Chapter-5

DISCUSSION
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To meet demand, agriculture in 2050 will need to produce almost 50 percent more food, feed and biofuel than it did in 2012. This FAO estimate takes into account recent United Nations (UN) projections indicating that the world’s population would reach 9.73 billion in 2050 (FAO, 2017). The world’s population is expected to grow to almost 10 billion by 2050, boosting agricultural demand – in a scenario of modest economic growth – by some 50 percent compared to 2013. Meeting the increased demand should not be a major challenge, if past achievements are a guide. Historically, much bigger increases in agricultural production have been recorded in comparable time frames. Between 1961 and 2011, global agricultural output more than tripled (Alexandratos and Bruinsma, 2012). Increased use of land, irrigation and agro-chemicals played a major role in the growth of agricultural production during the Green Revolution. However, it is now recognized that the gains were often accompanied by negative effects on agriculture’s natural resource base, including land degradation, salinization of irrigated areas, over-extraction of groundwater, the build-up of pest resistance and the erosion of biodiversity. Agriculture has also damaged the wider environment through deforestation, the emission of greenhouse gases and nitrate pollution of water bodies (FAO, 2011a). Climate-smart agriculture aims at sustainably increasing food security and incomes, and adapting and building resilience to climate change while capturing potential mitigation co-benefits. It connects other innovations, such as conservation agriculture, agroecology, agroforestry and the development of crop varieties that are more tolerant to pests, diseases, drought, waterlogging and salinity (FAO, 2013). The decline in the share of agriculture in total production and employment is taking place at different speeds and poses different challenges across regions. Although agricultural investments and technological innovations are boosting productivity, growth of yields has slowed to rates that are too low for comfort. Food losses and waste claim a significant proportion of agricultural output, and reducing them would lessen the need for production increases. However, the needed acceleration in productivity growth is hampered by the degradation of natural resources, the loss of biodiversity and the spread of transboundary pests and diseases of plants and animals, some of which are becoming resistant to antimicrobials. Over 20% of the irrigated land and more than 6% of the world’s total land are now within the ambit of the salt effects (Mickelbart et al., 2015). In
addition, about 1.5 Mha of arable land is lost and $27.5 billion is spent annually due to the salinity problem in the agricultural sector (FAO, 2010; Qadir et al., 2014). Furthermore, water scarcity in arid and semiarid regions, where more than 40% of the world population resides, is leading toward an increase in the amount of saline or brackish water used for irrigating essential food crops, such as wheat. All of these facts about salinity suggest that it is one of the most severe environmental stresses affecting human life.

The 68th UN General Assembly declared 2016 the International Year of Pulses (IYP). Because of the protein content of their seeds, grain legumes, pseudocereals, and other crops are candidates to satisfy the growing demand for plant protein for food and feed. Crop production worldwide is highly specialized and currently relies on a very small number of species, raising questions about the sustainability of farming (Tilman et al., 2002). The role of legumes in nutrition has been recognized as a relevant source of plant protein together with other benefits for health. Soybean, peanut, common bean, pea, lupine, chickpea, faba bean, lentil, grass pea, cowpea, and pigeon pea are currently the most important legumes for human consumption and animal feed (De Ron, 2015). The integration of legumes into agriculture could reduce the current protein deficit and contribute to the transition to more sustainable agricultural systems. Legumes contribute to the sustainable improvement of the environment due to their ability to fix nitrogen and their beneficial effects on the soil (Drevon et al., 2015), having a tremendous potential in the reclamation of poor and marginal lands for agriculture (Coba de la Pe-a and Pueyo, 2012). Salicylates play an important role in the regulation of the abiotic stress like salinity stress responses. Therefore, the present study is undertaken to have some insights into alleviating effects of salicylates (SA, ASA and SSA) on growth and development of chickpea under salinity stress. Plant growth regulators (PGRs) emerged as “magic chemical” that could increase agricultural production at an unprecedented rate and help in removing the barriers which is imposed by genetics and environment. These PGRs when added in small amounts can modify the natural growth regulatory system right from seed germination to senescence in several crop plants. The results of the tested parameters are discussed below:-

The growth parameters (fresh and dry weight of root and shoot, root and shoot length and number of branches per plant) decreased progressively with the rise of stress level, compared with the control (Table 4.1). Results obtained in the present study are in agreement with those of
Ghoulam et al. (2002), who reported that salinity caused a marked reduction in growth parameters of sugar beet plants. Similarly, Hasanuzzaman et al. (2009) noticed a remarkable reduction in plant height and tiller number in *Oryza sativa* plants grown in saline soil. In the present study, seed priming or foliar spray with salicylates enhanced root and shoot length considerably over control under salt stress (Tables 4.1-4.9 and 4.31-4.39). Likewise, SA treatment increased the growth of wheat plants under water stress (Singh and Usha 2003), barley (El-Tayeb 2005) and maize (Khodary 2004) under NaCl stress. Reductions in root length with salinity were also reported by Muharrem (2008). In another observation by Soltani et al. (2002), root length was diminished by increasing the sodium chloride (NaCl) concentration. Further, it was also observed that plants grown with SA and without salt had more root length as compared with other treatments. Dolatabadian et al. (2011) also demonstrated that salinity stress significantly reduced plant height, total biomass and shoot and root weight of Soybean. A number of studies also mentioned that salinity affects seed imbibitions and led reduction in growth and production of plants (Jamian et al., 2014; Sure et al., 2011). In *Suaeda salsa*, plant height, number of branches and diameter of shoot were significantly affected by salt stress which was due to increased content of Na⁺ and Cl⁻ (Guan et al., 2011).

