Effect of exogenous application of triacontanol on wheat 
(*Triticum aestivum* L.) under salt stress

By

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*An thesis submitted in partial fulfillment of the requirements for the degree of*

DOCTOR OF PHILOSOPHY
IN BOTANY

DEPARTMENT OF BOTANY
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Faisalabad 
2012
DECLARATION

“I hereby declare that the contents of the thesis, entitled “Effect of exogenous application of triacontanol on wheat (Triticum aestivum L.) under salt stress” are product of my own research and no part has been copied from any published source (except the references, standard mathematical or genetic models/equation/formulae etc.). I further declare that this work has not been submitted for award of any other diploma/degree. The University may take action if the information provided is found inaccurate at any stage”.

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We, the supervisory committee, certify that the contents and form of this thesis submitted by Shagufta Perveen D/O Muhammad Ibrahim, Reg. # 2005-ag-47 have been found satisfactory, and recommend that it be processed for evaluation by the external examiner(s) for the award of the degree.

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DEDICATION

Dedicated to
My
Mother and Father
May Allah my father’s soul rest in peace (Aamin)
ACKNOWLEDGEMENT

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ABSTRACT

In order to investigate the effect of exogenous application of triacontanol (TRIA) on two wheat (Triticum aestivum L.) cultivars [S-24 (a salt tolerant) and MH-97 (a moderately salt sensitive)] under salt stress, two independent experiments were conducted in a greenhouse. Both wheat cultivars were grown in full strength Hoagland’s nutrient solution under non-saline (0 mM NaCl) and saline (150 mM NaCl) conditions. Three optimized TRIA levels (0, 10 and 20 µM) were used both as foliar sprayed at three growth stages i.e. vegetative, boot and veg. + boot stages and as seed-priming. In foliar-spray set of experiments ninety two-day old, while in seed-priming experiment twenty four-day old plants were subjected to data analysis for various growth, physiological and biochemical attributes. Salinity stress adversely affected growth and yield (shoot and root fresh and dry weights, total leaf area per plant, shoot and root length, grain yield, number of grains and number of tillers per plant, 100-seed weight), photosynthetic rate (A), transpiration rate (E), stomatal conductance (gs), chlorophyll contents (chl. a, b and a/b ratio), leaf water relations (water potential, osmotic potential and turgor potential), relative water content (%), electron transport rate (ETR), shoot and root K+ and Ca2+ ions and K+/Na+ ratios, superoxide dismutase (SOD) activity, peroxidase (POD) activity (in cv. MH-97 only), Rooting medium salinity stress did not alter sub-stomatal internal CO2 concentration (Ci), Ci/Ca ratio, water use efficiency (WUE), efficiency of PS II (Fv/Fm), photochemical quenching (qP) and non-photochemical quenching exciton (NPQ) of both wheat cultivars, but increased co-efficient of non-photochemical quenching (qN), membrane permeability (%), hydrogen peroxide (H2O2), malondialdehyde (MDA), shoot and root Na+ and Cl- contents, activity of catalase (CAT), leaf soluble proteins and total free amino acids, free proline, glycinebetaine and total phenolic contents. Exogenous application of TRIA as a foliar spray significantly increased all growth and yield attributes, photosynthetic rate (A), stomatal conductance (gs), chl. a and b contents and ETR value, leaf water potential, decreased leaf osmotic potential at vegetative stage, while increased at boot stage of both cultivars at all growth stages, shoot and root K+ (cv. S-24) and Ca2+ in both cultivars, root K+/Na+ ratios in cv. S-24 under non-saline conditions, increased Cl- contents under non-saline, while decreased under saline conditions. Foliar spray of TRIA increased photochemical quenching at boot and veg. + boot stages under non-saline conditions, while decreased non-photochemical quenching exciton (NPQ) value in both cultivars at all growth stages, qN values only in cv. MH-97 at vegetative + boot stages, total phenolics at boot
and veg. + boot stages and shoot Na⁺ in both wheat cultivars under saline conditions. Pre-sowing seed treatment of TRIA did not improve growth or yield attributes, gas exchange characteristics, leaf osmotic and turgor potentials, and enzyme activities of SOD and CAT. However, TRIA application increased stomatal conductance under non-saline conditions and net CO₂ assimilation rate under saline conditions of both cultivars, while POD activity (both cultivars) and water potential (only cv. S-24) under both saline and non-saline conditions. Performance of salt tolerant cultivar S-24 was good in growth (shoot fresh and dry weights, and shoot length), stomatal conductance (gs), chl. a/b ratios and ETR value, leaf water relations, shoot and root K⁺, Ca²⁺ and Cl⁻ ions, K⁺/Na⁺ ratios, soluble proteins and free amino acids, free proline, in terms of foliar spray experiment. The design of both experiments was completely randomized with four replicates in each set.
CHAPTER 1
INTRODUCTION

Plant hormones are natural products that play a key role in the regulation of various plant responses to abiotic stresses and also known as plant growth regulators (PGRs) as they can be synthesized synthetically (Javid et al., 2011). These can be divided into two main classes: class one is called plant hormones as they control and regulate plant growth and development in all plants in very small quantities. The second group is called secondary plant growth substances some of which although present in small quantity but show high growth specificity (Mandava, 1979). Due to their role in the modulation of various physiological and biochemical processes, new plant growth regulators has been extensively studied in abiotic stress tolerance (Peleg and Blumwald, 2011).

Triacontanol (TRIA) is considered as a secondary plant growth compound/or an endogenous growth regulator (Mandava, 1979; Ries and Houtz, 1983) as within 10 minutes of its foliar application (10^{-15} M) the metabolic processes of maize and rice seedlings are changed (Ries, 1985). It is a long chain 30-carbon primary alcohol and is thought to promote growth and final productivity when exogenously applied to various crop species (Ries et al., 1977; Naeem et al., 2011). Chibnall et al. (1933) found it as a natural component of alfalfa (Medicago sativa L.) epicuticular waxes. It is also present in crops e.g., in wheat it comprises 3% of the total free alcohols (Kolattukudy and Walton, 1972; Tulloch and Hoffman, 1974).

Stimulation of photosynthetic processes, CO₂-fixation, increased uptake of water and nutrients, increased cell division and permeability of membranes are among major contributors to regulate plant growth by TRIA application (Ries, 1991; Savithry et al., 1992; Ivanov and Angelove, 1997; Kumaravelu et al., 2000; Khan et al., 2007). Effect of exogenous application of triacontanol can be tested in several different ways e.g. rooting medium, seed pre-treatment and foliar spray (Ries et al., 1978). The foliar application of TRIA between 1200 and 1700 h at three and four-leaf stage and anthesis has been found to increase growth and final yield of economically important crops such as rice, maize and wheat (Ries, 1991). Octacosanol, a 28-carbon analog of triacontanol did not show any
stimulatory effect (Ries et al., 1977; Hangarter et al., 1978). On the other hand, at very low concentration $2.4 \times 10^{12}$ M octacosanol inhibit the effect of triacontanol (Jones and Ries, 1979; Ries et al., 1978). In algae, cell number (21-35%), chlorophyll contents (7-31%) and CO$_2$ fixation (20-100%) increased when treated with 1-1000 µg L$^{-1}$ TRIA (Houtz et al., 1985a). In vitro treatment of isolated protoplast of pea plants with $10^{-6}$ M TRIA increased CO$_2$-fixation by 166% after 60 min, while in vivo treatment of pea seedling showed 119% increased CO$_2$ uptake compared to control (Ivanov and Angelov, 1997).

It has been suggested that increased growth and final productivity of various crop species due to TRIA application could be due to increased absorption and uptake of nutrients, enhanced photosynthetic rate, nitrogen fixation and finally increased translocation of photosynthetic products from site of synthesis to site of utilization (source-sinks relationship) (Singh et al., 2011; Naeem et al., 2011). The processes of photosynthesis and photorespiration are maintained in a balance way by treatment with TRIA as increased uptake of CO$_2$ (photosynthesis) and inhibition of O$_2$ (photorespiration) enhanced cell number and chlorophyll contents after 4 and 3 days, respectively (Haugstad et al., 1983). The primary photochemical reactions, leaf stomatal regulation, and activity of Calvin cycle’s enzymes like rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) are influenced by treatment with TRIA that leads to enhanced photosynthetic efficiency per unit surface area of leaf (Moorthy and Kathiresan, 1993; Borowski et al., 2000).

TRIA is involved in modulating the physical status and chemical composition of membrane lipids by differentially altering the lipid organization (Shripathi and Swamy, 1994; Swamy et al., 2009). TRIA is known to inhibits lipid peroxidation of thylakoid membranes of chloroplast (Ramanarayan et al., 2000) and increases the level of polyunsaturated fatty acids, monoglactosyldiacylglycerol (MGDG) and diglactosyldiacylglycerol (DGDG) after 24 h of treatment in cotton plants (Shripathi and Swamy, 1994), while MGDG is involved in the packaging of PS I proteins (Dominy and Williams, 1987; Shripathi and Swamy, 1994). It has a role in the down regulation of jasmonic acid-induced proteinase inhibitors (Ramanarayan et al., 2000).
Malik et al. (1990) are of the view that foliar application of aliphatic alcohols increase Calvin cycle enzyme PEP carboxylase activity and yield by increased supply of photoassimilates to developing kernels in peanut. Activity of certain other enzymes like nitrate reductase, tyrosinase (polyphenol oxidase) and carbonic anhydrase in hyacinth beans (Naeem et al., 2009), membrane bound ATPases of barley roots (Lesniak et al., 1986; 1989) and NADPH-oxidase in soybean hypocotyls has also been found to be increased by exogenous application of TRIA (Morre et al., 1991). Several other enzymes activities like isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase (increase 89% and 39%, respectively over control), peroxidase, acid phosphatase, alkaline phosphatase (remain relatively constant) and malate dehydrogenase (increase parallel with proteins) are regulated by TRIA after three day treatment in corn seedlings (Lesniak and Ries, 1983). Foliar application of TRIA effectively enhances the enzyme RUBPCase (ribulos-1,5-bisphosphate carboxylase) activity (11% over control), improve chlorophyll contents, cytoplasmic ribosomal RNAs, activity of thylakoid polypeptide, and chlorophyll-protein complexes of 4 day old radish seedling (Jin and Hong, 1988). TRIA response is very quicker as it increase increase dry weight, free amino acids, reducing sugars and soluble proteins of rice and maize plants within five minutes of application (Ries, 1991).

A combined foliar application of triacontanol (1.5 mg L⁻¹) and gibberellic acid (75 mg L⁻¹) increased the net photosynthetic rate (25.4%), stomatal conductance (14.1% ), internal CO₂ (15.4%) and promoted nitrate reductase (25.9%) and carbonic anhydrase (21.5%) enzymes activities compared to non-sprayed plants (Aftab et al., 2010). The cation Ca²⁺, Mg²⁺, and K⁺ concentrations are increased in tomato, maize and cucumber shoots by application of picomole amount of (+)-adenosine (Ries et al., 1993). L(+) -adenosine is appeared in the roots of plant, shoots of which are sprayed with nanomolar TRIA levels within one minute (Ries and Wert, 1988). Triacontanol treatment elicits i(+)-adenosine, a secondary messenger which acts by eliciting a signal that moves rapidly throughout the plant and increases the apoplastic ion concentration especially Ca²⁺ ions. The electrochemical gradient across plasmamembrane is thought to be developed due to change in ionic concentrations of either leaf or root cells in response to a stimulus (Ries et al., 1993) which stimulate the influx of Ca²⁺ into the cytoplasm (Ries et al., 1993) that bind to some receptor proteins like
calmodulin (Evans et al., 1991; Marme, 1986) and consequently regulate the activities of metabolic enzymes and growth to certain external stimuli (Ries et al., 1993). TRIA elicit several specific responses indirectly such as ATP production in rice and rape crops (Ries, 1991). TRIA is also involved in up and down regulation of many genes like it up-regulates the genes for photosynthetic process and suppress stress-related genes in rice plants (Chen et al., 2002). It is reported that TRIA elicits the second messenger TRIM (9-β-L (+)-adenosine), derived from AMP, ADP or ATP at the tonoplast in the root tissue of rice (Oryza sativa L) seedlings at low concentration (Ries et al., 1990; Ries, 1991). Gatica et al. (2008) found that this TRIA induced increase in adenosine level enhance cytokinins synthesis that results in cell division.

Foliar spray of TRIA has been reported to reduce the effect of environmental stresses on various physiological and biochemical processes like fresh and dry biomass, photosynthetic pigments, CO2-assimilation, carbohydrates, soluble proteins, activities of photosystem I and II, electron transport rate and rubisco that increase in Erythrina variegata seedlings subjected to various stresses including salt stress (Muthuchelian et al., 1994, 1995, 1996, 1997, 2001, 2003). Soyabean plants that are subjected to moderate salinity stress regained normal metabolic processes by foliar application of TRIA (Krishnan and Kumari, 2008). Pre-sowing TRIA treatment has also been found to be non-effective on promoting growth of various weed species that has been suggested to be lack of its penetration through seed coat. Furthermore, its role in very low concentration at various growth stages of different plant species and inhibitor effect at higher doses elucidate the TRIA properties (Hoagland, 1980). It has been suggested that optimal concentration of triacontanol and plant age are among important factors that control growth and final yield of various plant species (Sagaral et al., 1978).

A soil is said to be saline when the soluble salt content reaches to a level which is harmful for the crops (Frary et al., 2010). Salinity stress is one of the major abiotic stresses which decreases plant growth and severely reduces the productivity and yield of many crops globally (Grewal, 2010; Mehta et al., 2010) and especially in semi-arid regions of the world (Al-Karaki, 2006). According to FAO (2010) out of 6.8 billion current world’s population about 925 million (13.6 %) people are undernourished mainly due to poor agriculture. Over
800 million hectares of land throughout the world is salt affected (FAO, 2008) that covers more than 6% of total land area of world (Munns and Tester, 2008). About 2 Mha (1%) agricultural land of world deteriorates every year due to salinity (Tuteja, 2007). Pakistan is situated within the subtropical region and has semi-arid to arid climate (Ashraf and Fatima, 1994; Lin et al., 1997; Khan et al., 2001). The salinity problem is serious in Pakistan as about 5.7 Mha arable lands are turned to saline due to use of poor irrigation system (Mujtaba et al., 2003).

