimica et Biophysica Acta (BBA)*-Lipids and Lipid Metabolism. 664(1): 156-173.

77. Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 227(5259): 680-685.

78. Larque-Saavedra, A. (1979). Stomatal closure in response to acetylsalicylic acid treatment. Zeitschrift für Pflanzenphysiologie. 93(4): 371-375.

79. Lee, H.I. and Raskin I. (1999). Purification, cloning and expression of a pathogen inducible UDP-glucose: salicylic acid glucosyltransferase from tobacco. *Journal of Biological Chemistry*, 274, 36637–36642.

80. Légeret, B., Schulz-Raffelt, M., Nguyen, H.M., Auroy, P., Beisson, F. Peltier, G., Blanc, G. and Li-Beisson, Y. (2016). Lipidomic and transcriptomic analyses of *Chlamydomonas reinhardtii* under heat stress unveil a direct route for the conversion of membrane lipids into storage lipids. *Plant Cell Environ*. 39:834–847.

81. Leslie, C. A. and Romani, R. J. (1986). Salicylic acid: a new inhibitor of ethylene biosynthesis. *Plant Cell Reports*. 5(2): 144-146.

82. Leslie, C.A. and Romani, R.J. (1988). Inhibition of ethylene biosynthesis by salicylic acid. *Plant Physiol*., 88: 833–837.

83. Liljenberg, C. and Von Arnold, S. (1987). Effects of physiological and ontogenetical ageing on membrane lipid levels in pea leaves (*Pisum sativum*). *Journal of plant physiology*. 130(2-3): 255-265.

84. Maas, E.V. and Grattan, S.R. (1999). Crop yields as affected by salinity. In: Pessarakli M. (ed.). *Handbook of Plant and Crop Stress*. Marcel Dekker, New York: 55–108.

85. Malamy, J., Carr, J.P., Klessig, D.F. and Raskin, I. (1990). Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science*, 250: 1002-1004.

86. Manivannan, A., Soundararajan, P., Arum, L.S., Ko, C.H., Muneer, S.; Jeong, B.R. (2015). Silicon-mediated enhancement of physiological and biochemical characteristics of *Zinnia elegans* 'Dreamland Yellow' grown under salinity stress. *Hort. Enviro. Biotechnol*. 56:721–731.

87. Mansour, M.M.F., Stadelmann, E.J. and Lee-Stadelmann, O.Y. (1993). Salt acclimation of *Triticum aestivum* by choline chloride: Plant growth, mineral content and cell permeability. *Plant Physiol. Biochem*. 31: 341–348.

88. Marsalis, M.A., Angadi, S.V. and Contreras-Govea, F.E. (2010). Dry matter yield and nutritive value of corn; forage sorghum and BMR forage sorghum at different plant populations and nitrogen rates. *Field Crop. Res*. 116: 52–57.

89. Maslenkova, L. T. and Toncheva, S. R. (1998). SALICYLIC ACID-INDUCED CHANGES IN PHOTOSYSTEM II REACTIONS IN BARLEY PLANTS. *Dokladi na B lgarskata akademiâ na naukite*. 51(11-12): 101-104.

90. Mehta, P., Jajoo, A., Mathur, S. and Bharti, S. (2010). Chlorophyll a fluorescence study reveling effects of high salt stress on photosystem II in wheat leaves. *Plant Physiol. Biochem*. 48:16–20.

91. Metraux, J.P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W. and Inverardi, B. (1990). Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science*, 250: 1004-1006.

92. Minnick, L. and Kilpatrick, M. (1939). Acid base equilibria in aqueous and nonaqueous solutions. *J. Phys. Chem*. 43:259-68.

93. Mishra, A. and Choudhuri, M.A. (1999). Effects of salicylic acid on heavy metal-induced membrane deterioration mediated by lipoxygenase in rice. *Biol. Plant*, 42: 409–415.

94. Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*., 7: 405-410.

95. Moore, A.L., Albury, M.S., Crichton, P.G. and Affourtit, C. (2002). Function of the alternative oxidase: is it still a scavenger? *Trends in Plant Science,* 7, 478–481.

96. Munns, R. and Termaat, A. (1986). Whole-plant responses to salinity. *Aust. J. Plant Physiol*., 13: 143–160.

97. Munns, R. and Tester, M. (2008). Mechanism of salinity tolerance. *Annu. Rev. Plant Biol*. 59:651–681.

98. Nazir, N., M. Ashraf and Ejaz, R. (2001). Genomic relationships in oilseed Brassicas with respect to salt tolerance-photosynthetic capacity and ion relations. *Pak. J. Bot*. 33:483-501.