Reduction in root and shoot lengths of plants is one of the most commonly observed responses of salinity as also reported by Bernstein and Hayward (1958). Bruggeman et al. (2003) reported that increasing levels of salinity adversely affected both root and shoots length of chickpea seedling and decreased with salinity. Depression of the shoot:root ratio in response to salinity of wheat plants when different N sources were applied (Drihem and Pilbeam, 2002). Excess of salt concentrations in the root zone causes substantial changes in various morphological and physiological traits at various organizational levels in the plant (whole plant, tissue and cellular levels). This is achieved through osmotic stress, specific ion toxicities, and ion imbalance (Ashraf and Harris, 2004; Parida and Das, 2005). High salt concentrations in the root zone result in an increased osmotic stress in the soil solution and a consequent decrease in water availability to the plant, in a similar manner to drought stress. Specific ion toxicities, correspond to the excessive buildup of Na⁺ and Cl⁻ in the leaf blade, reaching toxic levels (Nawaz et al., 2010). The antagonism that exists between Na⁺ and essential cations, particularly K⁺ and Ca²⁺, in the site of ion uptake in the roots, causes ion imbalance at cellular and tissue levels by reducing the ratios of K⁺/Na⁺ and Ca²⁺/Na⁺ (Elhamid et al., 2014; De Leon et al., 2015). Salt-tolerant
genotypes can overcome the negative impacts of these events by generating distinct salt tolerance mechanisms. Since various morphological and physiological characteristics can contribute to salt tolerance mechanisms, several studies have demonstrated that using the specific morphological and physiological traits underlying salt tolerance mechanisms as screening criteria will contribute to making the evaluation of salt tolerance among genotypes more effective (El-Hendawy et al., 2005a,b; Munns and Tester, 2008; Nawaz et al., 2010; Zhang et al., 2014; Ashraf and Ashraf, 2015).

Plant growth inhibition under high salinity conditions is related not only to disturbances and imbalances of ions in the soil solution, but also to poor water relations. High salt concentrations in the soil solution depress the soil water potential, which subsequently results in increased leaf water potential. Therefore, the success of genotypes in adapting to low soil water potential is associated with their ability to lower the leaf water potential sufficiently to increase water uptake. The genotypes can reduce their water potential by decreasing the leaf osmotic potential, either through the accumulation of inorganic ions, or through the synthesis of organic osmolytes (Hasegawa et al., 2000; Nawaz et al., 2010; Singh et al., 2010). Active accumulation of osmotic adjustment components under salt stress helps the plant to maintain higher leaf turgor, which is one of the main mechanisms for ensuring water uptake and enhancing plant growth under salinity conditions (Pérez-López et al., 2009; Ashraf and Ashraf, 2015). Therefore, the salt tolerance of genotypes can be efficiently evaluated using leaf water relations parameters due to the close correlation between salt concentrations in soil solutions and plant water relations parameters.

When plants grow under salt stress conditions, the physiological drought stress, which is a result of a difficulty in withdrawing water from the soil due to a decreased osmotic and matric potentials of the soil, results in a marked reduction in the leaf water potential (becomes more negative). Disrupting the balance between the leaf water potential and the leaf osmotic potential leads to dramatic changes in leaf turgor pressure, which is considered as the major factor, along with K⁺, in the control of stomatal opening. Since stomata are the main entrance points for CO₂ uptake for photosynthesis and water evaporation from the leaves (transpiration), disturbed stomatal conductance leads to trouble in rates of photosynthesis and transpiration (Saqib et al., 2013). Because of this closed circuit between these physiological process, most parameters
related to leaf water relations and photosynthesis have been routinely and effectively used as selection criteria for evaluating salt tolerance in a number of crops, such as barley and faba bean (Jiang et al., 2006; Tavakkoli et al., 2010), chickpea (Flowers et al., 2010), rice (Sanni et al., 2012), quinoa (Razzaghi et al., 2011) and cotton (Zhang et al., 2014). The four reasons that were usually introduced as solely responsible for reduction of plant growth under salt-stressed conditions, were reviewed by Al-Yassin (2004) as: osmotic stress caused by lowering the availability of external water, specific ion toxicity effects caused by metabolic processes in the cell, nutritional imbalances caused by these ion-toxicity effects and combination of any two of the above mentioned factors.