A large number of metabolic disorders occur in plants as a result of salinity stress which are due to ion toxicity, osmotic stress, nutrient deficiency and inhibition of gas exchange which adversely affect various physiological and biochemical processes (Ashraf, 1994; Munns, 2005; Frary et al., 2010). Salinity stress affects plant growth in two ways (i) osmotic effect that reduces availability of water to plants and reduce growth of young leaves (ii) ionic effect that increase the senescence rate of older leaves (Munns and Tester, 2008). Both type of damages are caused by two primary ions such as sodium (Na⁺) and chloride (Cl⁻) that decrease growth and yield of crops significantly (Munns and Tester, 2008). The influx of excess Na⁺ induces K⁺ leakage by depolarization of cell membrane (Shabala et al., 2006; Volkov and Amtmann, 2006) and severely inhibits the uptake of K⁺ and Ca²⁺ (Grattan and Grieve, 1999). The deficiency of K⁺ and Ca²⁺ ions reduces stomatal regulation, photosynthesis and growth under salinity stress (Tavakkoli et al., 2010). However, in wheat and other crop species low level of Na⁺ uptake and its accumulation in roots and leaves tissues and high K⁺/Na⁺ is considered to be associated with salt tolerance (Dogan et al., 2010).

Photosynthesis is one of the key physiological processes of plant growth which is directly affected by salinity (Munns et al., 2006; Liu and Huang, 2008; Chaves et al., 2009; Gorai et al., 2010; Mathur et al., 2010; Mehta et al., 2010). The osmotic phase can be determined by stomatal conductance measurements (James et al., 2008) as stomatal aperture decreased under salinity stress which reduces CO₂ availability for photosynthetic process in plants (Seemann and Critchley, 1985; Flexas et al., 2004, 2007, Chaves et al., 2009).
In addition to impairment of photosynthesis, salinity stress induces changes in leaf water relations and osmotic homeostasis which decreases water uptake by plants (Boursiac et al., 2005; Munns, 2005; Silva et al., 2010). However, plants have developed mechanisms to maintain photosynthetic capability and to escape fluctuations in their water potential under stressful conditions (Ashraf, 1999, 2004; Koryo, 2006). As salinity stress damage and tolerance is a multigenic response which involves the regulation of a large number of physiological and biochemical processes (Niknam and Mccomb, 2000; Munns, 2002; Flowers, 2004; Sairam and Tyagi, 2004; Kumar et al., 2009; Shabala et al., 2010).

One such tolerance mechanism is the osmotic stress tolerance by accumulation of various compatible osmolytes such as proline and glycinebetain under salinity stress (Moghaieb et al., 2004; Koka et al., 2007; Munns and Tester, 2008; Moller et al., 2009; Silveira et al., 2009; Teakle and Tyerman, 2010) that protect plant by scavenging oxygen-free radicals and also protects enzymes when plants subjected to salt stress (Ashraf, 2004; Munns and Testor, 2008; Huang et al., 2009a, 2009b). Glycinebetaine and proline protect the quaternary structure of proteins; maintain enzyme activity, membranes structure and photosynthetic machinery from the damaging effects of salinity stress (Yoshiba et al., 1997; Silva et al., 2009; Chen and Murata, 2011). The relative contribution of these osmolytes varies among species or even among cultivars of a same species (Chen et al., 2007; Ashraf and Foolad, 2007; Cha-um et al., 2007).

The disturbance of ionic homeostasis produces a chain of oxidative stress in plants under salinity stress (Zhu, 2001; Neto et al., 2006; Miller et al., 2010). Peroxidation of membrane lipid is an indication of membrane damage and malondialdehyde (MDA) is a product of oxidative damage at cellular level under salt stress (Wang et al., 2010). However, plants have natural tendency to induce a complex antioxidative defense system that reduces the level of oxidative stress in plants (Azooz et al., 2009). Plants also synthesize phenolic compounds through phenylpropanoid pathway and can be induced by biotic and abiotic stresses (Kim et al., 2006). Under salt stress, the contents of phenolic compounds changed depending upon the sensitivity of plant species (Kim et al., 2008). However, plants can protect themselves by an active antioxidant enzyme system under salt stress (Wang et al., 2010).
Cereal crops all over the world’s cultivated area are adversely affected by subsoil salinity or sodicity (Rengasamy, 2010). In Pakistan, wheat yield is low on account of many biotic and abiotic factors and about 60% yield loss occurs due to soil salinity and associated problems of sodicity (http://www.technologytimes.pk/2011/06/18/critical-issues-in-agriculture-of-pakistan/). Wheat is a major food crop used as staple diet of the people of Pakistan. It is grown on all kinds of soils and is considered as a salt tolerant with a salinity tolerance threshold (ECe) level between 6-8.6 dS m⁻¹ (Francois and Maas, 1999).

Development of salinity tolerance in wheat is an important goal to fulfill the needs of growing world’s population with limited agricultural area to support it (FAO, 2010). Salinity stress tolerance trait is multigenic and complex that could be controlled and engineered (Poltronieri et al., 2011). Up till now many strategies are examined to increase plant growth under salinity stress and to develop salt tolerant genotypes is one of those strategies. To develop salt tolerance in plants through methods of conventional breeding is time taking and laborious. Lot of effort has also been done through management practices. Similarly to alleviate salinity stress effect in crop species various strategies has been adopted in different reports (Turkan and Demiral, 2009; Ashraf, 2009). However, exogenous application of plant growth regulators by different ways (seed priming, rooting medium and foliar spray) is an alternative and effective approach to improve crop salt tolerance (Ashraf et al., 2008; Javid et al., 2011).

It has been suggested that salinity stress decrease the synthesis or cause degradation of plant growth regulators (PGRs) (Kuiper et al., 1988; Debez et al., 2001). However, exogenous application of plant growth regulators as foliar spray or through seed priming can improve their deficiency (Ashraf et al., 2008). As foliar spray PGRs improved growth, physiological and biochemical processes of different crop species under salt stress in a number of studies. For example, brassinosteroids in wheat (Shahbaz et al., 2008; Eleiwa et al., 2011), salicylic acid in tomato and faba bean (Stevens et al., 2006; Khafaga et al., 2009), 28-homobrassinolide in mustard (Hayat et al., 2000), 24-epibrassinolide in pepper (Houimli et al., 2008, 2010), 5-aminolevulinic acid in Brassica napus (Naeem et al., 2010), gibberellic acid in green gram (Akbari et al., 2008), and putrescine, ascorbic acid and thiamine in gladiolus plants (Nahed et al., 2009).
Through pre-sowing seed plants can be made more resistant to environmental stresses (Conrath et al., 2006) and have advantage to combat a specific stress effect more rapidly through increased defense responses (Ton et al., 2007). The role of plant growth regulators to combat various abiotic stresses in plants has been investigated when applied exogenously (Hasan et al., 2008; Hayat et al., 2008). In wheat, many plant growth regulators like cytokinins, brassinolide, auxin, polyamines, gibberellic acid has been used as seed priming agent to promote growth and yield under salt (NaCl) stress (Iqbal et al., 2006; Shahbaz et al., 2008; Iqbal and Ashraf, 2005, 2007, 2010).

However, there is no information available on the effect of exogenous application of triacontanol on wheat as foliar spray and as pre-sowing seed treatment under saline conditions. So the present study was made to assess the effect of TRIA on wheat with the following objectives.

- To assess whether triacontanol could ameliorate the adverse effects of salt stress on wheat.
- To examine the pattern of changes under different levels of exogenously applied triacontanol in wheat under salt stress.
- To determine which way of triacontanol application (Foliar spray, seed priming) could be most effective in modulating growth of wheat under salt stress.
CHAPTER 2
REVIEW OF LITERATURE

Soil is a valuable resource with a key productive role in agriculture, as it is required to produce crops, vegetables, fruit and other economically important items (Mane et al., 2010). Salinity is a general term that is used for different soluble salts in soil and water (Mane et al., 2010). The major ions are sodium (Na\(^+\)), chloride (Cl\(^-\)), magnesium (Mg\(^{2+}\)), calcium (Ca\(^{2+}\)), sulphates and bicarbonates. Soil salinity is due to Na\(^+\) and Cl\(^-\) anions that contribute 50-80% of total soluble salts in soil (Rengasamy, 2010). It is mainly divided into three types: low salinity (ECe 2-4 dS m\(^{-1}\)), moderate salinity (ECe 4-8 dS m\(^{-1}\)) and high salinity (ECe > 8 dS m\(^{-1}\)) (Rogers et al., 2005).

Soil salinity occurs by two processes natural or primary, and human-induced or secondary soil salinity. Primary soil salinity originates from the nature by weathering of parent rocks that contain soluble salts and deposit to form the basin of oceans through wind and rain carriers. Secondary salinity occurs through human based activities (Ghassemi et al., 1995). For example, excess irrigation, exploitation of natural resources such as overgrazing and land clearing lead to salinization and desertification of already productive land throughout the world (Rengasamy, 2006; Li et al., 2007; Turkan and Demiral, 2009). Secondary soil salinity problem become more sever due to poor quality water usage in agriculture with inadequate soil management practices (Ferreira-Silva et al., 2009). Out of 1500 million ha (Mha) about 32 Mha (2%) are affected by secondary soil salinity (FAO, 2008).

Abiotic stress is the negative impact of nonliving factors on the living organisms in a specific environment (Mane et al., 2010). According to a recent report released by FAO (2010) of the current 6.8 billion world’s population about 925 million (13.6 %) people are undernourished due to poor agriculture. Crop yield losses are mainly due to abiotic stresses (Munns and Tester, 2008; Reynolds and Tuberosa, 2008). Salinity contributes to yield loss by 40%, drought 17%, high and low temperature 20% and 15% respectively and some other factors 8% (Rehman et al., 2005; Ashraf et al., 2008). Rapidly growing population growth especially in the developing countries and resulting increased demand for agricultural commodities have made salinity problem more sever (Hamayun et al., 2010).
2.1 Status of salinity in the world

Soil salinity is a major problem throughout the world especially in arid and semi-arid regions (Al-Karaki, 2006; Tuncturk et al., 2011). Saline water occupies 71% (FAO, 2007), while dryland more than 6% (800 Mha) of planet earth (FAO, 2008; Munns and Tester, 2008). According to an estimate about 20% (45 Mha) out of 230 Mha of irrigated land is salt affected, while 1% (2 Mha) is being deteriorate every year due to salinity (Tuteja, 2007). There are reports which show that by the middle of 21st century about 50% arable land will become saline (Wang et al., 2003; Mahajan and Tuteja, 2005; Manchanda and Garg, 2008). In Pakistan, approximately 6.3 Mha of land is salinity affected (Alam et al., 2000) and irrigation contributes approximately 3.45 Mha (Anonymous, 2005). According to another survey, of total 16.795 Mha irrigated land, 73% is non-saline, 10% slightly saline, 4% moderately saline, 7% strongly saline and 6% is miscellaneous type land (Anonymous, 2007). Every year about 40,000 ha productive land in Pakistan is destroyed by salinity that is equal to 110 ha per day (Alam et al., 2000). According to a report by World Bank (1992) soil salinity is decreasing 25% potential productive loss of major crops and cause loss to about two billion US$ that are 5% of the gross national productivity of Central Asia (Mashali et al., 2005).

2.2 Effects of salt stress on plants

At whole plant level salinity leads to death of plants as it exerts 3-fold effect on growth of plants due to osmotic, ionic and nutrients imbalance effect (Munns and Tester, 2008; Tavakkoli et al., 2011). The most negative effect of salt stress is the reduction in growth, yield and quality of products as in Brassica (Kumar, 1995). Salt affected plants show symptoms of small leaves, leaf necrosis (Malirao et al., 2008). Premature leaf senescence is due to decreased PSII and PSI activities induced by high Na+ in leaf tissues that ultimately result in reduced growth and yield (Grover and Mohanty, 1992; Zhu et al., 2001; James et al. 2002, Husain et al. 2003). Salinity decreases photosynthetic pigments (Beltagi, 2008) and ethylene production in roots (Nandwal et al., 2007) and visual scores of necrosis could be used as a salt resistant criterion suggested by these authors. Salinity exerts toxic effects on plant growth by five ways e.g.
2.2.1 Osmotic stress

Rooting medium salinity causes osmotic effect because due to high concentration of salts outside the roots responsible for growth reduction in plants (Munns, 1993; Munns and Tester, 2008). Osmotic stress is the condition when soluble salts in the soil solution decreased plant’s ability to absorb water leading to drastic decrease in plant growth and yield (Ahmad et al., 2011). High salt concentration in soil solution decreases the water potential of leaf tissues ultimately reducing plant water contents and photosynthetic rate due to stomatal closure (Munns and Tester, 2008; Flowers et al., 2010). Large accumulation of Na$^+$ and Cl$^-$ in cell chloroplast leads to severe metabolic disorders (Nivas et al., 2011). Cell dehydration due to osmotic effect and ion toxicity is the major effect of soil salinity on plant growth and development (Joseph and Jini, 2011). Osmotic adjustment plays key role in regulation of turgor that increases growth and yield under water limiting conditions (Farouk, 2011). Adaptations in plants for osmotic stress tolerance under saline conditions are similar with those involved in drought stress tolerance (Munns and Tester, 2008).