99. Netondo, G.W., Onyango, J.C. and Beck, E. (2004). Sorghum and salinity. II. Gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Sci*. 44:806–811.

100. Noreen, S. and Ashraf, M. (2008). Alleviation of adverse effects of salt stress on sunflower (*Helianthus annuus* L.) by exogenous application of salicylic acid: growth and photosynthesis. *Pak. J. Bot*. 40: 1657–1663.

101. Noreen, S., Ashraf, M., Hussain, M. and Jamil, A. (2009). Exogenous application of salicylic acid enhances antioxidative capacity in salt stressed sunflower (*Helianthus annuus* L.) plants. *Pak. J. Bot*. 41: 473–479.

102. Ohshima, M., Itoh, H., Matsuoka, M., Murakami, T. and Ohashi, Y. (1990). Analysis of stress-induced or salicylic acid-induced expression of the pathogenesis-related la protein gene in transgenic tobacco. *Plant Cell*, 2: 95-106.

103. Pancheva, T. V. and Popova, L. P. (1998). Effect of salicylic acid on the synthesis of ribulose-1, 5-bisphosphate carboxylase/oxygenase in barley leaves. *Journal of plant physiology*. 152(4-5): 381-386.

104. Pancheva, T.V., Popova, L.P. and Uzunova, A.L. (1996). Effects of salicylic acid on growth and photosynthesis in barley plants. *J. Plant Physiol*. 149:57–63.

105. Parida, A.K., Das, A.B. and Mohanty, P. (2004). Investigations on the antioxidative defense responses to NaCl stress in a mangrove, *Bruguiera parvi flora*: differential regulations of isoforms of some antioxidative enzymes. *Plant Growth. Regul*. 42: 213–226.

106. Park SW, Kaimoyo E, Kumar D, Mosher S, Klessig DF. (2007). Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science*, 318, 113–116.

107. Radwan, S.S. and Mangold, H.K. (1976). The lipids of plant tissue cultures. *Adv. lipid Res*. 12: 171–211.

108. Raskin, I., Ehmann, A., Melander, W. R., Meeuse, B.J.D. (1987). Salicylic acid- a natural inducer of heat production in *Arum* lilies. *Science*, 237: 1545-56.

109. Rhoads, D. M. and McIntosh, L. (1991). Isolation and characterization of a cDNA clone encoding an alternative oxidase protein of *Sauromatum guttatum* (Schott). *Proceedings of the National Academy of Sciences*. 88(6): 2122-2126.

110. Richards, L.A. (1954). Origin and nature of saline and alkali soils. pp, 1-6. In: Diagnosis and improvement of saline and alkali soils. Agricultural Handbook No: 60, USDA, Washigton, D.C., USA.

111. Rivas-San Vicente, M. and Plasencia, J. (2011). Salicylic acid beyond defence: its role in plant growth and development. *Journal of experimental botany*. 62(10): 3321-3338.

112. Rodas-Junco, B. A., Nic-Can, G. I., Muñoz-Sánchez, A. and Hernández-Sotomayor, S. M. (2020). Phospholipid signaling is a component of the salicylic acid response in plant cell suspension cultures. *International Journal of Molecular Sciences*, 21(15), 5285.

113. Roháček, K. (2002). Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning and mutual relationship. *Photosynthetica*. 40:13–29.

114. Salama, K. H., Mansour, M. M. F., Ali, F. Z., and Abou-Hadid, A. F. (2007). NaCl-induced changes in plasma membrane lipids and proteins of *Zea mays* L. cultivars differing in their response to salinity. *Acta Physiologiae Plantarum*, 29(4), 351-359.

115. Schuler, I., Milon, A., Nakatani, Y., Ourisson, G., Albrecht, A.M.; Benveniste, P. and A Hartman, M. (1991). Differential effects of plant sterols on water permeability and on acyl chain ordering of soybean phosphatidylcholine bilayers. *Proc. Natl. Acad. Sci. USA*. 88: 6926–6930.

116. Seemann, J.R. and Critchley, C. (1985). Effects of salt stress on the growth, ion content, stomatal behavior and photosynthetic capacity of a salt-sensitive species (*Phaseolus vulgaris*). *Planta*. 164: 151-162.