In the present study, seed priming and foliar application of derivatives of salicylic acid led to significant enhancement in plant height, fresh weight and dry weight of plant and number of branches per plant (Tables 4.1-4.9 and 4.31-4.39). Similarly, Hayat et al. (2005) also reported that seed priming significantly increased number of leaves, fresh and dry weight per plant of wheat seedlings. Khodary (2004) and stevens et al. (2006) observed that SA treatments ameliorated the negative effect of salt stress on fresh and dry weight of maize and tomato plants respectively. Hamid et al. (2008) reported that SA also increased shoot and root dry weight of wheat under salt stress conditions. In addition, Azooz (2009) investigated that SA enhanced the dry weight of root and shoot in Vicia faba under salt stress. Likewise, Kovacik et al. (2009) reported that SA stimulates the growth of leaf rosettes and roots of chamomile plants. Also, Iqbal et al. (2001) noticed similar results spraying with Gibberellic acid in chickpea. In another study on black gram, Jeyakumar et al. (2008) demonstrated that SA increased dry matter production. The increase in fresh and dry matter of stressed plants in response to SA might be related to the induction of antioxidant response that increased the tolerance of plants to damage (Gunes et al., 2005). Wheat seedlings treated with SA develop larger ears and enhanced cell division is observed within the apical meristem of seedling roots (Shakirova et al., 2003). SA treatments enhanced the level of cell division by stimulating the mitotic system of the apical meristem of seedling roots which caused an increase in plant growth (Sakhabutdinova et al., 2003). Previous study by Nagasubramaniam et al., (2007) demonstrated that SA increased plant height, total dry matter production and crop growth rate in babycorn. Also, Singh and Usha (2003) reported that SA increased plant dry weight and RUBISCO carboxylase activity in wheat. SA regulated growth and productivity of plants by affecting physiological and biochemical activities of plants
Salicylic acid ameliorates the adverse effects of salt stress on strawberry (Karlidag et al., 2011). Similarly, Zamaninejad et al., (2013) demonstrated the effect of SA on morphological characteristics of Zea mays L. under drought condition. Khan et al., (2003) showed that plants treated with SA survive more, exhibit higher relative growth rate and enhanced dry mass production. Our study revealed that lower concentration of SA, ASA and SSA were able to enhance morphological traits more effectively as compared to higher concentrations of salicylates. The results of many other studies with SA under salt stress have shown that lower concentrations of SA is often more effective than higher concentrations (Khan et al., 2010; Najafian et al., 2009). Moreover, Khan et al. (2003) observed that the application of ASA and SSA elevated the dry matter and leaf area of corn and soybean. Also, Noreen and Ashraf (2008) demonstrated that foliar application of SA led to significant increase in dry weight of sunflower under drought stress and non-stress conditions. Abd El-Wahed et al. (2006) confirmed the stimulatory effect of ASA or SA on growth of yellow maize plants. Furthermore, Metwally et al. (2003) found that SA alleviated the cadmium toxicity in barley seedlings by improving dry weight which might be due to increased lateral growth.

The reduction in chlorophyll content of chickpea leaves observed under NaCl stress (Table 4.9) might be ascribed to the destruction of chlorophyll pigments, decreased chlorophyll synthesis and the vulnerability of the pigment-protein complexes (Rasool et al., 2013). Our results showed that salinity decreased the photosynthetic pigments which are in conformity with Baber et al. (2014) who reported that salinity caused a marked reduction in photosynthetic pigments in fenugreek which might be due to the possible oxidation of chlorophyll and other chloroplast pigments coupled with instability of the pigment protein complex under salt stress. Also, the decrease in photosynthetic pigments might have been due to salt-induced destruction of the structure of chloroplast and the fluidity of pigment protein complexes (Jamil et al., 2012). This decrease in chlorophyll content might partially cause a decrease in growth and biomass yield. Interestingly, the negative impacts of salt stress on photosynthetic performance can be detected even under mild salinity conditions or short-term salinity stress before the irreversible morphological damage becomes visible (Zarco-Tejada et al., 2003). It is possible to detect these negative impacts by measuring different chlorophyll fluorescence parameters, which are sensitive to any changes that directly affects the plant metabolism and photosynthesis apparatus (Baker and Rosenqvist, 2004; Murchie and Lawson, 2013). Carotenoids have been known to
play a key role in photosynthetic reaction center in which they are involved in mechanisms regulating photo protection against photo-oxidation (Yang et al., 2013; Gururani et al., 2015). Another important effect that inhibits the growth and photosynthetic abilities of plants is the loss of balance between the production of reactive oxygen species (ROS) and the antioxidant defense (Fu Jinmin and Huang, 2001; Reddy et al., 2004), causing accumulation of ROS which induces oxidative stress in proteins, membrane lipids and other cellular components. Likewise, Parida and Das (2005) reported that the total chlorophyll content decreases in tomato under higher salt stress. Reduction in uptake of minerals (e.g. Mg$^{2+}$) needed for chlorophyll biosynthesis also reduces the chlorophyll concentration in the leaf (El-Desouky and Atawia, 1998). Further in another study, Fahad and Bano (2012) also noticed that salt stress significantly decreased the total chlorophyll content of leaves of maize plant. El-Hendawy et al. (2005 a) reported that the substantial reduction in the growth of salt tolerant wheat genotypes under salinity stress was primarily related to a decline in photosynthetic capacity, rather than a reduction in leaf area. Zarco-Tejada et al. (2003) also mentioned that the negative impact of salinity stress on the photosynthetic apparatus, especially photosystem II (PSII), could be detected before the irreversible morphological damage is visible. Moreover, the buildup of Na$^+$ and Cl$^-$ in the leaf blade to toxic concentrations is known to reduce the photosynthesis rate through the destruction of chlorophyll ultrastructures and an inhibition of PSII at both donor and acceptor sites (Chen and Murata, 2011). Furthermore, any environmental stress that has a negative effect on the operating efficiency of PSII was shown to have an effect on chlorophyll fluorescence (Naumann et al., 2008). Zhang et al. (2011) also mentioned that the early detection of chlorophyll fluorescence could be used as an indicator to avoid loss of plant biomass under high salinity conditions. This close relationship between salinity stress and photosynthesis efficiency allows us to use all parameters related to the photosynthetic apparatus as useful screening criteria for distinguishing salt-tolerant genotypes from salt-sensitive ones.