2.2.2 Specific ion toxicity

Sodium (Na$^+$) is the basic major cause of specific ion toxicity in crops (Nemati, 2011). The movement of Na$^+$ ions into the root is passive (Cheeseman, 1982), so the net Na$^+$ uptake increased due to unidirectional influx of Na$^+$ (Kronzucker et al., 2006). In root cells, Na$^+$ is readily absorbed due to its small size and transported to all plant tissues resulting in ion toxicity, osmotic stress and nutrient imbalance (Siringam et al., 2009; 2011). High Na$^+$ concentration disturbs stomatal regulation by competing K$^+$ and Ca$^{2+}$ ions, while high Cl$^-$ contents degrade chlorophyll pigments and interrupt photosynthetic processes by causing leaf necrosis (Maliro et al., 2008; Tavakkoli et al., 2011). Excess Na$^+$ and Cl$^-$ ions toxicity
damage the membranous system and decreased growth (Siringam et al., 2011). Chloride (Cl\(^-\)) also accumulates under salinity stress and compartmentalize in vacuoles to balance the water status (Van der Boon et al., 1990). If vacuole becomes saturated with Na\(^+\) and Cl\(^-\) these ions start to build up in cytoplasm and cause enzyme inhibition and cell dehydration (Munns, 2002). Shoot dry matter, leaf area, plant height, relative water content, chlorophyll and K\(^+\) decreased due to increased accumulation of Na\(^+\) and Cl\(^-\) (Bybordi, 2010; Hossain et al., 2011). However, the mechanism of Na\(^+\) and Cl\(^-\) exclusion is different in different plant genotypes (Tavakkoli et al., 2011). Soil Cl\(^-\) content plays key role in growth and yield reduction by altering non-stomatal factors effect like chlorophyll degradation and impaired chlorophyll fluorescence and photosynthetic processes (Dang et al., 2008; Tavakkoli et al., 2011). Genotypic variation in Na\(^+\) exclusion and salt tolerance exist in bread wheat when tested on biomass and yield basis (Cuin et al., 2009) that leads to genetic variation in K\(^+\) accumulation because both ions transport through same pathways (Munns et al., 2003). In wheat and other crop species the mechanism of salt tolerance is considered to be associated with plants ability to reduce excess toxic ions accumulation in leaves (Praxedes et al., 2010; Rahnama et al., 2011). Under salinity AtNHX\(_{1}\) and AtSOS\(_{1}\) Na\(^+\) transporters work in Na\(^+\) tolerance mechanism (Hauser and Horie, 2010). A new class of HKT transporters (AtHKT\(_{1}\); 1 and OSHKT\(_{1}\); 5) has also been reported to be involved in salt tolerance mechanism in monocots including wheat. These transporters help in excess Na\(^+\) exclusion and prevent shoots to accumulate high Na\(^+\) under salinity stress (Hauser and Horie, 2010). In glycophytes ion exclusion capacity become saturated when plants are exposed for long period of time to salt stress (Gorham et al., 1985). There are some reports which show that salt tolerance trait has no relationship with Na\(^+\) accumulation (Cramer et al., 1994). Humidity level affects the Na\(^+\) accumulation in leaves e.g., decreased atmospheric humidity increased the Na\(^+\) to same level in two chickpea cultivars (Flowers et al., 2010).

### 2.2.3 Nutritional imbalance

Salinity stress disturbs the normal uptake and distribution of essential nutrients in plants. Balanced absorption of essential nutrients is disrupted due to excess salts in the external soil solution (Tester and Devenport, 2003). Interference of excess Na\(^+\) in root cells of plant occurs as Na\(^+\) competes with K\(^+\) for uptake and causes ionic imbalance (Munns and Tester,
Potassium and \( \text{Ca}^{2+} \) uptake decreased, while that of \( \text{Na}^{+} \) increased under salt stress conditions (Bavei et al., 2011). Different plants have different nutrient essentiality depending on internal ionic status of plants (Nivas et al., 2011). Sodium is also an essential micronutrient that is a major cation in salt affected soils and is passively entered in different plant tissues (Nivas et al., 2011). Nutrients imbalance i.e. increase in \( \text{Na}^{+} \) and \( \text{Cl}^{-} \) contents, while decrease in \( \text{K}^{+} \) and \( \text{Ca}^{2+} \) ions and \( \text{K}^{+}/\text{Na}^{+} \) ratio under \( \text{NaCl} \) stress has been studied in various crops such as wheat (Colmer et al., 2006; Shafi et al., 2010), barley (Tavakkoli et al., 2011), maize (Turan et al., 2010), tomato (Taffouo et al., 2010), cowpea (Patel et al., 2010) and brassica species (Atlassi Pak et al., 2009). As physiochemical properties of \( \text{Na}^{+} \) and \( \text{K}^{+} \) are similar, uptake of \( \text{K}^{+} \) is affected by high \( \text{Na}^{+} \) level (Murata et al., 1994; Hossain et al., 2011). Under high salinity, excess \( \text{Na}^{+} \) reduces \( \text{K}^{+} \) accumulation that leads to reduced growth (Hossain et al., 2011). Potassium-dependent key metabolic processes like protein synthesis, stomatal regulation and photosynthesis are changed due to high accumulation of \( \text{Na}^{+} \) concentration (Munns and Tester, 2008). Under salinity stress \( \text{K}^{+} \) plays role in osmotic adjustment more than any other inorganic or organic solutes (Ashraf and Sarwar, 2002) and increase salt stress tolerance in plants (Cuin et al., 2008; Shabala et al., 2010). Excess in \( \text{Na}^{+} \) concentration in all plant tissues decreased \( \text{K}^{+} \) and \( \text{Ca}^{2+} \) contents and \( \text{K}^{+}/\text{Na}^{+} \) ratio in tomato cultivar (Taffouo et al., 2010). In bread wheat high \( \text{Na}^{+} \) and \( \text{Cl}^{-} \) accumulation and \( \text{K}^{+} \) and \( \text{Ca}^{2+} \) reduction cause reduced \( \text{K}^{+}/\text{Na}^{+} \) ratio in leaf tissues (Raza et al., 2007). Similarly, low shoot \( \text{Na}^{+} \) and high \( \text{K}^{+} \) selectivity is linked with salt tolerance in wheat (Gorham et al., 1987; Sarwar et al., 2003). However, genetic variation exist in \( \text{K}^{+}/\text{Na}^{+} \) discrimination and transport to shoot in favor of \( \text{K}^{+} \) in crop species (Gorham, 1990). In hexaploid wheat, salt tolerance capability and discrimination in \( \text{K}^{+}/\text{Na}^{+} \) ratio is carried out by a 4D chromosome that is derived from \textit{Arabidopsis squarrosa} wild grass (Shah et al., 1987).

### 2.2.4 Hormonal imbalance

Impairment in appropriate supply of photosynthates and hormones to growing tissues is another adverse effect of salinity (Munns, 1993). Salt stress changes the endogenous level of plant hormones that is the first step in controlling growth reduction. For example, salinity stress induces decrease in salicylic acid, auxin, gibberellins (GA) and cytokinin (CKs) level, while increase in abscisic acid (ABA) and jasmonic acid (JA) in the plant tissues (Javid et al.,
However, exogenous application of plant growth regulators can be used to ameliorate the NaCl-induced growth reduction (Javid et al., 2011). It is thought that hormonal changes are sometime necessary for plant growth regulation under environmental stresses (Keskin et al., 2010). Plant hormones regulate entire plant growth by dramatically influencing the cell division and cell elongation (Fercha et al., 2011), while decreased plant growth rate under salt stress might be due to altered endogenous hormonal level (Iqbal and Ashraf, 2010). Rice tolerant cultivars show high endogenous ABA level than sensitive cultivars (Saedipour, 2011). In wheat exogenous application of brassinosteroids decreased the negative effects of salinity stress (Eleiwa et al., 2011). In another study on pea, Shabala et al. (2007) found that polyamines treatment is effective in inhibiting leaf mesophyll K+ efflux under NaCl stress. Jasmonates are thought to be accumulated under salt stress and increase stress tolerance in plants e.g. salt tolerant tomato variety accumulated more jasmonates than salt sensitive variety under salinity stress (Pedranzani et al., 2003; Hilda et al., 2003). Kang et al. (2005) also suggested that tolerant cultivar possess more jasmonates than sensitive cultivars. Seed treatment with jasmonates reduced the negative effects of salinity stress on barley (Tsonév et al., 1998). Salicylic acid is (SA) is a phenolic compound and endogenous growth regulator which regulate various processes in plants such as growth and photosynthesis under salt stress (Hayat et al., 2010), membrane permeability and peroxidation of lipid (Horvath et al., 2007), defence system (Hussein et al., 2007) and decrease accumulation of Na+ and Cl− ions (Gunes et al., 2007). Salicylic acid is known to improve stress tolerance in number of plants like wheat (Shakirova et al. 2003), maize (Escobar et al., 2010), pea (Barba-Espin et al., 2011), thyme (Najafian et al., 2009) and lemongrass (Idrees et al., 2011). Under salinity stress, endogenous SA level increased in rice seedlings (Sawada et al., 2006). In wheat, increased salt tolerance was associated with increased antioxidant defense system with exogenous application of salicylic acid (Erdal et al., 2011).

2.2.5 Oxidative stress

Salinity stress produces oxidative stress in plants by producing reactive oxygen species (ROS) such as superoxide radical (O2−), hydrogen peroxide (H2O2) and hydroxyl radical (•OH) (Joseph and Jini, 2010). Oxidative stress is a limiting factor for plant growth and productivity. Reactive oxygen species are produced during metabolic processes and disrupt...
normal metabolic processes and consequently induces senescence and death of plant cell. Salt stress induced stomatal closure reduces CO₂ availability for photosynthetic CO₂-fixation, consequently, excesses excitation energy of chloroplasts lead to the generation of ROS (Parvaiz and Satyawati, 2008). Garnczarska et al. (2004) found that chloroplasts, mitochondria and peroxisomes are the sites where ROS are produced in plant cells. The ROS play dual role in plants responses to environmental stresses. For example, ROS can function as a toxic by-product during metabolism under stress conditions or they can play key role in signal transduction pathway and regulate many metabolic processes (Miller et al., 2010), cause membrane and other cellular damages (Gao et al., 2008) and are toxic and must be scavenged by cellular response for successful survival (Gratao et al., 2005). The major cause of ROS is the over-reduction of electron transport chain together with decreased CO₂ assimilation (Miller et al., 2010). Several important biomolecules such as nucleic acids, amino acids, proteins and lipids are disrupted due to excess production of ROS (Luna et al., 1994), that leads to irreparable metabolic dysfunction and consequently cell death in plants (Shahbazi et al., 2011). However, plants escape the oxidative damage by developing an efficient defense system comprising of enzymatic and non-enzymatic antioxidant enzyme system. The enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Ashraf, 2009; Joseph and Jini, 2010). ROS detoxification and scavenging depends upon the sequential and simultaneous action of antioxidant enzymes (Azooz et al., 2009).

2.3 Responses of plants to salinity stress

2.3.1 Morphological responses of plants to salt stress

Growth inhibition a morphological marker under salinity stress has been studied in different crop e.g., wheat (Shafi et al., 2010), rice (Summart et al., 2010; Cha-um et al., 2010), canola (Bybordi, 2010), soybean (Neves et al., 2010) and faba beans (Tavakkoli et al., 2010). The most typical morphological effect of salinity stress is reduction in plant growth (Jaleel et al., 2008). Plant responses to salinity stress is very complex as they vary not only among different cultivars of same species but also at different growth stages (Aghaie et al., 2009; Panda and Khan, 2009; Ghanem et al., 2009). In wheat vegetative stage is more sensitive
than other growth stages (Khayatnezhad and Gholamin, 2010; Bhutta and Hanif, 2010). However, Munns et al. (2006) reported that barley shows more disorders at reproductive stage under salt stress. Tomato cultivar Xewel, Jaguar Nadira and Mongal has been shown salt-sensitive with salt tolerance level upto 50 mM NaCl, while Ninja moderately salt-tolerant and Lindo relatively tolerant at 100 mM NaCl concentration when tested on the basis of growth and photosynthetic pigments (Taffouo et al., 2010). Plants ability to tolerate salt stress depends upon their absolute and reletave growth and yield (Mass, 1986). Reduced grain number under salt stress also depends upon the salt tolerance ability of a particular genotype (Rahnama et al., 2011). Reduced growth and yield is due to osmotic stress and specific ion toxicity such as Na\(^+\) and Cl\(^-\) (Munns and Tester, 2008). High Na\(^+\) ions level in salt sensitive crops is the main reason of low yield under saline conditions (Tester and Davenport, 2003).

2.3.2 Physiological responses of plants to salt stress

The direct physiological effects of salt stress on plant growth is (i) reduced water potential (ii) specific ion (Na\(^+\), Cl\(^-\)) toxicity and (iii) interference in essential mineral nutrients uptake (Carpici et al., 2010). However, due to mobile reserves of nutrients the third factor is not too much important (Flowers and Flowers, 2005; Carpici et al., 2010). Adaptations to these effects include firstly, osmotic stress tolerance that is not specific for salinity stress but also related to water deficit effect or water stress, secondly, Na\(^+\) exclusion that protect leaves by accumulating excess ions in the roots and thirdly, tissue tolerance that include compartmentation at intercellular and cellular level to avoid toxicity in cytoplasm (Munns and Tester, 2008).

2.3.2a Photosynthetic pigments and gas exchange characteristics

Salinity stress induced negative effects on plant growth, chlorophyll pigments, photosynthetic efficiency in bread wheat (Eleiwa et al., 2011). Photosynthetic pigments decreased under salt stress in various plants for example in wheat (Akhkha et al., 2011), tomato (Doganlar et al., 2010; Taffouo et al., 2010), rice (Cha-um et al., 2010), brassica species (Atlassi Pak et al., 2009) and Catharanthus ruseus (Jaleel et al., 2008). Salinity stress
decreased the growth attributes and chlorophyll contents of *Morinda pubescens*, while in *Morinda citrifolia* chl. *a* and total chl. remain stable while chl. *b* increased under increasing salinity levels (Nivas *et al.*, 2011). Photosynthetic pigments did not undergo any major change under 150 mM salinity stress in durum wheat (Fercha, 2011). In Silver buffaloberry plant leaf, total chlorophyll contents and K⁺ decreased with increasing salinity level (Qin *et al.*, 2010). Salinity stress induces disruption in gas exchange characteristics (Munns and Tester, 2008). Photosynthesis is a primary process that is affected by salinity stress. Harmful effects of salinity stress on the gas exchange and other physiological processes has been studied extensively on various crop species like grape (Hatami *et al.*, 2010), wheat (Ashraf and Bashir, 2003; Zheng *et al.*, 2008; Rahnama *et al.*, 2010; Akhkha *et al.*, 2011), sunflower (Steduto *et al.*, 2000), rice (Cha-um *et al.*, 2010), commonbean (Gama *et al.*, 2007) and rice etc (Moradi and Ismail, 2007). The decreased photosynthetic process in most of the plants might be directly affected by salinity. For example, stomatal limitation to CO₂ diffusion or change in the metabolic processes of photosynthesis may also be due to direct or indirect effect of NaCl (Chaves *et al.*, 2009). Stomatal closure and reduced level of sub-stomatal CO₂ concentration under salinity stress directly lead to growth reduction (Netondo *et al.*, 2004). Reduction in CO₂ due to stomatal closure is not the only reasons of decreased photosynthesis but non-stomatal factors are also involved (Qin *et al.*, 2010). For example, in durum wheat net photosynthetic rate remained often unchanged inspite of decrease in stomatal conductance under salt stress (James *et al.*, 2002). In grape genotypes gas exchange reduced under salt stress (Hatami *et al.*, 2010). At the onset of salt stress substomatal CO₂ decreased due to stomatal closure but later on, increased due to limitation in CO₂ consumption in the photosynthetic process (Hatami *et al.*, 2010). Salinity stress exerts a negative effect on leaf area per plant that is due to decrease in photosynthetic rate (Munns and Tester, 2008). Reduction in photosynthetic rate due to high salinity stress decrease the final yield and productivity of several crop species as in wheat (Dubey, 2005; Eleiwa *et al.*, 2011). Salt tolerant blue panicgrass was high in leaf K⁺/Na⁺ ratio and net photosynthetic capacity than the sensitive population (Ahmad *et al.*, 2010).
2.3.2b Chlorophyll fluorescence

Salt stress induced decreased in actual efficiency of PS II photochemistry ($\Phi_{PS\ II}$) and increased non photochemical quenching (NPQ) in salt-sensitive rice plants than in salt-tolerant cabbage plants (Zhu et al., 2011). Electron transport membrane system is impaired in plants that are subjected to abiotic stresses (Smirnoff, 1993; Navari-Izzo and Rascio, 1999). Photosystem II (PSII) activity is differentially influenced by the salinity stress e.g. in wheat (Mehta et al., 2010), okra (Saleem et al., 2011), radish (Jamil et al., 2007).