117. Senaratna, T., Merrit, D., Dixon, K., Bunn, E., Touchell, D. and Sivasithamparam, K. (2003). Benzoic acid may act as the functional group in salicylic acid and derivatives in the induction of multiple stress tolerance in plants. *Plant Growth Regul*., 39: 77-81.

118. Shakirova, F.M., Sakhabutdinova, A.R., Bezrukova, M.V., Fatkhutdinova, R.A. and Fatkhutdinova, D.R. (2003). Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. *Plant Sci*. 164: 317–322.

119. Shao, R.X., Xin, L.F., Zheng, H.F., Li, L.L., Ran, W.L., Mao, J. and Yang, Q.H. (2016). Changes in chloroplast ultrastructure in leaves of drought-stressed maize inbred lines. *Photosynthetica*. 54(1):74–80.

120. Sharma, P. K. and Singhal, G. S. (1992). Influence of photoinhibition on photosynthesis and lipid peroxidation. Journal of Photochemistry and Photobiology B. *Biology*. 13(1): 83-94.

121. Sharma, P. K., Shetye, R. and Bhonsle, S. (1997). Effect of supplementary ultraviolet-B radiation on young wheat seedlings.

122. Shi, H., Ishitani, M., Kim, C. and Zhu, J. K. (2000). The Arabidopsis thaliana salt tolerance gene SOS1 encodes a putative Na^+/H^+ antiporter. *Proceedings of the national academy of sciences*. 97(12): 6896-6901.

123. Shirasu, K., Nakajima, H., Rajashekar, K., Dixon, R.A. and Lamb, C. (1997). Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signal in the activation of defense mechanisms. *Plant Cell*, 9: 261-270.

124. Shulaev V. and Silverman P, Raskin I. (1997). Airborne signaling by methyl salicylate in plant pathogen resistance. *Nature*, 385, 718–721.

125. Silva, C., Aranda, F.J., Ortiz, A., Carvajal, M., Martıńez, V. and Teruel, J.A. (2007). Root plasma membrane lipid changes in relation to water transport in pepper: a response to NaCl and $CaCl_2$ treatment. *J Plant Biol*. 50:650–657.

126. Silva, J.V., Lacerda, C.F., Costa, P.H.A., Eneás-Filho, J., Gomes-Filho, E. and Prisco, J.T. (2003). Physiological responses of NaCl stressed cowpea plants grown in nutrient solution supplemented with $CaCl_2$. *Braz J Plant Physiol*. 15:99–105.

127. Singh, B. and Usha, K. (2003). Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul*. 39:137-141.

128. Sinha, R., & Khare, S. K. (2014). Protective role of salt in catalysis and maintaining structure of halophilic proteins against denaturation. *Frontiers in Microbiology*, 5:165.

129. Skórzyńska, E., Urbanik-Sypniewska, T., Russa, R. and Baszyński, T. (1991). Galactolipase activity of chloroplasts in cadmium-treated runner bean plants. *J. Plant Physiol*. 138: 454–459.

130. Somerville, C., Browse, J., Jaworski, J.G. and Ohlrogge, J.B. (2000). Lipids. In: Buchanan B, Gruissem W, Jones R (eds) *Biochemistry and molecular biology of plants*. American Society of Plant Physiologists, Rockville. 456–527.

131. Sudhir, P. and Murthy, S.D.S. (2004). Effects of salt stress on basic processes of photosynthesis. *Photosynthetica*. 42: 481- 486.

132. Sui, N. and Han, G. (2014). Salt-induced photoinhibition of PSII is alleviated in halophyte *Thellungiella halophila* by increases of unsaturated fatty acids in membrane lipids. *Acta Physiol. Plant*. 36: 983–992.

133. Szalai, G., Tari, I., Janda, T., Pestena´cz, A. and Pa´ldi, E. (2000). Effects of cold acclimation and salicylic acid on changes in ACC and MACC contents in maize during chilling. *Biol. Plant.* 43: 637–640.

134. Szepesi, A., Csisza´r, J., Bajka´n, S., Ge´mes, K., Horva´th, F., Erdei, L., Dee´r, A.K., Simon, M.L. and Tari, I. (2005). Role of salicylic acid pre-treatment on the acclimation of tomato plants to salt- and osmotic stress. *Acta Biol. Szeged*. 49: 123–125.

135. Takahashi, M.A. and Asada, K. (1983). Superoxide anion permeability of phospholipid membranes and chloroplast thylakoids. *Archives of Biochemistry and Biophysics*. 226:558–566.