In contrast to salinity stress, application of SA, ASA and SSA to salinity stressed C. arietinum increased photosynthetic pigments. In our study, reduction in photosynthetic pigments was mitigated by the foliar application as well as seed priming with salicylates (Tables 4.10-4.18 and 4.40-4.48). Similarly, Abreu and Munne-Bosch (2009) also revealed that SA deficiency is associated with reduced damage to the photosynthetic apparatus as well as chlorophyll levels. It has been reported that pretreatment of Brassica napus with SA following drought stress caused
significant increases in chlorophyll content (Maryam et al., 2012). El Tayeb (2005) found that SA application to barley induced a pre-adaptive response to salt stress, enhanced the synthesis of chl a, chl b and carotenoid and maintains membrane integrity, leading to improvement of plant growth. SA-pretreated plants exhibited less Ca\(^{2+}\) and more accumulation of K\(^+\) and soluble sugars in roots under saline conditions. Zea mays treated with SA exhibited increased growth, decreased lipid peroxidation and membrane permeability, which were increased by salt stress (Gunes et al., 2007). Tomato plants treated with SA via root drenching increased the accumulation of photosynthetic pigments, the K\(^+\) concentration and the soluble sugar concentration (Wasti et al., 2012). In addition, Khan et al. (2003) demonstrated that foliar application of SA increased photosynthetic rate in corn and soybean. Our results are in concordance with Moharekar et al., 2003 for wheat and Yildirim et al., 2008 for cucumber. Furthermore, Nazar et al., 2011 reported that SA alleviates salt-induced decrease in photosynthesis and minimizes the leaf Na\(^+\), Cl\(^-\) and H\(_2\)O\(_2\) content in mungbean plants. In addition, exogenous application of SA improves grain yield under salt stress in T. aestivum (Afran et al., 2007). Application of SA via root drenching protected Lens esculentum plants against NaCl stress and increased photosynthetic rates under salt stress (Stevens et al., 2006; Poor et al., 2011). The inhibition of salt-induced plant growth and photosynthetic capacity of the Medicago sativa were alleviated by pre-treatment with SA (Palma et al., 2013). Strawberry plants treated with SA exhibited greater growth, as did higher chlorophyll concentrations under salt stress (Karlidag et al., 2009).

The role of legumes in nutrition has been recognized as a relevant source of plant protein together with other benefits for health. Soybean, peanut, common bean, pea, lupine, chickpea, faba bean, lentil, grass pea, cowpea, and pigeon pea are currently the most important legumes for human consumption and animal feed (De Ron, 2015). Soluble protein content of chickpea plants as influenced by various levels of salt treatment and salicylates application is presented in Tables 4.19-4.27 and 4.49-4.57. Protein accumulations are important for cell survival under salt stress and causes membrane stabilizations. In response to salt stress, plants make new proteins that help them to grow and develop under saline conditions. Sarkar et al. (2013) reported the occurrence of degradation and oxidation of proteins under salt stress. Furthermore, Song et al. (2011) added that alleviation of degradation of proteins occurs by interactions between SA, NO and ABA.
under salt stress. Noctor and Foyer (1998) reported that free radicals produced under salt stress conditions may damage the proteins and reduces its content. The reduction in protein content under salinity stress might be due to decrement in photosynthetic activity, leading to inhabitation of some essential material for protein synthesis resulting in dramatically reduction or even inhibition of protein synthesis (Mohammadkhani, 2008; Karimi et al., 2012). The gradual decrease in protein content during water deficiency was induced by proteolysis or decline in some essential mineral for protein synthesis which uptake with water as nitrogen compounds (Lqbal and Bano, 2009; Costa et al., 2011). Salicylic acid increases the uptake of potassium in salinity conditions, potassium being essential for protein synthesis. Moreover, salinity causes production of ROS in plants lead to oxidative damage which causes the loss of proteins and nucleic acids (Kim et al., 2005). Higher protein concentration could be due to higher efficiency of the osmotic regulation mechanism in mungbean plants which in turn prevents protein reduction under salt stress (Flowers and Yeo 1995; Kumar et al., 2010) and induces the synthesis of osmotin like protein structure. This protein increment lead to membrane stabilization and helps plants to grow and develop under saline conditions (Goudarzi and Pakniyat, 2009). One of the adverse effects of salt stress on plants is disrupting the balance and quality of proteins (Cachorro et al., 2003). Soluble proteins play a main role in osmotic adjustment under NaCl stress and can provide storage form of nitrogen (Singh et al., 1987). Increase in soluble protein content under stress may be the result of enhanced synthesis of specific stress-related proteins (Doganlar et al., 2010).