2.3.2c Water relations

Sodium ion concentration between 100 to 2380 mM is equivalent to water potential range from −0.5 to −11 MPa. However for survival under saline conditions plants must adjust osmotically to a water potential range between -2 to -3 MPa (Flowers, 1985; Flowers et al., 1977; 2010), while marine photosynthetic organisms balance their water relations with seawater that is about −2.3 MPa (Harvey, 1966). Plant water relations are severely interrupted by the salinity stress in various crops such as in wheat (Zheng et al., 2008; Shafi et al., 2010), rice (Summart et al., 2010; Cha-um et al., 2010), faba beans (Tavakkoli et al., 2010), soyabean (Neves et al., 2010), canola (Bybordi, 2010), Catharanthus roseus (Jaleel et al., 2008), Bambara groundnut (Taffouo et al., 2010) and Phragmites australis (Gorai et al., 2010). The first sign of salinity stress on plants exerts its effect by water deficit effect or drought effect (Nemati, 2011). Homeostasis in water relations is disrupted due to high salt accumulation and alter the distribution of ions at both cellular and whole plant levels that lead to molecular disorders, arrested growth and cell death (Bastias et al., 2004; Qin et al., 2010). Under salt stress leaf water relations are severely inhibited (Raza et al., 2007). Under normal environments water enters plant roots through water channel proteins referred as aquaporines due to lower external water potential of soil solution (Katsuhara et al., 2008). However, under salinity stress the water potential difference between plant roots and soil water become reverse that result in reduced uptake of water or even water loss (Boursiac et al., 2005). Reduced external water potential of soil solution is the potential effect of salinity stress that is due to excess salt in the soil solution outside the root (Akram et al., 2007; Carpici et al., 2010). Salt-induced osmotic effect reduces photosynthesis, creates water
deficit and disturbance in water balance and consequently reduction in seedling growth (Poljakoff-Mayber, 1982). As salinity stress hampers plant growth and metabolic processes due to decreased plants ability to utilize water (Munns, 2002), decrease in relative water content is among early responses to salinity stress (Walker et al., 1993; Alarcon et al., 1993). Salt-resistant chickpea genotypes possess higher water content than those of salt-sensitive under saline conditions (Flowers et al., 2010). In silver buffaloberry plants, leaf water potential decreased with increasing salinity level (Qin et al., 2010). Decrease in relative water content under salt stress can also induce reduction in stomatal conductivity (Lawlor and Cornic, 2002). However, plants cope with salinity-induced water deficit effect by high uptake of mineral nutrients and synthesis of compatible solutes which helps in osmotic adjustment (Singh et al., 2010; Nemati, 2011). The failure in osmotic balance under saline conditions leads to dehydration of cells, turgid loss and consequently cell death of plant tissues (Gorham, 1995). Brassica species treated with 150 mM NaCl exhibit two phase model. In first phase rapid change in turgor pressure occurs, while in second phase accumulation of solutes occurs that causes a decrease in transpiration rate and altered turgor pressure (Singh et al., 2010). More reduction in leaf water and osmotic potential and less in turgor potential were exhibited by salt tolerant wheat genotypes (callus) than sensitive genotypes (Javed, 2002).

2.3.3 Biochemical responses of plants to salt stress

2.3.3a Activities of antioxidant enzymes

Salinity stress brings about significant changes in the activities of catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) for detoxification of reactive oxygen species (ROS) (Wang et al., 2010). Catalase is up made of four polypeptide chains (a tetramer) and each polypeptide chain contains more than five hundred amino acids (Boon et al., 2007) and is found in the peroxisome cell organelle and has a role in scavenging of plants from oxidative stress by decomposition of H₂O₂ into H₂O and O₂ (Mittler, 2002; Garg and Manchanda, 2009; Mane et al., 2010). In salt tolerant barley cultivar, a consistent increase in CAT activity has been observed (Khosravinejad et al., 2008). The high induction of antioxidant enzymes like catalase and peroxidase antioxidant enzyme has been thought as one of the mechanism
plant salt tolerance (Hernandez et al., 2003; Azooz et al., 2009) and shown to be high in salt tolerant cultivars than sensitive ones e.g., in wheat (Athar et al., 2009) and maize (Azooz et al., 2009). Increased catalase, peroxidase and superoxide dismutase activity had been observed in wheat (Meneguzzo et al., 1999; Sairam et al., 2002; Mandhania et al., 2006; Raza et al., 2007; Ma et al., 2011). A decrease in activity of CAT and POD (Gadalla, 2009), and SOD (Mandhania et al., 2006; Raza et al., 2007) had also been observed in wheat under salt stress. Superoxide dismutase (SOD) activity increased in salt tolerant wheat (Sardari) cultivar, while remained constant in salt sensitive (Alvand) cultivar, CAT activity showed same trend in the two wheat cultivars under salt stress (Esfandiari et al., 2007). In another report, Esfandiari et al. (2011) found that in salt tolerant Egypt 449 durum wheat cultivar activity of superoxide dismutase (SOD) and catalase (CAT) enzymes did not changed, while in that of salt-sensitive Syria 371 cultivar SOD and CAT activity decreased under salt stress of 200 mM. Ma et al. (2011) found that under 150 mM NaCl stress POD and CAT show similar trend in scavenging H2O2 in wheat. Similarly, activity of CAT, SOD and POD increased in tolerant (SC 129 and SC 13) cultivars, while decreased in sensitive (SC 155) maize cultivar (Azooz et al., 2009). SOD activity increased under low salinity stress but at high salt level no significant difference was observed in potato leaves (Rahnama and Ebrahimzadeh, 2005). Salt tolerant maize cultivars (SC 129 and SC13) exhibit increased SOD, POD and CAT activity, while salt sensitive cultivar (SC 155) has been depressed in the activities of all these enzymes (Azooz et al., 2009). SOD that has been considered as a first line of defense against reactive oxygen species (Zhang et al., 2005) generates H2O2 that increase transiently in response to salt stress (Hernandez et al., 2010). In salt-sensitive pea (Hernandez et al., 1993) decreased activity of SOD isoenzymes has been reported that could be due to their different subcellular location affected under various levels of salinity stress. There are some other reports which show that SOD activity was high in salt tolerant and low in salt sensitive cultivars of various crop plants like e.g. tomato (Shalata and Tal, 1998), maize (Neto et al., 2006), cucumber (Furtana and Tipirdamaz, 2010) and wild beet (Bor et al., 2003). Dionisio-Sese and Tobita (1998) was of the view that low SOD activity in rice is associated with salt induced damage resulting in increased lipid peroxidation and membrane permeability, which will further cause decrease in peroxidase activity and ultimately growth. In cucumber, SOD is unaffected (Lechno et al., 1997), while decreased in barley under high
salt stress (Seckin et al., 2010). In tomato, H$_2$O$_2$ increased, while SOD decreased in root mitochondria and peroxisomes (Mittova et al., 2004).

2.3.3b Membrane permeability (%), Malondialdehyde (MDA), Hydrogen peroxide (H$_2$O$_2$)

A long time exposure to salinity stress reduces the anti-oxidant activities and cause more lipid peroxidation, membrane permeability and H$_2$O$_2$ accumulation (Seckin et al., 2010). Regulation of membrane permeability and active antioxidant potential are two major mechanism of protection against oxidative damage (Lushchak, 2010). In Brassica species differences in the oxidative stress and the antioxidant response had been reported (Hernandez et al., 2010). Plasmamembrane is the primary site of stress injury (Levitt, 1980). The plasmamembrane damage results in increase in cell permeability that induces electrolyte leakage (Blum and Ebercon, 1980). Collado et al. (2010) suggested that cell membrane stability trait could be used as a criterion for salt tolerance. Salt induced lipid peroxidation has been studied in number of crops including wheat (Esfandiari et al., 2011) and maize (Azooz et al., 2009). Peroxidation of membrane lipids increased with the increasing level of salinity stress particularly in salt sensitive varieties as compared to tolerant ones (Arora et al., 2008).

In addition to its action as ROS, H$_2$O$_2$ functions as intercellular signal to stimulate stress responsive genes for CAT and POD synthesis (Prasad et al., 1994). Hydrogrn peroxide is produced by the enzymatic action of SOD on superoxide radical (O$_2$). Hydrogrn peroxide is then scavenged by ascorbate peroxidase, catalase or peroxidase and converted into water and oxygen (Mittler, 2002; Masood et al., 2006; Azooz et al., 2009). Chickpea leaves treated with 100 mM NaCl contain 170% H$_2$O$_2$ (Eyidogan and Oz, 2007). In wheat flag leaf salinity increased leaf senescence, H$_2$O$_2$ and MDA contents (Gadalla, 2009). In durum wheat H$_2$O$_2$ contents increased at 150 mM salinity (Fercha, 2011).
2.3.3c Total soluble proteins

The N-contents and total soluble protein usually increased under high salt concentrations in glycophytes (Abed El- Baki, 1996; Shaddad et al., 2005). In wheat at 115 mM (NaCl) amino acids increased (Farouk, 2011) that could be due to protein degradation (Yadav et al., 1999) and for synthesis of new protein synthesis under stressful conditions (Yadav et al., 1999; Farouk, 2011). Increased soluble proteins accumulation under salt stress has been studied in various crops like wheat (Javed, 2002; Tammam et al., 2008) and tomato (Isfahani cv.) (Amini and Ehsanpour, 2005). As salt adapted wheat plants divert synthesized carbohydrates into insoluble proteins (building materials) and soluble proteins (osmoregulation) that is an interconversion between carbohydrates and nitrogen metabolism, whereas this equilibrium is disturbed under sever saline injury (Tammam et al., 2008).

2.3.3d Phenolics

Plants can synthesize and accumulate total phenolics under stress that protect plants from ROS due to their antioxidative properties like H-donating ability and scavenger of singlet oxygen species (Dixon and Paiva, 1995; Rice-Evans et al., 1996, 1997; Rivero et al., 2001; Posmyk et al., 2009). In Catharanthus ruseus secondary metabolites (alkaloids) increased under salt stress (Jaleel et al., 2008). Production of phenolics under saline conditions is reported in various crops like wheat (Abd El-Baky, 2009). A positive relationship had been reported between antioxidant activity and accumulation of total phenolic contents (Verzellon et al., 2007; Liu et al., 2007; Naciye et al., 2008). Accumulation of phenolics depends upon the growth stage of plant (Barros et al., 2007). A differential response exists in wheat genotypes at different growth stages in phenolics accumulation under salinity stress (Ashraf et al., 2010).

2.3.3e Free amino acids and free proline

For osmotic adjustment the accumulation of ions is more favorable in terms of energy cost, however, under osmotic stress many plants also accumulate organic osmolytes such as proline and glycinebetaine (Chen and Murata, 2000; Gurmani et al., 2007; Shafi et al., 2011). Compatible organic solutes are low molecular weight, non-toxic water soluble organic
molecules also known as osmoprotectants (Chen and Murata, 2002) that play role in osmotic adjustment. Plants accumulate free proline and glycinebetaine to maintain osmotic adjustment under salinity stress (Hayashi et al., 2000; Kumar et al., 2003; Farouk, 2011). It not only acts as an indicator of salt tolerance but also indicate a sign of reaction to salt induced damage (Carpici et al., 2010). Proline play role in maintaining turgor potential, stabilizing membranes integrity, enzymes regulation and detoxification of ROS in plants under salt stress (Misra and Saxena, 2009). Growth and photosynthetic pigments improved by exogenous application of proline under salinity stress (Nawaz et al., 2010). Hossain et al. (2011) investigated that high proline accumulation in peanut seedlings could not be related to salinity (200 mM NaCl) tolerance. According to Greenway and Munns (1980) proline role is more related to survival than simply maintaining growth. However, excess proline accumulation was a product of impaired photosynthetic machinery whereas it did not maintain cell turgidity or relative water contents effectively (Hossain et al., 2011). Proline synthesis under salt stress might be due to increased synthesis of proline synthesizing enzymes or decreased activity of proline degrading enzymes (Amini and Ehasapour, 2005; Misra and Saxena, 2009). Proline is among most frequently occurring compounds that play key role in osmotic adjustment (Gilbert et al., 1998; Ueda et al., 2007). In wheat proline acts as an endogenous osmotic regulator and its level in plant tissues indicates plants ability to adapt to environmental conditions especially salt stress (Munns et al., 2006). Proline can act as a radical scavenger and protect cytoplasmic enzymes (Nikolopoulos and Manetase, 1991; Hoque et al., 2007). Over accumulation of proline under salt stress has been shown by various researchers (Farouk, 2011; Zhu et al., 2011a, 2011b).