136. Tang, Y., Sun, X., Wen, T., Liu, M., Yang, M. and Chen, X. (2017). Implications of terminal oxidase function in regulation of salicylic acid on soybean seedling photosynthetic performance under water stress. *Plant Physiology and Biochemistry*. 112:19-28.

137. Tari, I., Csisza´r, J., Szalai, G., Horva´th, F., Pe´csva´radi, A., Kiss, G., Szepesi, A´., Szabo´, M. and Erdei, L. (2002). Acclimation of tomato plants to salinity stress after a salicylic acid pretreatment. *Acta Biol. Szeged*. 46: 55–56.

138. Tasseva, G., Richard, L. and Zachowski, A. (2004). Regulation of phosphatidylcholine biosynthesis under salt stress involves choline kinases in *Arabidopsis thaliana*. *FEBS Lett.* 566: 115–120.

139. Turnham, E. and Northcote, D. H. (1984). The incorporation of [1-14C] acetate into lipids during embryogenesis in oil palm tissue cultures. *Phytochemistry*. 23(1): 35-39.

140. Ulery A.L. and Ernst F.F. (1997). Sorghum response to saline industrial cooling water applied at three growth stages. *Agronomy Journal*, 89, 392–396.

141. Upchurch, R. (2008). Fatty acid unsaturation, mobilization and regulation in the response of plants to stress. *Biotech. Lett*. 30: 967–977.

142. Wang, S., Uddin, M.I., Tanaka, K., Yin, L., Shi, Z., Qi, Y., Mano, J., Matsui, K., Shimomura, N., Sakaki, T., *et al*. (2014). Maintenance of chloroplast structure and function by overexpression of the rice monogalactosyldiacylglycerol synthase gene leads to enhanced salt tolerance in tobacco. *Plant Physiol*. 165: 1144–1155.

143. Waseem, M., Athar, H.U.R., and Ashraf, M. (2006). Effect of salicylic acid applied through rooting medium on drought tolerance of wheat, *Pak. J. Bot*. 38: 1127–1136.

144. Wildermuth, M.C., Dewdney, J., Wu, G. and Ausubel, F.M. (2001). Isochorismate synthesis is required to synthesize salicylic acid for plant defense. *Nature*, 414 (6863): 562-565.

145. Williams, W.P. (1998). The physical properties of thylakoid membrane lipids and their relation to photosynthesis. In *Lipids in Photosynthesis: Structure, Function and Genetics*; Paul-André, S., Norio, M., Eds.; Springer: Dordrecht, The Netherlands. 6p.

146. Wu, D., Shen, Q., Qiu, L., Han, Y., Ye, L., Jabeen, Z., . . . Zhang, G. (2014). Identification of proteins associated with ion homeostasis and salt tolerance in barley. PROTEOMICS, 14(11): 1381–1392.

147. Xie, Z., Fan, B., Chen, C. and Chen, Z. (2001). An important role of an inducible RNA-dependent RNA polymerase in plant antiviral defense. *Proceedings of the National Academy of Sciences*, USA 98, 6516–6521.

148. Yalpani, N., Enyedi, A.J., Leo´n, J. and Raskin, I. (1994). Ultraviolet light and ozone stimulate accumulation of salicylic acid and pathogenesis related proteins and virus resistance in tobacco. *Planta*, 193: 373–376.

149. Yamamoto, R., Aminaka, M., Yoshioka, M., Khatoon, K., Komayama, D., Takenaka, A., Yamashita, N., Nijo, K., Inagawa, N., Morita, T., Sasaki, Y., Yamamoto. (2008). Quality control of photosystem II: impact of light and heat stress. *Photosynthesis Research*. 98:589–608.

150. Yancey, P.H. (2005). Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *Journal of experimental biology*. 208(15): 2819- 2830.

151. Zhang, S. and Liu, Y. (2001). Activation of salicylic acid-induced protein kinase, a mitogen-activated protein kinase, induces multiple defense responses in tobacco. *The Plant Cell*. 13:1877–1889.

152. Zhao, H.J., X.W. Lin, H.Z. Shi and S.M. Chang. (1995). The regulating effects of phenolic compounds on the physiological characteristics and yield of soybeans. *Acta Agron. Sin*., 21: 351–355.

153. Zhu, J.K. (2001). Plant salt tolerance. *Trends Plant Sci*., 2: 66-71.