In the present study, seeds primed with SA significantly affected the amount of protein under stressed as well as non-stressed conditions. SA enhances antioxidant system and reduces the destructive effects of ROS on proteins in soybean plants (Ghorbani et al., 2004). Application of SA in soybean would increase protein content (Kumar et al. 1996). Previous reports by Zhang et al. (2015) showed that exogenous SA application could improve N remobilization efficiency by up-regulating protein degradation and amino acid transport in leaves of B. napus. Also, Jakhar and Sheokand (2015) revealed the effect of foliar application of SA on protein content of soybean plant. Kumar et al. (2000) showed that SA increase the protein content inside the plant cells that make the plant have the ability to tolerate salt stress. Naz (2008) noticed that sunflower plants produce proteins in response to abiotic and biotic stresses and many of these proteins are
induced by phytohormones including salicylic acid. Arfan et al. (2007) showed that salinity significantly reduced the mean protein in wheat under salinity stress, but application of salicylic acid significantly increased protein content in plants. SA affected the processes related to seed quality including protein biosynthesis, seed primary metabolism and transport of seed storage proteins which were increased like seed vigour (Rajjou et al. 2006). Furthermore, Akbari et al. (2011) investigated that increasing the protein content of bacterial inoculation of seeds and grow through increased efficiency due to phytohormones are produced. Similar findings were obtained by Abd El-Wahed et al. (2006), working in yellow maize, SA application led to significant effect on biochemical constituents such as crude protein, oil and carotenoid content also. The present study established that priming of chickpea seeds with SA could encompass appropriates results under salt stress.

Salt-stressed chickpea leaves accumulated higher levels of H$_2$O$_2$ and MDA contents, thereby increasing electrolyte leakage, which might be due to membrane destruction caused by ROS-induced oxidative damage and exogenous application of salicylates reduced electrolyte leakage and the levels of MDA in NaCl-treated chickpea plants (Tables 4.19-4.27 and 4.49-4.57). Our experimental results indicated that the end product of lipid peroxidation i.e. MDA content accumulated under salinity stress and exogenous application of SA lowered the MDA content. Our findings are in agreement with those of Kukreja et al. (2005) who noticed significant enhancement in lipid peroxidation in Cicer arietinum roots under salinity stress. Additionally, stress-induced enhanced lipoxygenase was shown to be responsible for higher lipolytic activity and oxidation of lipid moieties in membranes (Kazemi et al., 2010). Similar increase in MDA content has also been noted in Cicer arietinum L.cv. Gocke (Eyidogan and Oz, 2007). Membrane damage is sometimes taken as a single parameter to determine the level of lipid destruction (i.e. lipid peroxidation ). The peroxidation of lipids is considered as the most damaging process known to occur in every living organism. Small hydrocarbon fragments such as ketones, MDA are formed by lipid peroxidation (Weckx and Clijsters 1996). Consistent rise in lipid oxidized products were also measured in salt-affected seedlings of C. sativus L. (Naliwajski and Sklodowska, 2014). Lipid peroxidation increased with salinity levels on different plant species (El-Beltagi et al., 2008, Sadak et al., 2010). Zhang et al. (1996) stated that salinity could stimulate O$_2$ production and modify the membrane structure, which facilitates lipid peroxidation.
Moreover, SA reduces MDA after the priming of faba bean under saline conditions (Azooz, 2009). Our results are in harmony with that of Agami (2013) who has observed the alleviation in adverse effects of NaCl stress in maize seedlings by pretreating seeds with salicylic acid. Like ours, significant fall in lipid peroxidation reaction was also noticed following exogenous application of SA in salt-stressed seedlings of Vigna. radiata L. (Khan et al., 2014). The similar results were also observed by Efimova et al. (2014) on B. napus L. with brassinosteroids under salt stress. Seed priming neutralized the harmful effects on lipid peroxidation of membranes (Hsu et al., 2003). Another study by Sairam and Saxena (2000) also suggested that oxidative stress caused by water deficit conditions leads to increase in lipid peroxidation which will be subsequently terminated to membrane injury. Membrane damage is suggested as a consequent incident caused by reactive oxygen species (Alscher et al., 1997; Becana et al., 1998). The application of SA improved barley plant growth by maintaining membrane integrity (El-Tayeb, 2005). The lipid peroxidation and membrane permeability were decreased by SA in maize under salinity stress, leading to the enhancement of plant growth (Gunes et al., 2007). Basra et al. (2004) reported that priming increased the SOD and CAT activities in plants. Lipid peroxidation and membrane permeability, which were increased by salt stress, were lowered in SA treated plants (Horvath et al., 2007). The decrease of MDA in tomato leaf tissues under SA application is consistent with that of reported by Stevens et al. (2006). Kabiri et al. (2014) mentioned that pretreatment with SA was evident by a reduction in the level of lipid peroxidation and leakage of electrolytes from plant tissues as well as by more intensive growth processes as compared to control plants. In addition, SA can diminish the injuries in cell membranes through enhancing the antioxidant potential of plant under stress conditions and partly maintained membrane permeability as well as reduced the amount of ion leakage (Tagin et al. 2006; Orabi et al. 2010; 2013). Azevedo Neto et al. (2005) reported that addition of H₂O₂ to the nutrient solution induced salt tolerance by enhanced activities of antioxidants and reduced peroxidation of membrane lipids in leaves and roots of maize. Seed priming also neutralized the harmful effects on lipid peroxidation of membranes (Bailly et al., 2000; Hsu et al., 2003).

One of the main consequences of salinity stress is the loss of intracellular water. Plants accumulate many metabolites that are also known as “compatible solutes” in the cytoplasm to increase their tolerance against salt stress-induced water loss from the cells. According to Hong et al. (2000), proline is synthesized by the enzyme pyrroline-5-carboxylate synthetase and
pyrroline-5-carboxylate reductase and is subsequently metabolized by the enzyme proline dehydrogenase. In response to salinity, proline accumulation protects the cells by balancing the osmotic strength of cytosol with that of vacuole and the external environment [Kavi et al., 2005, De-Lacerda et al., 2003]. Our study showed that proline content varied in chickpea plants at all the levels of NaCl treatment. Data of the proline content of chickpea plants as predisposed by various treatments of NaCl treatments are presented in Tables 4.19-4.27 and 4.49-4.57. In the present investigation, the proline content was found to be more at highest level (150 mM) of salinity. Similar results were obtained by Garg and Baher (2013) in C. arietinum under salt stress. Under saline conditions, plants accumulate proline as a non-toxic and protective osmolyte to maintain osmotic balance under low water potentials (Ashraf and Foolad, 2007; Parida et al., 2002).