2.3.3f Glycinebetaine

Glycinebetaine (GB) (N, N, N-trimethylglycine) is ubiquitous (widely occur in plants, animals and microorganisms) in nature, extremely soluble in water and an effective compatible solute for protection against osmotic stress (Le Rudulier et al., 1984; Rhodes and Hanson, 1993; Ohnishi and Murata, 2006). It maintains and stabilizes the complex molecules such as antioxidants, membrane proteins and oxygen-evolving functional units of PSII under osmotic stress (Rhodes and Hanson, 1993; Ma et al., 2006; Raza et al., 2007). Glycinebetaine had been reported to enhance salt tolerance in crops e.g. in maize (Rhodes et al., 1989;
Saneoka et al., 1995) and barley (Grumet and Hanson, 1986). Glycinebetaine protects photosynthetic system such as rubisco and oxygen-evolving complex of PSII from salt stress injuries (Papageorgiou and Murata, 1995). Glycinebetaine protect macromolecular structure by forming a hydration cell around proteins surface (Arakawa and Timasheff, 1983) or by directly stabilizing the proteins (Schobert, 1977). Since NaCl stress disrupts intermolecular association of protein subunits and hamper activity of translational machinery GB may have capability to enhance such type of association and competes NaCl to reduce its effect (Ohnishi and Murata, 2006). Glycinebetaine play role in improving the antioxidant defense system that scavenge ROS produced under salt stress (Raza et al., 2007). Rajendrakumar et al. (1997) reported that GB might play role in enhancing transcription and replication under salt stress.

### 2.4 Approaches to solve salinity problems

Plants possess capability to cop salinity induced injuries by adopting various physiological, biochemical and molecular mechanisms (Munns and Tester, 2008). It has been proposed by Epstein et al. (1980) that it is more feasible to choose biotic approaches rather than technological methods to eradicate salinity induced problems. Biotic approaches include selection and conventional breeding, marker assisted selection and genetic engineering of plants (Ashraf, 1994, 1999, 2009; Munns and Tester, 2008; Ashraf et al., 2008).

#### 2.4.1 Screening and Conventional breeding

Physiological trait identification and associated key genes for salt tolerance at cellular and whole plant level should be kept at central position to all approaches (Munns et al., 2002). Different physiological traits could be used as selection criteria to screen genotypes. Measuring shoot growth, dry weights and grain yield is important selection criteria for salt tolerance in screening wheat genotypes (El-Hendawy et al., 2011). Root and shoot length is rapid indices to determine the salt tolerance and is used as selection criteria to pool out tolerant genotypes (Saeed et al., 2011; Collado et al., 2011). There are reports which show that shoots are more sensitive than roots (Moud and Maghsoudi, 2008; Kaya et al., 2008) in contrast to Ogawa et al. (2006) and Jamil et al. (2006) who indicate that roots are more
sensitive than shoots. In tomato, 72 accessions are screened on the basis of shoot and root length and shoot and root fresh weight basis (Saeed et al., 2011). Leaf area although decreased but is not too much effective in this way (Cicek and Cakir, 2002). Grain crops like rice, wheat, barley, maize, sorghum and cowpea are tolerant to salt stress at germination stage, sensitive at seedling and early vegetative growth stage and again become tolerant at maturity stage (Ashraf, 1994). At cellular level changes in membrane properties like structure and composition of membranes could be used for evaluating salt tolerance as salt sensitive genotypes show high membrane permeability than salt tolerant genotypes (Collado et al., 2010).

Through conventional breeding approach salt tolerant lines/cultivars of some crop species have been developed. For example, two salt tolerant lines/cultivars of bread wheat such as KRL1-4 (Hollington, 2000) and S-24 (Ashraf and O’Leary, 1996) were tested on natural salt affected soils. Although some progress has been made in developing salt tolerant cultivars/lines through conventional breeding and selection (Ashraf, 2002), this approach encounter some problems for transferring salt tolerance to targeted species (Sairam and Tyagi, 2004). Furthermore, difference in physiological mechanism, a complex interaction between genotypes and environment (Arzani, 2008) and a limited access to genetic variation make it difficult for developing salt tolerant plants through conventional breeding (Flowers, 2004; Colmer et al., 2005; Munns et al., 2006; Cuartero et al., 2006; Munns, 2007). There are many reasons for limited success in developing salt tolerance through conventional breeding (1) it is time consuming and laborious (2) many undesirable genes are transferred along with the gene of interest and (3) the reproductive barriers reduces transfer of favorable alleles from inter-specific and inter-generic sources (Ashraf and Akram, 2009). Uptill now very few cultivars/lines of different crop species showed success in field inspite of too much expenditure on developing for producing such transgenic lines (Ashraf and Akram, 2009).

2.4.2 Biotechnological/Molecular approaches/Genetic Manipulation

Genetic variation exist in various crop species for salt tolerance e.g., cotton (Abbas et al., 2011), maize (Collado et al., 2011), rice (Bhowmik et al., 2009; Arshadullah et al., 2011), mungbean (Win et al., 2011), wheat (Abdelmalek and Khaled, 2011) and tomato (Saeed et
These genetic variations within crops can be assessed by using various agronomic, physiological and biochemical selection criteria (Ashraf, 2004; Munns, 2007). Similarly, genetic variation exist in wheat under sever salinity stress and genotypes that show more resistance to salt stress can be used for cultivation e.g. wheat cultivar S-78-11 show high resistance to salinity (Eskandari and Kazemi, 2011). Salt tolerant trait that is a quantitative trait (QTL) as in rice could be used for marker assisted selection in improving crop salt tolerance under saline conditions (Islam et al., 2011). Marker assisted selection helps in locating salt tolerant genes that could be introduced into high yielding genotypes through genetic engineering or conventional breeding techniques (Sankar et al., 2011). A quantitative trait locus (SKC1) has been identified in rice that encode Na$^+$ transporter (Zhong et al., 2005). In transgenic rice, tobacco OPBP1 gene of tobacco improves salt tolerance and increases disease resistance (Chen and Guo, 2008). Transgenic rice plants in which yeast Na$^+$/H$^+$ antiporter SOD2 gene over express accumulate less Na$^+$ and more K$^+$, Ca$^{2+}$ and Mg$^{2+}$ in shoots compared to non-transgenic plants (Zhao et al., 2006). In wheat more QTLs (36) are expressed under saline conditions than under control conditions (Diaz De Leon et al., 2011). Quantitative trait loci have been expressed in several cereal crops (Semikhodskii et al., 1997; Flowers et al., 2000).

### 2.4.3 Shotgun approaches

Ashraf and Foolad (2007) and Ashraf et al. (2008) suggested shotgun approaches to overcome the limitation of biotechnological approaches. These shotgun approaches include exogenous application of different osmolytes, osmoprotectants and plant growth regulators. Exogenous application can be done by following three ways.

2.4.3a Seed priming

2.4.3b Foliar spray

2.4.3c Rooting medium
2.4.3a Seed Priming

Among different strategies, seed priming is an easy, simple, low risk and low cost approach to combat salinity induced damages in agricultural crops (Tavili et al., 2011). From agronomic point of view seed vigor is an important quality that determine embryo growth and seedling emergence (Farahani and Maroufi, 2011). The process of seed priming involves making sensitive genotypes to grow faster under salt stress conditions and improve the performance of tolerant genotypes (Kaya et al., 2006). Maiti (2011) was of the view that seed priming is effective in all crops to improve seedling growth, seedling vigor and yield. It includes imbibition of seed upto radical emergence followed by redrying to original state (McDonald, 2000). Pre-germinated seeds allow rapid seed emergence and metabolic repair of seeds occur during imbibitions in priming process as hydration level of seeds is controlled to allow necessary metabolic activity for seed germination (Maiti, 2011). Priming reduces phytochrome-mediated secondary dormancy (Vlades et al., 1985). Seed priming exerts stimulating effects on the germination process by mediating cell division (Hassanpouraghdam et al., 2009). It has been suggested that seed priming helps in repairing process of damaged membranes of seeds (Arif et al., 2008). Fastened germination rate and uniformity of seed emergence reduces metabolic phase (Bewley and Black, 1978; Maiti, 2011). Seed priming increases speed and uniformity of germination rate (Khalil et al., 2010), break seed dormancy, imbibitions, mobilization of reserve food materials and activation of several enzymes (Asgedom and Becker, 2001). Rapid seed germination leads to increased germination percentage, seedling establishment and final yield (Rogers et al., 1995; Ghassemi-Golezani et al., 2011). This is achieved by faster seed emergence, early flowering, more vigorous plants and higher yield under stressful conditions (Amooaghaie et al., 2010; Amooaghaie, 2011). Priming are of different types e.g. (i) hydropriming that is seed soaking in water as is reported in sunflower (Farahani et al., 2011) and wheat (Jafar et al., 2011) (ii) osmopriming in which seeds are soaked in polyethylene glycol, glycerol and mannitol etc. (Maiti, 2011) (iii) Matrix priming seed soaking in insoluble solid matrix with less amount of water in solution (Maiti, 2011) (iv) halopriming that include seed soaking in some inorganic salt solution (Nawaz et al., 2011) and (v) hormone priming (Iqbal and Ashraf, 2010). Hydropriming of seed increased improved seed vigor and increased uniformity of
germination under salinity stress (Farahani and Maroufi, 2011). Osmotic priming induces mobilization of reserve food materials, synthesis of enzymes, RNA and DNA and improves seed vigor, seed emergence and yield (Sadeghi et al., 2011). In osmo-priming Bromus seeds were soaked in polyethylene glycol solution for 12 h at 25°C that increased speed of seed germination (Tavili et al., 2011). Halopriming (25 mM KNO₃) for 24 h increased germination percentage, germination index, shoot length and shoot fresh weight of tomato (Nawaz et al., 2011). Seed soaking in PEG (10%), KCl (2%), and KH₂PO₄ (0.5%) increased leaf area, dry mass and seed size in wheat (Yari et al., 2011). Seed treatment with KNO₃ (0.8%) for 4 h improved grain yield in Isabgol under salt stress (Ghassemi-Golezani et al., 2011). Hormone seed priming ameliorate adverse effects of salt stress on various physiological and biochemical processes like growth, yield, ionic status, photosynthesis and hormonal homeostasis in spring wheat e.g. cytokinins (Iqbal and Ashraf, 2005) and GA₃ (Iqbal and Ashraf, 2010) and triacontanol (Perveen et al., 2010, 2011). Hormonal priming with salicylic acid increased emergence and seedling growth in cucumber (Rehman et al., 2011). Wheat seeds treated with ascorbic acid, kinetin and GA₃ under salinity increased growth and yield (Khan et al., 2011). Pre-sowing seed treatment with ascorbic acid increased salt tolerance in Brassica species (Khan et al., 2010). Gibberellic acid seed soaking increased germination rate in chickpea (Thakare et al., 2011). Seed soaking for 48 hours in aerated solution of salicylic acid, ascorbic acid, kinetin and GA₃ with 20 mgL⁻¹ solution of each proved to be effective for wheat under salt stress (Khan et al., 2011).

2.4.3b Foliar application of osmolytes, osmoprotectants and plant growth regulators

Exogenous application of a plant growth regulator salicylic acid ameliorated the adverse effects of salt stress in violet (Hussain et al., 2011). Foliar spray of brassinosteroids increase uptake of micro and macro nutrients, enhance nutritional status and improve salt resistance in wheat (Shahbaz et al., 2008). Salt resistance in wheat increased due to increased antioxidant defense system when ascorbic acid applied through rooting medium, as a foliar spray and as seed priming treatment (Athar et al., 2009). Foliar spray of inorganic or mineral ions increased growth and yield in potato under salt stress (Azeem and Ahmad, 2011). Plant growth regulators like spermidine and putrescine polyamines increased growth of pomegranate under salt stress (Amri et al., 2011). Gas exchange characteristics and yield of
bread wheat increased when thiourea was applied as foliar spray (Anjum et al., 2008). Salinity stress (2000, 4000 and 6000 ppm NaCl solution) induced negative effects on plant growth, chlorophyll pigments, photosynthetic efficiency and grain yield in bread wheat were ameliorated by foliar application of plant growth regulator brassiosteroids (50, 100 and 200 mg L\(^{-1}\)) (Eleiwa et al., 2011). Shoot mineral contents did not much affected by foliar spray of brassinosteroids in wheat (Shahbaz and Ashraf, 2007). Growth and photosynthesis increased by exogenous application of salicylic acid under salinity stress (Noreen and Ashraf, 2008). In pepper exogenous application of 24-epibrassinolide as foliar spray improved growth, relative water content and chlorophyll fluorescence under salt stress (Houimli et al., 2008). Salicylic acid treatment increased salt tolerance in pearl millet (Hussain et al., 2010). Foliar application of osmoregulator glycinebetaine increase antioxidant defense system in wheat (Raza et al., 2007) and alleviated adverse effects of salt stress on egg plant (Abbas et al., 2010). In sorghum growth and photosynthetic pigments adversely affected by salt stress (100 mM) but ameliorated by exogenous application of proline (Nawaz et al., 2010). In bread wheat (KRL-1-4) NaCl stress decreased the chlorophyll; however, plant growth regulators (maleic hyderazide, cycocel, mixtalol and benzyle adenine) reduced the effect of salt stress on all these parameters (Bagdi et al., 2011). At vegetative stage foliar spray of antioxidant ascorbic acid protected photosynthetic machinery and enhanced salt tolerance in wheat (Khan et al., 2006). Wheat antioxidant defense system was enhanced by foliar application of glycinebetaine (Ma et al., 2006; Raza et al., 2007). Foliar application of inorganic nutrients such as Fe, Zn and Mn increased salt stress tolerance in wheat (El-Fouly et al., 2011). Exogenous application of proline as foliar spray improved photosynthetic machinery and enhanced antioxidant defense system of two-year old olive tree that was subjected to salinity stress (Ben Ahmad et al., 2010). Foliar application of glycinebetaine (GB) increased growth and improved water relation attributes in maize (Nawaz and Ashraf, 2007). Exogenous application of GB on plants under salt stress induces the expression of stress-responsive genes including for those enzymes that play role in ROS scavenging (Chen and Murata, 2011). Antioxidant like ascorbic acid nullified the effect of 115 mM NaCl on wheat flag leaf area and yield/plant due to increased accumulation of osmolytes, turgor maintenance and osmotic adjustment (Farouk, 2011).
2.5 Triacontanol

Plant hormones play an important role in growth and development of plants (Lucas et al., 2001). Such plant hormones, growth regulators and inhibitors are used for many purposes (Kefeli and Dashek, 2008). Triacontanol (TRIA) is a newly discovered plant hormone that has stimulatory effects at very low concentrations when exogenously applied to plants (Ries et al., 1977). Crosby and Vlitos (1959) reported the plant growth regulating property of TRIA and later by Stoutemyer (1981), and Stoutemyer and Cook (1987). It is a 30-carbon aliphatic alcohol with the molecular weight (438.8), molecular formula C$_{30}$H$_{62}$O and structural formula CH$_3$-(CH$_2$)$_{29}$-OH. Chibnall et al. (1933) isolated n-triacontanol from Lucerne leaf wax. Lucerne is a common name of *Medicago sativa* and also known as alfalfa. It was confirmed by x-ray analysis that long-chain primary alcohol found in alfalfa leaf wax was *n*-triacontanol.

Plant cuticular wax forms a hydrophobic layer which protects the aerial parts of plants from stomatal water loss and biotic and abiotic stresses (Riederer and Schreiber, 2001; Marcell and Beattie, 2002; Kunst and Samuels, 2009). Many environmental and developmental factors are involved in the wax production in plants. For example, developmental stage, tissue type, and humidity changes are involved in accumulation of wax (Jenks et al., 1996; Jetter and Schaffer, 2001).

2.5.1 Role of triacontanol in plant growth

There are many reports on the growth enhancing property of TRIA (Prasad and Prasad, 1991) and is used on several million hectares to promote crop yield in Asia (Devakumar et al., 1986). The major role TRIA plays in plants is regulation of various physiological and biochemical processes such as increase in net CO$_2$ assimilation rate (Eriksen et al., 1981; Houtz et al., 1985a; Haugstad et al., 1983) cell division (Hangarter et al., 1978), enzymes activity (Naeem et al., 2009, 2010, 2011) and leaf mesophyll protoplast and chloroplast membranes viscosity and fluidity (Shripathi et al., 1997; Ivanov and Angelov, 1997). In addition, it also promotes mineral nutrients absorption, uptake and stress tolerance of several
crops (Muthuchelian et al., 2003; Cavusoglu and Kabar, 2007; Krishnan and Kumari, 2008; Karacakaya et al., 2009; Kilic et al., 2010).

The major role of TRIA is the regulation of photosynthesis as it increases net CO₂ assimilation rate by enhancing the specific activity of rubisco enzyme (Erikson et al., 1981; Houtz et al., 1985a, b) and exerts a positive effect on complexes focusing light in PSI and PSII (Moorthy and Kathiresan, 1993). The regulation of photosynthesis is a complex mechanism in which rbcS isogene expression profile is involved and promoted by TRIA application. As TRIA up-regulate the genes involved in photosynthesis and down-regulate abscisic acid and wound-related genes and proteins in rice (Chen et al., 2002). TRIA can also be used as an effective growth regulator in the micropropagation (Malabadi et al., 2005; Parimalan et al., 2009), somatic embryogenesis (Malabadi et al., 2005), tissue culture (Tantos et al., 1999), essential oil yield (Fraternale et al., 2003), morphogenetic responses under in vitro conditions (Giridhar et al., 2004), shoot and root multiplication (Reddy et al., 2002) and secondary metabolites (Giridhar et al., 2005). TRIA has been found effective in reducing peroxidation of membrane lipids (Ramanarayan et al., 2000). Triacontanoic acid is the main metabolite of 1-triacontanol oxidation (Haim et al., 2009) as long chain fatty alcohols are converted to their fatty acids in fatty alcohol cycle (Rizzo et al., 1987).

2.5.2 Signal transduction

Triacontanol influences enzymes which regulate metabolic (Ries and Houtz, 1983) and growth processes (Ries et al., 1990). It has been suggested that TRIA-mediated change in membrane bound enzymes level might be involved in the regulation of biosynthetic pathway of various lipids oleoresin (29.4%) and essential oil yield (18.7%) over control (Singh et al., 2011). Since plasmamembrane has been suggested as the initial site of signal transduction pathways in plant growth regulation (Morre et al., 1991; Trewavas and Gilory, 1991), TRIA-mediated increase in membrane-bound enzyme activities e.g. Ca²⁺/Mg²⁺ ATPases in barley roots (Lesniak et al., 1986) and fluidity of membranes to several solutes can be considered as a possible mechanism of TRIA action in plants, that leads to dephosphorylation of L (+) form of AMP, ADP and ATP that induce the formation of second messenger 9-β-L (+)adenosine that triggers a cascade of rapid physiological responses to TRIA action (Ries et al., 1990). It
has been reported that 9-β-L (+)adenosine is similar in structure to cytokinins (Ries, 1991) that can increase Ca^{2+}, Mg^{2+} and K^{+} ions in the tomato, maize and cucumber shoot up to 60% within 5 s of application (Ries et al., 1993). Although exogenous application of cytokinins are effective in inducing flowering when applied at the rate of 10^{-6} M (He and Loh, 2000; Bonhomme et al., 2000). In another study, on Arabidopsis thaliana treatment with 0.3 μM TRIA increased endogenous level of isopentenyl adenosine (iPAdos) which is also a type of cytokinins (He and Loh, 2002). In rice, foliar spray with a combination of cytokinin (120 mg L^{-1}) and TRIA (10 mg L^{-1}) at tillering and panicle initiation growth stages, increased growth and yield (Pandey et al., 2001). Cavusoglu and Kabar (2007b) found that all growth regulators like gibberellic acid, kinetins, benzyladenine, ethelene, brassinosteroids overcome the abscisic acid-induced inhibition on germination of radish seeds except TRIA. Fincher (1989) was of the view that ABA and GA_{3} work in antagonistic way. Similarly, TRIA reversed the effect of jasmonic acid (JA) by down-regulating JA induced proteinase inhibitors in tomato leaves (Ramanaryan and Swamy, 2004), reversed the effect of JA on chlorophyll a fluorescence (Ramanaryan, 2004) and differentially modulated membrane lipids (Swamy et al., 2009). In another study, IAA nullified the effect of TRIA in cotton plants when tested for MGDG and DGDG membrane lipids (Shripathi and Swamy, 1994).

Singh et al. (2011) was of the view that increased growth and productivity of ginger could be due to TRIA-induced increase in absorption and utilization of nutrients, enhanced photosynthetic rate and translocation of photosynthetic products and metabolites to the sinks or reproductive parts. Other researchers also reinforced these facts on various plant species (Naeem et al., 2009, 2010, 2011).

### 2.5.3 TRIA and abiotic stresses

There are many reports which show the ameliorating effect of TRIA under different environmental stresses. As increase in water uptake, cell elongation and cell density is the major role that TRIA plays in the regulation of plant growth (Hangarter et al., 1978). For example, the negative influences of heavy metal (cadmium) stress (1000 μmol L^{-1}) on fresh and dry weights, effective leaf area, chlorophyll and carotenoid pigments, photosynthesis, activity of photosystem-II (PSII), electron transport rate, rubisco and nitrate reductase
enzyme activities were ameliorated by foliar spray of TRIA (1 mg L\(^{-1}\)) on *Erythrina variegata* seedlings (Muthuchelian *et al.*., 2001). Shoot growth, O\(_2\) evolving complex (thylakoid polypeptide of 33, 23 and 17 kDa), activity of small (15 kDa) and large (55 kDa) subunits of rubisco, chlorophyll \(a\) contents and chlorophyll fluorescence were improved with treatment of TRIA under water stress of *Erythrina variegata* seedlings (Muthuchelian *et al.*, 1997). In another study by Muthuchelian *et al.* (1994), foliar spray of TRIA improved the thylakoid polypeptide contents (33, 23 and 17 kDa), CO\(_2\)-fixation and activities of PSI and PSII of flooded *Erythrina variegata* seedlings. In betelvine, that was subjected to water stress total yield and essential oil contents increased by foliar spray with TRIA (Chatterjee, 1999). Foliar application of TRIA (1 mg kg\(^{-1}\)) protected growth, carbon dioxide fixation, activities of rubisco, PSII and electron transport chain in *Samanea saman* (Jacq.) seedling when undergone under acidic mist stress of pH 4.0 and pH 2.0 (Muthuchelian *et al.*, 2003). TRIA treatment prevented decline in stomatal conductance, maintained photosynthetic process and membrane integrity in drought-stressed jack pine seedling (Rajasekaran and Blake, 1999). Chilling stress-induced negative influence on electrolyte leakage, chlorophyll contents, efficiency of PSII and gas exchange characteristics were ameliorated by foliar spray with TRIA of 0.1 mg dm\(^{-3}\) in sweet basil plants (Borowski and Blamowski, 2009). TRIA also reduced wastewater pollutants by increasing biomass of common duckweed on culture media which contain brilliant blue R dye (Kilic *et al.*, 2010).

There are some other reports also available which show no stimulatory or inhibitory effect of TRIA under different stress conditions. For example, triacontanol applied as a soil supplement at early heading stage to soil water stressed wheat plants did not improved yield components under both control or stressed conditions (Bole and Dubetz, 1978).

### 2.5.4 TRIA and Salt stress

Foliar application of TRIA on soybean plants subjected to salt (10, 15, 20, 25 and 30 mM NaCl) stress proved to be effective in restoring normal metabolic processes and increased chlorophyll, total soluble sugars, nucleic acids and proteins contents in treated plants (Krishnan and Kumari, 2008). Salt stress induced negative effects on growth, physiological and biochemical processes of *Erythrina variegata* seedlings were ameliorated with foliar
application of TRIA (Muthuchelian et al., 1996). Pre-sowing seed treatment with TRIA regulated the gas exchange characteristics of salt-stressed wheat plants (Perveen et al., 2010). Seed treatment with some plant growth regulators including TRIA improved the water conducting vascular tissues of radish seedlings under salt stress (Cavusoglu et al., 2008). Furthermore, Cavusoglu and Kabar (2007a), found that the negative influence of salt stress on radish seed germination, water uptake and percentage of hypocotyle were improved by treatment with plant growth regulators including TRIA.

2.5.5 Mode of application of triacontanol

Exogenous application of triacontanol can be done by three ways

2.4.5a) Pre-sowing seed treatment

2.4.5b) Foliar application

2.4.5c) Root-medium application

2.5.5a Presowing/seed treatment

Seed priming is a technique that is used to increase crop yield and uniformity of seed emergence under saline conditions during last few decades. It is divided into osmopriming, thermopriming, hydropriming, halopriming and hormone-priming; osmopriming is a processes that involves seed-soaking with some solution of polyethylene glycerol (PEG), sugars, sorbitol, glycerol or mannitol followed by redrying; thermopriming is a process of treating seed with low or high temperature treatments; hydropriming involves seed-soaking in water; halopriming is the process of soaking seeds in the solution of some inorganic ions; hormone-priming involves seed soaking with some optimal concentration of plant hormones or plant growth regulators (Ashraf et al., 2008). Presowing seed treatment with plant growth regulators has gained much importance to increase crop growth and yield under salt stress conditions. For example, gibberellic acid (GA₃)-induced seed priming reduced the damaging effects of salinity stress on wheat by maintaining shoot and root ionic and hormonal homeostasis (Iqbal and Ashraf, 2010). In wheat plant negative effects of salt stress has been
attenuated by using various plant growth regulators as seed priming agents e.g. brassinolides (Fariduddin et al., 2008), auxins (Iqbal and Ashraf, 2007), kinetin, polyamines, and cytokinins (Iqbal et al., 2006; Iqbal and Ashraf, 2005, 2006), and gibberellic acid (Iqbal and Ashraf, 2010). Pre-sowing seed treatment with TRIA 5-10 mg L⁻¹ followed by 5 mg L⁻¹ foliar spray at 30 days after sowing enhance seed germination, seedling growth and shoot and root length of some tree species (Raichur et al., 2001). In barley and radish seedlings pre-soaking seed treatment with triacontanol in combination with some other growth regulators ameliorated salinity effect (Cavusoglu et al., 2007, 2008). However, Charlton et al. (1980) found that seeds of Leeds durum wheat treated with triacontanol and its derivatives did not improve germination and growth.

2.5.5b Foliar-application

TRIA has stimulatory effects when applied as a foliar spray to plants (Ries et al., 1977). For example, Singh et al. (2011) studied the effect of foliar application of TRIA (10⁻⁶ M) at three growth stages on the growth, quality and productivity of ginger at 25 day interval and postulated that all growth attributes, photosynthetic pigments, leaf and rhizome nutrients, protein and carbohydrate contents were improved, while the higher concentrations of TRIA (5 x 10⁻⁵ M) inhibited the growth. Foliar spray of 0.2% TRIA at vegetative, flowering and poding stages increased plant height, fresh and dry biomass in mungbean (Jayarami et al., 2002). Similarly, in another study on mungbean Reddy et al. (2002) found that foliar spray of 0.2% TRIA at vegetative, flowering and poding stage increased seed yield under rained conditions. In rice two time foliar spray of TRIA at tillering and initiation of panicle stage were proved to be effective in enhancing grain yield (Pandey et al., 2001).

In Opium poppy, Srivastava and Sharma (1990) found that three time (at different growth stages) foliar spray of TRIA (0.01 mg L⁻¹) at 4 week interval increased shoot fresh and dry weights, plant height, total chlorophyll and morphine contents, CO₂ exchange rate and shoot nutrients (Fe, Mn, Cu) content that were decreased at higher concentration. Foliar spray of 10⁻⁶ M TRIA at 15-day interval increased the fresh and dry weight per plant, number of leaves, leaf area per plant and seed-yield by 49.2, 53.3, 45.4, 38.5 and 56.3%, respectively as compared to non-treated plants (Naeem et al., 2009). In Pearl millet foliar spray of 10 mg L⁻¹
TRIA at vegetative growth stage increased the grain yield, total sugar contents, total chlorophyll, soluble proteins, and activity of nitrate reductase and IAA oxidase (Sivakumar et al., 2006). A consistent increase in the foliar applied dose of TRIA from 0-50 mg L\(^{-1}\) increased leaf area in groundnut (Deotale et al., 1994), while a dose of 2.5 mg L\(^{-1}\) proved effective for fresh and dry biomass in okra (Nargisjahan et al., 1997). Foliar application of 4 mg L\(^{-1}\) TRIA at pre-flowering stage increased seed weight, seed yield and protein contents in chickpea (Singh et al., 1991).