To overcome the negative impacts of salt stress-induced osmotic stress, plants produce higher levels of osmolytes in the cytosol and other organelles (Abdel Latef and Miransari, 2014). In the present study, a similar accumulation trend of proline and total soluble proteins was recorded in chickpea leaves under NaCl stress. Increased accumulation of total soluble proteins in response to saline stress was reported by Liu et al. (2016) in Nitraria tangutorum. Proline also act as a reservoir of energy and nitrogen for utilization during salt stress conditions (Goas et al., 1982). Proline was also reported to accumulate under salt stress in B. juncea (Siddiqui et al., 2008), linseed (Linum usitatissimum) (Khan et al., 2010) and mulberry (Morus alba) (Ahmad et al., 2014). Proline and glycine betaine (GB) are important osmolytes that help in cell osmoregulation under salt stress (Ahmad et al., 2010, 2015). Several roles have been attributed to this supraoptimal level of proline; for instance, osmoregulation and detoxification of free radicals (Kaul et al., 2008). Hoque et al. (2008) showed that proline improves salt tolerance in N. tabaccum by increasing the activity of enzymes involved in the antioxidant defense system. Proline is also reported to protect photosynthetic machinery and act as energy storage under NaCl stress (Khan et al., 2013; Reddy et al., 2015). Proline has the ability to scavenge ROS and shield the cell from the oxidative damage (Ahmad et al., 2010; Khan et al., 2010; Jogaiah et al., 2013). Verdoy et al. (2006) have reported that proline accumulation enhanced the N₂ fixation in Medicago truncatula plants under salt stress. Thus, application of NO to salt-stressed chickpea plants provoked a remarkable increase in levels of proline, perhaps to provide a better protection to plants exposed to stress. Proline also induces the expression of salt-stress-responsive proteins
and may improve the plant adaptation to salt stress (Khedr et al., 2003). Proline stabilizes many functional units such as complex II electron transport by protecting the photosynthetic apparatus (Ashraf et al., 2008), by functioning as an oxygen radical scavenger (Heuer, 2003) and by displaying an antioxidant activity (Okuma et al., 2004). Ghoulam et al. (2002) also suggested an increase in the level of proline under NaCl stress in sugar beet. It is also reported that proline content of leaves in drought sensitive and tolerant chickpea cultivars may be increased (Mafakheri et al., 2010).

Priming with SA in chickpea plants under saline conditions caused a considerable increase in proline content (Asadi et al., 2013). It is also noteworthy that follow up treatment of the salinity stressed plants with SA caused upto 112% increase, over control in the proline pool (Tables 4.19-4.27). These observations are in conformity with those of El-Tayeb (2005) who suggested that proline can be considered as an important component in the spectra of SA-induced protective reaction of plants to salinity. Results showed that priming with SA caused a considerable increase in proline which are in agreement with those obtained by Sahar et al. (2011) for Salvia officinalis. Janda et al. (2007) attributed the increased level of ABA and proline to the development of anti-stress reactions, induced by SA. Shakirova et al. (2003) reported that SA improved wheat plant growth and promoted the accumulation of ABA and proline. Also, Sakhabutdinova et al. (2003) investigated the effect of SA on plant resistance against environmental stress factors. Hayat et al. (2005) reported that SA application increases plant proline content; this is in accordance with our results, where foliar SA application increased proline synthesis and thus content in leaves. Salicylic acid has a protective role and induces high proline accumulation under drought stress conditions (Umebese et al., 2009). Baghizadeh et al. (2009) observed that SA application accumulated more proline in the leaves of okra in comparison to ascorbic acid application.

Salt stress induces the generation of huge amount of ROS, leading to the abnormalities at cellular level due to oxidation of proteins, lipids and nucleic acids (Schutzendubell and Polle, 2002; Ahmad et al., 2008, 2010; Hayat et al., 2012). However, plants are capable to deal with such stressful conditions through increasing synthesis of antioxidant metabolites, including proline, and antioxidant enzymes, such as SOD, CAT, APX and GR (Schutzendubell and Polle, 2002; Ahmad et al., 2008, 2010; Hayat et al., 2012). In many plant studies, it was observed that
production of ROS is increased under saline conditions (Hasegawa et al. 2000). In the present study, the increase in the activities of SOD, CAT, APX and POD content in chickpea plants due to salinity was observed (Figures 4.1-4.72). NaCl stress is the generation of oxidative stress that results from increased level of ROS in cells exposed to stress (Schut zendubel and Polle 2002). The increase in activity of antioxidant enzymes (CAT, POD and SOD) following SA application could be the indicator of buildup of a protective mechanism to reduce oxidative damage induced by salt stress. Salinity causes oxidative stress by stopping the carbon dioxide assimilation, exposing chloroplasts to excessive excitation energy, which in turn increases the generation of ROS from triplet chlorophyll (Gosset et al., 1994). Heidari (2009) reported that antioxidant enzymes activity was induced by salinity in sorghum leaves. Fahad and Bano (2012) reported that the saline condition resulted in significantly higher SOD activity of leaves in maize plants. Salinity tolerance supported the activity of antioxidant enzymes, such as SOD and with the accumulation of non-enzymatic antioxidant compounds (Gupta and Huang, 2014). APOX is the most important peroxidase in H$_2$O$_2$ detoxification operating both in cytosol and chloroplasts (Mittova et al., 2000). Higher activities of SOD and CAT in wild tomato correlated with higher salt tolerance (Shalata et al., 2001)

Our results are supported by the observations reported by Hayat et al. (2012) in Solanum lycopersicum, Kausar et al. (2013) in T. aestivum and Manai et al. (2014) in S. lycopersicum. Biochemical and molecular studies of salt stress responses in plants have revealed significant increases of ROS, including singlet oxygen, superoxide, hydroxyl radical and hydrogen peroxide (Tanou et al., 2009; Ahmad et al., 2010a, 2012; Ahmad and Umar, 2011). Furthermore, co-application of NO with NaCl markedly increased the activities of SOD, CAT, APX and GR in chickpea plants, which is in harmony with previous findings reported in mustard (Khan et al., 2012), tomato (Hayat et al., 2012; Manai et al., 2014) and in cotton (Dong et al., 2014). However, the effect of salt stress on plants depends on the concentration and time of exposure of salt, plant genotypes and environmental factors. ROS are highly reactive and may cause cellular damage through oxidation of lipids, proteins and nucleic acids (Pastori and Foyer, 2002; Apel and Hirt, 2004; Ahmad et al., 2010a, b).

Consistent with the accumulation of antioxidant enzymes in chickpea plants under salt stress, the activities of SOD, CAT, POD and APOX enzymes examined was also up-regulated in
treated chickpea plants by salicylates (Figures 4.1-4.7). This result suggested that up-regulation of SOD, CAT, POD and APX genes might enhance activities of the SOD, CAT and APX enzymes, thereby providing better protection to the cells against oxidative stress triggered by high salinity (Lu et al., 2007; Yamane et al., 2010; Hu et al., 2012). Torabian (2011) reported that pretreatment with SA induced adaptive responses in Medicago sativa plant under salinity stress and consequently, encouraged protective reactions in biotic membranes which improved the growth of seedlings. In support of our finding, several studies also reported the up-regulation of antioxidant enzyme-encoding genes under stress with or without SA treatment. For instance, Hernandez et al. (2000) have reported the enhanced expression of SOD and APOX genes in a NaCl-tolerant Pisum sativum variety in comparison with the sensitive variety. Senaratna et al. (2000) have suggested a similar mechanism to be responsible for SA-induced multiple stress tolerance in bean and tomato plants. SA can play a critical role in modulating the cell redox balance, thereby protecting the plants against the oxidative damage (Yang et al. 2004). Catalase seems to be a key enzyme in salicylic acid induced stress tolerance since it was shown to bind salicylic acid in vitro (Chen et al., 1993). Peroxidase activity was increased by SA application in plants subjected to various abiotic stresses (Kang and Salveit, 2002; Popova et al., 2003). SA enhanced the antioxidant enzymes activities (POD, SOD and CAT) when sprayed exogenously to the salinity stressed plants (Szepesi, 2008; Yusuf, 2008). However, Tasgin et al., 2006 found that with SA treatment, catalase enzyme activity decreased, peroxidase activity increased in winter wheat leaves. Also, Chen et al. (1997) reported that SOD and POD activity increases while catalase activity decreases in response to SA application in rice under salinity. SA application during seed priming has been associated with enhanced SOD, CAT and APX activities in maize under chilling stress (Farooq et al., 2008).