Sarada et al. (2008) found that two foliar sprays of TRIA (1.0 ml L\(^{-1}\)) at 40 and 60 days after sowing (DAS) produced maximum seed yield as compared to one or three sprays recorded at 40 DAS or 40, 60 and 80 DAS respectively in coriander. Foliar spray of TRIA (1µM) along with soil application of potassium increased fruit yield, lycopene, β-carotene and ascorbic acid contents by 57.5, 9.5, 8.3 and 6.7% over control in tomato (Khan et al., 2009). In rice, foliar application of TRIA increased chlorophyll contents by 20-30% in field experiments (Debata and Murthy, 1981). Photosynthetic rate, transpiration rate and stomatal conductance increased 26.3, 14.2 and 15.6% over control at 90 days after sowing in hyacinth beans (Naeem et al., 2009). It was suggested that increased photosynthesis due to foliar spray with TRIA was associated with increased chlorophyll contents (Ivanov and Angelove, 1997), while increased transpiration rate is associated with increased stomatal conductance (Jarvis and Davies, 1998). The nutrient content level remained unaffected at 120 days after sowing plants (Naeem et al., 2009). In two tomato varieties (Hyb-SC-3 and Hyb-Himalata) growth, yield, beta-carotene and lycopene contents increased with increasing level of TRIA upto 1 mg L\(^{-1}\) (0, 0.25, 0.5, 1.0 and 2.00 mg L\(^{-1}\)), while the ascorbic acid contents were not changed by foliar applied TRIA (Khan et al., 2006). Exogenous application of TRIA along with aliphatic alcohols (Mixtalol) at the rate of 1-2 mg L\(^{-1}\) increased photosynthesis and yield of various crops, while percentage increase in paddy yield was 14-27%, wheat 13-27%, maize 33%, pearl millet 20%, potatoes 21-29%, groundnuts 15-20% and sorghum fodder 48% (Menon and Srivastava, 1984). In chilli, TRIA at the rate of 5 mg L\(^{-1}\) produced 25.70% more yield over control (Chaudhary et al., 2006).

In corn seedlings, specific activities of metabolic enzymes isocitrate dehydrogenase and 6-phosphogluconate dehydrogenase increase by 89% and 39% respectively over control
Lesniak and Ries (1983) studied that peroxidase enzyme activity remained unaffected on per plant basis and slightly decreased on per protein basis with treatment of TRIA in corn seedlings. Increased nitrate reductase has been suggested to be due to increased uptake of nutrients particularly of nitrogen or nitrates due to TRIA application (Muthuchelian et al., 1994). Jin and Hong (1988) studied the effectiveness of TRIA in 4-day old radish seedling and found increased chlorophyll contents, cytoplasmic ribosomal RNAs and activities of rubisco, chloroplast and chlorophyll-protein complexes and thylakoid polypeptide. Exogenously applied TRIA between 1200 and 1700h at three and four-leaf stage and at anthesis enhanced growth and yield in rice, maize and wheat (Ries, 1991). Among different concentrations of TRIA (0, 0.5, 1.0, 2.0 mg L⁻¹) foliar spray of 0.5 mg L⁻¹ increased the growth, photosynthetic pigments, cellular metabolites like saccharides, starch, soluble proteins, amino acids and phenolics in green gram seedlings when sprayed at 15 and 25 days after sowing (Kumaravelu et al., 2000). In strawberry, it enhanced the flowering, yield and fruit composition of spring cultivars (Hashim and Lundergan, 1985). In mangrove’s seedlings TRIA treatment enhanced the shoot and root growth, chlorophyll and carotenoid contents and protein and energy contents in leaves as well as chlorophyll contents of light harvesting complex of PSI and PSII of chloroplast (Moorthy and Kathiresan, 1993). In pea plants, in vitro treatment of isolated protoplast with TRIA increased CO₂-fixation by 166% after 60 min., while in vivo studies showed increased CO₂-fixation by 119% over control (Ivanov and Angelov, 1997). In algae, treatment of TRIA with 1-1000 µg L⁻¹ increased cell number by 21-35%, chlorophyll content 7-31% and photosynthetic CO₂-fixation by 20-100% (Houtz et al., 1985a). In Chlamydomonas cell density and chlorophyll contents increased by treatment of TRIA (Haugstad et al., 1983).

In tissue culture studies, the response of various plant species such as oat, lettuce, soybean or wheat plants was proved to be non-effective to TRIA application (Marcelle and Chrominski, 1978). Similarly, no stimulatory effect of TRIA on germination and growth (somatic embryogenesis) of wheat callus cultures (Charlton et al., 1980) and germination and early seedling growth of various weed and horticultural crops was observed (Hoagland, 1980). Effect on photosynthetic process and final yield in dark adapted rice seedlings was also observed non-significant (Bittenbender et al., 1978).
2.5.5c Application in root growing medium

Rooting medium applied TRIA increased tomato yield at concentration of 0.3µg (Borowsky et al., 2000). Two time triacontanol (0.228 µM) soil drenching with a 60 days gap enhanced growth, yield and flavor contents in swallow roots (Giridhar et al., 2005).

2.6 Wheat

Wheat (Triticum aestivum L.) is an hexaploid wheat belongs to family Poaceae (Gramineae) (Schreiber et al., 2009). It is a major staple food crop of about 35% world population (Shirazi et al., 2001). In South Asia it is cultivated in rabbi (post-monsoon season) after kharif (rainy season) crops harvest (Hobbs and Morris, 1996). Globally, wheat is grown in European Union, China, India, United States, Russia, Canada, Pakistan, Ukraine, Australia and Turkey. Pakistan ranked 8th in position for wheat production and out of 3.72% wheat growing land in Pakistan it contributes 3.17% of the world wheat production (Khan et al., 2007; Shuaib et al., 2007; 2010).

Wheat is the leading food crop occupying the central position in agriculture of Pakistan (Shuaib et al., 2007, 2010) and cultivated on wide range of climatic conditions (Chowdhry et al., 1995). In Pakistan it is grown on an area of 8.805 Mha 3.6 percent decreases than last year’s area of 9.132 Mha with total annual production of 24.2 million tons, which was 3.9 percent more than previous year (Anonymous, 2011). In agriculture of Pakistan wheat contribute 13.1% and to the gross domestic production (GDP) 2.7 (Anonymous, 2011). Yield losses of wheat on moderately saline areas are about 65% (Quayyum and Malik, 1988).

Wheat is very important due to its nutritional value. It is one of the few staple food crops that can be used as a source of flour and semolina etc., which are basic ingredient of bread and other bakery products and pastas (Sramkova et al., 2009). It is the major caloric food that contains protein, carbohydrates, minerals (Mg, P, Cu, Fe and Zn) and vitamins like riboflavin, thiamine, vitamin E and niacin (FAO, 1985; El-Bassiouny, 2005; Zhang et al., 2007; Jaiswal, 2009). The protein (gluten) contents of wheat seeds possess unique physical and chemical properties (Jaiswal, 2009).
2.6.1 Salinity effects and wheat responses

Among abiotic stresses, salinity stress is the major factor that reduces wheat crop production in arid and semi-arid regions (Fercha et al., 2011). Salinity stress reduced the life cycle of wheat crop leading to low yield (Grieve et al., 1994) and could be harvested 1 to 2 week before the non-stressed plants (Francois et al., 1986). Salt tolerant genotypes can efficiently overcome salinity-induced injury and regulate physiological and biochemical processes (Zheng et al., 2008a). Genetic variations exist in wheat genotypes. Wheat cultivars show a variable level of salt tolerance (Ma et al., 2006). For example, Elytrigia intermedia classified as salt tolerant, while E. trichophora sensitive at varying levels of NaCl due to different tissue Na\(^+\) and K\(^+\) contents (Zan et al., 2011). Under non-saline conditions wheat genotypes show a similar behaviour, while at 150 mM NaCl salt tolerant (DK 961) genotype exhibit high growth and metabolic activity than salt sensitive (JN17) genotype (Zheng et al., 2009). Decrease in wheat growth was mainly due to ion toxicity, ROS production and decrease in photosynthetic rate (Zheng et al., 2008a).

In wheat, early vegetative growth stage is more sensitive to salt stress as compared to later growth stages (Khayatnezhad and Gholamin, 2010; Bhutta and Hanif, 2010). Impairment at vegetative growth stage can significantly decrease final yield (El-Hendawya et al., 2011), while final yield is associated with numbers of tillers per plant that are main components of the final grain production of wheat and originated at early vegetative growth stages (Naseer et al., 2001). In bread wheat salinity stress delay germination processes due to increased Na\(^+\) accumulation in seed (Akbarimoghaddamh et al., 2011). Shoot and root dry weight decreased antagonistically with increased salinity level and depends upon the length of shoot and root in wheat (Salim, 1991; Akbarimoghaddamh et al., 2011).

Increase in salt stress from 50, 100, 150 and 200 mM NaCl has decreased germination (%) and growth attributes in all eight wheat cultivars used i.e. Anmol-91, Sarsabz, Mehran-89, Kiran-95, Moomal, TJ-83, Marvi and Abadgar-94 (Ghaloo et al., 2011). Salt effect was more prominent in reduction of shoot as compared to roots (Salim, 1991; Begum et al., 1992; Akbarimoghaddamh et al., 2011). In durum wheat, 150 mM of salinity significantly decreased the leaf growth, leaf area and fresh and dry weights (Fercha, 2011), while in bread
wheat shoot and root length, and dry weight reduction was 26%, 31%, and 20%, respectively at 150 mM NaCl (Ma et al., 2011). In another study, 100 mM NaCl decreased shoot and root dry weight by 56% and 47% respectively over control in wheat (Carillo et al., 2005). All yield attributes including number of tillers per plant, 1000-grain weight, spike per plant, grain yield and chlorophyll contents decreased in eight durum wheat cultivars under salinity stress (Chaabane et al., 2011). At reproductive stag, winter wheat is more sensitive for high yield production (Yu et al., 2001).

Gas exchange characteristics are not altered markedly under normal or low salt level in salt tolerant or sensitive wheat cultivars (Kafi, 2009). Photosynthetic response in wheat genotypes alters with alteration in salinity levels, plant developmental stages and duration of salinity (Kafi, 2009). Long exposure of wheat plants to salinity stress causes decrease in stomatal conductance and net photosynthetic rate (Kafi, 2009). In bread wheat (KRL-1-4) NaCl stress decreased the photosynthetic rate and chlorophyll contents (Bagdi et al., 2011).

Under high salinity stress wheat plants also suffer in decreased maximum efficiency of PSII (Kafi, 2009). Photosynthesis and chlorophyll fluorescence attributes e.g. photochemical quenching (qP), non photochemical quenching (NPq) and maximum quantum yield ($F_{v}/F_{m}$) were adversely affected by increasing NaCl level (0, 80, 120 and 160 mM) in four bread wheat cultivars (fong, chamran, star and kharchia) (Abdeshahian et al., 2010). Water relation parameters like total water content, relative water content, water potential and osmotic potential decreased, while turgor potential increased in wheat flag leaf under salinity stress (Farouk, 2011). In another report, salinity stress of 150 mM did not change relative water content in durum wheat (Fercha, 2011). Increase in proteins, sugars, antioxidant activity but decrease in $K^{+}/Na^{+}$ ratio, chlorophyll content, photosynthesis and yield has been observed in wheat genotypes (Zheng et al., 2009). In wheat, 150 mM NaCl increased MDA content by 1.5 times more than control (Ma et al., 2011).

Sodium (Na$^+$) exclusion from leaf mesophyll tissues is related with salt tolerance in many cereal crops such as bread wheat (Cuin et al., 2009, 2010), *Triticum tauschii* (Schachtman et al., 1991), tall wheatgrass (Colmer et al., 2006), rice (Asch et al., 2000; Ul Haq et al., 2010), pearl millet (Krishnamurthy et al., 2007) and *Hordeum* species (Garthwaite et al., 2005).
However, wheat tolerant lines had also been reported to possess high shoot Na⁺ contents than sensitive ones (Schachtmann and Munn, 1992). Salt tolerance of wheat cultivar (KRL 19) could be due to high K⁺/Na⁺ ratio (Mandhania et al., 2010). Under normal conditions wheat genotypes are not different, while at 150 mM NaCl salt tolerant (DK 961) genotype exhibit high osmoregulation and antioxidative ability than salt sensitive (JN17) genotype (Zheng et al., 2009).

To improve salt tolerance many attempts have been made including conventional breeding, interspecific hybridization, pooling physiological traits and marker-assisted selection. Through conventional breeding approach salt tolerance in crop plants achieved little success due to complexity of trait at physiological and genetic level. In rice and barley salt tolerance is linked with a multigenic trait Quantitative Trait Loci (QTL) that is identified in these crops. In field trials transgenic approaches did not show much progress (Flowers, 2004). Due to multigenic nature plants salt tolerance traits cannot be genetically engineered (Poltronieri et al., 2011). So, the objectives of the present study is to use some shotgun approaches like pre-sowing seed treatment and foliar spray with some plant growth regulator (that is triacontanol in this case) which could enhance growth and yield of wheat crop under salt stress.
CHAPTER 3
MATERIALS AND METHODS

In order to examine whether exogenous application of triacontanol could mitigate the adverse effects of salt stress on spring wheat (*Triticum aestivum* L.), two experiments were conducted during November-April, 2009-2010 and 2010-2011 under natural climatic conditions in a net-house of the old Botanical Garden, University of Agriculture, Faisalabad.

**Meteorological data**

The meteorological data has been shown the form of figure that indicates average temperature, relative humidity, rainfall and radiation.

![Meteorological data graph](image)

Fig.3.1 Meteorological data during two experimental years i.e. November-April, 2009-2010 and 2010-2011 respectively.
3.2 Cultivars

There were two wheat cultivars:

1. S-24 (salt tolerant)
2. MH-97 (moderately salt sensitive cultivar)

Seed of both cultivars were obtained from the Department of Botany, University of Agriculture, Faisalabad, Pakistan and Ayub Agricultural Research Institute, Faisalabad, Pakistan, respectively.