Up-regulation of SOD gene expression has also been reported in other plants, including Lycopersicon esculentum (Aydin et al., 2014) and Lotus japonicus (Rubio et al., 2009) under NaCl stress. Zhang et al. (2014) showed the up-regulation of CAT and APX genes in Limonium sinense under high salinity. Menezes-Benavente et al. (2004) and Shafi et al. (2015) reported enhanced expression of APOX gene in rice and Arabidopsis, respectively, under salt stress. Therefore, it is reasonable to conclude that SA may activate the expression of antioxidant enzymes-related biosynthetic genes, which leads to accumulation of antioxidant enzymes, thereby providing better tolerance to plants under stresses. SA-stimulated pre-
adaptation state was beneficial in the acclimation to subsequent salt stress in *Solanum lycopersicum* (Gemes *et al.*, 2011). Similar results have been obtained in *B. juncea* (Hayat *et al.*, 2003) and wheat (Hayat *et al.*, 2005). Vaseva *et al.*, 2012 reported the involvement of ROS in salinity damages to macromolecules and cellular compartments and the role of enzymatic scavengers is quite important for preserving cellular integrity against oxidative damages. Kovacik *et al.* (2009) reported that SA-induced protein synthesis leads to stimulation of antioxidants. Garratt *et al.* (2002) reported that plants containing high concentrations of antioxidants show resistance to oxidative damage caused by activated oxygen species. SA application was shown to increase peroxidase activity in maize, cucumber and rice subjected to chilling stress (Kang and Salveit, 2002). Aggarwal *et al.* (2005) noticed the changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. ROS production mediated by guaiacol peroxidases was induced by SA in guard cells (Mori *et al.*, 2001; Khokon *et al.*, 2011). In *Vicia faba*, the improvement in salinity tolerance due to application of SA during seed priming was associated with enhanced CAT, APX and POD activities (Azooz, 2009). Pretreatment with SA causes low levels of ROS accumulation (Harfouche *et al.*, 2008). Shakirova *et al.* (2003) suggested SA-induced SOD activation in wheat plants was consistent with the results obtained with other plants like cucumber, maize and rice. Exogenous SA could regulate the synthesis and activities of antioxidant enzymes and increase plant tolerance to biotic and abiotic stress (He *et al.*, 2002; Fayez and Bazaid, 2014). In *B. juncea*, SA enhanced the level of SOD and POD under stress and stress-free conditions (Yusuf *et al.*, 2012). On the other hand, SA has ability to inhibit the activity of CAT that lead to rise in the level of H₂O₂ in vivo and stimulate the defense genes (Boukraa *et al.*, 2014). Further results proved that SA significantly enhanced the activities of SOD and CAT, both of which separately contributed to delay of H₂O₂ accumulation in wheat leaves under salt stress (Simaei *et al.*, 2011). Zhao *et al.* (2006) had similar findings and expressed that the increase in SOD activity and decrease in oxidative damage were closely related. Similar results were recorded by Rasool *et al.* (2013) who reported that APX activity increased the effects in chickpea seedlings by salinity stress. However, Liu *et al.* (2014) described that salt stress significantly decreased POD activity in cotton seedlings, but when SA was added to plants in salinity stress, POD activity increased. Guo *et al.* (2009) showed that pretreatment with SA increased the activity of GPOX in Cd-exposed rice plants.
Study revealed that grain yield, the ultimate objective of crop production, was negatively affected by salt stress. Lowest grain yield plant\(^{-1}\) was recorded for plants that were grown without salicylates and were exposed to salt (Tables 4.28-4.30). The effects of salt stress on plants ultimately lead to reduction of yield of crop which is the most countable effect of salt stress in agriculture. In the present study, salt stress significantly reduced the growth and biomass yield of chickpea plants, which is in harmony with earlier reports on different crops, such as wheat (*Triticum aestivum*) (Kausar *et al*., 2013), tomato (*Lycopersicon esculentum*) (Abdel Latef and Chaoxing, 2011), pepper (*Capsicum annuum*) (Abdel Latef and Chaoxing, 2014) and rice (*Oryza sativa*) (Mostofa *et al*., 2015). Baiburdi (2009) in their experiment of salinity stress on canola plant, grain weight loss was reported. Reduction in chickpea seed yield by means of terminal drought stress has been previously reported (Behboudian *et al*., 2001; Fallah *et al*., 2005). Similar results obtained by Mafakheri *et al*. (2010) reported that drought stress had considerable effects on the number of pods in chickpea plants. Nahar and Hasanuzzaman (2009) reported that yield components of *V. radiata* were significantly affected by salinity stress. Number of pods per plant, seeds per pod and seed weight were negatively correlated with salinity levels. Grain yield reduction of rice due to salt stress is also reported by Linghe and Shannon (2000) and Gain *et al*. (2004).

Seed priming and foliar spray with PGRs has potential to improve yield and harvest quality under stress conditions (Halmer, 2004). Similarly, Zheng *et al*. (2002) showed that seed priming produced more number of tillers per unit area in wheat and rice over non-primed. Rashid *et al*. (2004) suggested the effects of seed priming in improving yield of mungbean. Basra *et al*. (2003) studied the effect of priming on yield and yield components of canola and observed that number of branches per plant was significantly affected by different priming treatments. Majd *et al*. (2006) showed that chickpea plants treated with SA significantly increased the yield parameters. Salicylic acid appears to increase stress resistance by increasing the activity of enzymes act to deal with stress which leads to an increase in yield components and grain yield can be increased accordingly. The effect of ASA on yield parameters has been reported by Canakc and Munzuroglu (2000) who mentioned that ASA administration to leaf caused an increase in fresh and dry weight gain of radish. These results are in agreement with those of Singh *et al*. (2002) on onion plants. Maddah *et al*. (2006) reported the application of SA in forms of spray and irrigation treatments showed positive effect on increasing the yield and its
components. Several studies pointed out to the positive response of yield to foliar fertilization (Amal et al., 2010 on fenugreek). Karim and Fattah (2004) found that number of pods per plant increased by Knap in chickpea. Similar results were also measured by Iqbal et al. (2001) by foliar application of gibberellic acid in chickpea plants. Likewise, Khan et al. (2001) studied the biological effect of gamma irradiation and its modulation with gibberellic acid in chickpea. The results are in good agreement with those reported by Hameed et al. (2004) who stated that exogenous application of H$_2$O$_2$ provided more vigorous root system in wheat, that can be used to increase nitrogen uptake resulting in better growth and yield (Liao et al., 2004). Number of pods per plant is an important yield feature for pulses. In the present study, the number of pods per plant showed a close similarity with the findings of Mut and Gulumser (2001) and Bozoglu and Ozcelik (2005). Regarding SA, foliar application of SA significantly increased yield and its components of maize (Abdel-Wahed et al., 2006) and wheat plants (Iqbal and Ashraf, 2006). Foliar application of SA significantly enhanced the fruit yield in tomato and cucumber (Martin-Mex and Larque-Saavedra, 2007). However, according to Bhatia et al. (1993), pods per plant was highly variable yield component compared to seeds per pod, which was relatively less variable.