3.3 Salinity (NaCl) levels

Two NaCl levels were:

1. 0 mM NaCl (control) + Hoagland’s nutrient solution (full strength)
2. 150 mM NaCl (salt stress) + Hoagland’s nutrient solution (full strength)

3.4 Triacontanol (TRIA) Levels

Triacontanol (M. wt. 438.8192) was obtained from SIGMA-ALDRICH USA (99% pure). TRIA solution was made by dissolving weighed quantity of solid TRIA in hot distilled water + 0.1% Tween-20 (as a solvent). In addition, Tween-20 (0.1%) was again added to each level to ensure effective of TRIA in the leaf tissues. There were three TRIA levels:

1. Control (0 µM TRIA, water spray)
2. 10 µM TRIA
3. 20 µM TRIA
3.5 Mode of TRIA application

Exogenous application of TRIA was made by following two modes:

3.5.1 Seed-Priming

3.5.2 Foliar spray

3.5.1 Seed priming

To assess the effect of exogenous application of TRIA as seed priming agent seed of both wheat cultivars were surface sterilized for 5 min. in sodium hypochlorite solution (5%). After that seeds were rinsed in distilled water and air-dried. Seeds of both cultivars were soaked for 12 h in different levels of TRIA solution (0, 10, and 20 µM). Triacontanol stock solution was made by dissolving weighed quantity of TRIA in hot distilled water and 0.1% Tween-20. Tween-20 in case was used as a solvent and diluted the stock solution to attained three levels of TRIA. After 12 h soaking period, seeds were re-dried under shade so that original weight was attained. Ten seeds per pot were shown in plastic pots containing thoroughly washed river sand. Thinning was done to ten day-old plants and maintained six plants in each pot. Salinity (150 mM NaCl) treatment was applied to twenty four day-old plants. There were two salt (NaCl) levels control (0 mM) and 150 mM (NaCl). Hoagland’s nutrient solution (full strength) was applied @ 2 litters/ pot every week. Salt treatment (in full strength Hoagland’s nutrient medium) was applied in an aliquot of 50 mM solution/pot every day so that desired level was attained. After that salt level (150 mM NaCl) was applied every week until the end of experiment. Moisture content of sand was maintained daily by adding 200 ml H₂O per pot. Experimental design was completely randomized with four replicates. Data was recorded of 45 day old plants.

3.5.2 Foliar spray

Ten seeds were shown per plastic pot that contained 10 kg thoroughly washed dry river sand. Two liters of full strength Hoagland’s nutrient solution was added to each pot so that to flush through all previously present salts in the sand. Thinning was done to ten day old seedling.
Twenty one day old seedling was subjected to salinity stress (150 mM NaCl). Salt (NaCl) treatment was applied in full strength Hoagland’s nutrient solution in aliquots of 50 mM per pot every day until attained the desired level. Treatment was applied every week at the rate of two liters per pot in the evening until the end of experiment. Foliar spray of TRIA was applied of 25 ml per pot at following three growth stages:

(i) Vegetative stage when plants were 30 day old.

(ii) Boot stage when plants were 78 day old.

(iii) Vegetative + boot stages

There were four replicates of each treatment. Data was recorded of 92 day old plants. Two plants were uprooted carefully after 14 days of foliar application of TRIA at boot stage and data for growth (shoot and root fresh weights) attributes was recorded. After taking fresh biomass samples were oven-dried at 65°C for one week and then recorded dry weight. Remaining four plants were left for attaining grain yield. Data for following physiological and biochemical attributes was recorded.

a- Growth attributes

b- Yield parameters

c- Leaf Water Relations

d- Membrane permeability (%)

e- Hydrogen peroxide (H₂O₂)

f- Malondialdehyde (MDA)

g- Chlorophyll fluorescence

h- Gas Exchange Characteristics
i- Biochemical attributes

3.6 a) GROWTH ATTRIBUTES

i) Shoot and roots fresh weights (g plant⁻¹)

Two plants per replicate were uprooted carefully, thoroughly washed with distilled and immediately recorded fresh biomass of both shoots and roots.

ii) Shoot and root dry weight (g plant⁻¹)

After one week of air dry under shade plant were kept for further one week in an oven at 65°C and recorded their dry biomass.

iii) Shoot and Root length (cm)

Shoot and root length were measured in centimeters with a meter rod. Shoot length was measured from the stem base to the tip of flag leaf. Average height of two plants per replicate was recorded.

iv) Total leaf area per plant (cm²)

Total leaf area per plant was calculated by using the formula of Carleton and Foote (1965).

Leaf area (cm²) = maximum leaf length x maximum leaf width x 0.75

0.75 = Correction factor

3.6 b) Yield parameters:

At maturity the data for the following yield parameter was recorded.

1. Grain yield (g plant⁻¹)

2. Number of grains per plant

3. 100-seed weight (g)
4. Number of tillers per plant

3.6 c) LEAF WATER RELATIONS

i) Leaf water potential (-MPa)

Second leaf from top was cut with scissor from petiole before sunshine to determine leaf water potential with a Scholander type pressure chamber (Arimad-2-Japan) according to the method of Scholander *et al.* (1964).

ii) Osmotic potential (-MPa)

The same leaf of which water potential was determined was frozen at -20°C in a freezer for one week and osmotic potential was determined with the help of vapor pressure osmometer (Model 5520, USA, VAPRO).

iii) Leaf turgor pressure (MPa)

The leaf turgor potential was calculated as the difference between osmotic potential and water potential values according to Nobel (1991).

v) Relative water contents (%)

By using Jones and Turner (1978) percentage relative water content were determined. Fresh leaf samples 0.5 g was weighed (Fw), kept in the dark for 24 h in deionized water and turgid weight (Tw) was calculated. Dry weight (Dw) of samples was measured by keeping the samples in oven at 80°C for 48 h. Percentage relative water content was measured by using the following formula:

\[
RWC (%) = \left(\frac{Fw - Tw}{Fw - Dw}\right) \times 100
\]

3.6 d) MEMBRANE PERMEABILITY (%)

Fresh leaf (0.5 g) tissue was chopped and put in 10 ml of distilled water, vortexed for 5s and measured the electrical conductivity (ECo). The test tubes containing leaf samples were
covered with aluminium foil and kept at 4°C for 24 h and determined the electrical conductivity (EC₁). Then the test tubes containing samples were autoclaved for 1 h cooled at room temperature and electrical conductivity (EC₂) of dead tissues was measured. The relative membrane permeability (%) was determined by applying the following formula:

\[ \text{RMP} \% = \left( \frac{\text{EC}₁ - \text{EC}_0}{\text{EC}₂ - \text{EC}_0} \right) \times 100 \]

e) **Hydrogen peroxide (H₂O₂)**

Hydrogen peroxide was determined by following Velikova et al. (2000). Fresh leaf tissue (0.5 g) was homogenized in an ice bath with 5 ml of 0.1 % (w/v) trichloroacetic acid (TCA) by using a pre-chilled pestle and mortar. Then homogenate was centrifuged for 15 min at 12,000 x g. To the 0.5 ml supernatant added 0.5 ml potassium phosphate buffer (pH 7.0) and 1 ml potassium iodide. After vortex the absorbance of supernatant was read at 390 nm using a UV visible spectrophotometer (IRMECO U-2020). By using standard curve constructed from 20, 40, 60, 80 and 100 µM H₂O₂ contents were determined.

f) **Malondialdehyde (MDA)**

Carmak and Horst (1991) method was used to measure the MDA contents. In finely grinded 0.5 g leaf tissues added 0.1% (w/v) 10 ml of trichloroacetic acid (TCA) solution and for 10 min centrifuged at 12000 x g. To 1 ml of the supernatant added 0.5 % 4 ml thiobarbituric acid (TBA) that was prepared in 20% TCA. For 30 min. the reaction mixture was kept in a water bath at 95°C. After that, samples were cooled by keeping the samples in an ice bath. After that samples were again centrifuged at 12000 x g for 10 min. and absorbance of samples was read with the help of spectrophotometer (U2020 IRMECO) at two wavelengths of 532 and 600 nm.

3.6 e) **CHLOROPHYLL FLOURESCENCE**

According to Strasser et al. (1995) the polyphasic rise of fluorescence transients was determined by using an OS5p Modulator Fluorometer (ADC BioScientific Ltd, Great Amwell Herts, UK). The fluorescence transients were inducted by red light of 3000 µmol m⁻²
s$^{-1}$ provided by an array of six light inducing diods (peaks 650 nm), that focused to give homogenous illumination over the exposed area of sample surface. Before measurements of chlorophyll fluorescence all leaf samples were kept in dark for half an hour. The data for following chlorophyll fluorescence attributes was determined like

\[ F_o = \text{Minimal fluorescence} \]

\[ F_v/F_m = \text{Maximum quantum yield of PSII} \]

\[ ETR = \text{Electron transport rate} \]

\[ q_P = \text{Photochemical quenching} \]

\[ q_N = \text{Co-efficient of non-photochemical quenching} \]

\[ NPQ = \text{Non-photochemical quenching} \]

### 3.6 f) GAS EXCHANGE CHARACTERISTICS

Gas exchange characteristics were determined by using an open system LCA-4 ACD a portable infrared gas analyzer (Analytical Development, Hoddesdon, UK). Following measurements were made photosynthetic rate ($A$), transpiration rate ($E$), internal CO$_2$ concentration ($C_i$) of leaf tissue, stomatal conductance ($g_s$) and water use efficiency ($A/E$) from 10:30 to 12:30 h on second leaf from top of each plant. Conditions during data measurements were: ambient pressure (P) 98.8 kPa; leaf chamber gas flow rate (U) 251 µmol s$^{-1}$; leaf surface area 11.25 cm$^2$; concentration of ambient CO$_2$ was 350 µmol mol$^{-1}$; water vapor pressure into the leaf chamber ranged from 6.0 to 8.9 mbar; temperature of leaf chamber varied from 28.4 to 32.4 °C; RH of the chamber 41.2%; molar flow of air/unit leaf area (Us) 22.06 mol m$^{-2}$ s$^{-1}$; \( PAR (Q_{leaf}) \) at the leaf surface was maximum up to 942 µmol m$^{-2}$ s$^{-1}$. 

\( P_{\text{PAR}} \)
3.6  

i) **BIOCHEMICAL ATTRIBUTES**

i) Chlorophyll contents

Chlorophyll ‘a’ and ‘b’ contents were determined by following Arnon (1949). Fresh leaves 0.5 g were cut into small pieces, added 10 ml of 80% acetone to it and kept overnight at 0-4°C. The extract was centrifuged at 10,000 × g for five min. and absorbance of the supernatant was read at 645 and 663 nm with spectrophotometer (Hitachi-U2001, Tokyo, Japan).

Chlorophyll ‘a’ and ‘b’ contents were calculated by using the following formulae

\[
\text{Chl. } a = [12.7 \text{ (OD 663)} - 2.69 \text{ (OD 645)}] \times \frac{V}{1000} \times W
\]

\[
\text{Chl. } b = [22.9 \text{ (OD 645)} - 4.68 \text{ (OD 663)}] \times \frac{V}{1000} \times W
\]

\[V = \text{volume of the extract (mL)}\]

\[W = \text{weight of the fresh leaf tissue (g)}\]

3.6 ii) Mineral Nutrients determination: Mineral ions (Na⁺, K⁺ and Ca²⁺) in shoot and root were determined by following Allen *et al.* (1985). Digestion mixture was made by properly mixing Se (0.42g) and LiSO₄.2H₂O₂ (14g) to H₂O₂ (350 ml), slowly added conc. H₂SO₄ (420 ml) to it by keeping it in an ice bath and stored at 2°C and used for plant tissue (shoot and root) digestion. To 100 mg dried ground shoots and root material added 2 ml digestion mixture in a digestion flask. After two hours flask was placed on a hot plate. Temperature of hot plate was raised gradually from 50°C to 200°C. Then added 1 ml HClO₄ to the reaction mixture and heated till colorless material gained. After that flasks were cooled down by removing from hot plate. Then mixture is diluted with distilled water, volume was maintained upto 50 ml, filtered and filtrate was used for Na⁺, K⁺ and Ca²⁺ ions determination with the help of a flame photometer (Jeneway, PFP-7).

**Chloride (Cl⁻) determination:** For Cl⁻ determination 100 mg dried ground leaf or root material was taken in a test tube and added 10 ml of distilled water to it. Then material was
extracted by placing test tubes in test tube stands in an oven at 80°C for 6 h. Then test tube stands were removed and cooled down. Then volume was maintained to original volume by adding distilled water. The concentration of Cl- was determined with a chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK).

iii) Antioxidant enzyme Assay

Antioxidant enzymes extraction

Antioxidant enzymes were extracted by finely grinding 0.5 g fresh leaves (0.5 g) in 10 ml of phosphate buffer (50 mM with pH 7.8) in an ice bath. The homogenate was then centrifuged at 12000 × g at 4ºC for 20 min and then centrifuged again at 15000×g for 10 min. The supernatant was stored at 20°C for determining the activities of antioxidant enzymes.

Superoxide dismutase (SOD)

Giannopolitis and Ries (1977) method was used for the determination of SOD activity by determining the enzyme ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT). The 3 ml reaction solution consists of 50 mM phosphate buffer of 7.8 pH, distilled water, methionine 13 mM, 50 µM NBT, 50 µl enzyme extract and 1.3 µM riboflavin. The reaction solutions were then kept under light (15 fluorescent lamps) for 15 minutes at 78 µmol m⁻² s⁻¹. Then Absorbance of reaction mixture was read at 560 nm with a UV-visible spectrophotometer (U-2100, Hitachi, Tokyo, Japan). One unit activity of SOD was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT photoreduction as compared to the sample that lacked plant enzyme extract.

Catalase (CAT) and peroxidase (POD)

Chance and Maehly (1955) method was used in the present study to determine the activities of CAT and POD on protein basis. The reaction solution for CAT enzyme contains phosphate buffer and H₂O₂ of 50 and 5.9 mM respectively and addition of 0.1 ml enzyme extract initiate the reaction. After every 20 seconds the changes in the absorbance of reaction mixture was observed at 240 nm. The reaction mixture for POD consists of phosphate buffer,
guaicol, H₂O₂ and enzyme extract with molar values as 50, 20 and 40 mM respectively and enzyme extract of 0.1 ml. At 470 nm the absorbance was taken after every 20 seconds. The enzyme activity was assessed on protein basis, while one unit of CAT was considered equivalent to 0.01 units per min. change in absorbance and one unit of POD was defined as the 0.01 units per min. absorbance change.

iv) Total soluble proteins

Fresh leaves 0.5 g were homogenized in 10 ml of 50 mM phosphate buffer, centrifuged at 6000 x g for 5 minutes at 4°C and extract was used for total soluble proteins determination by following Bradford (1976).

j) Osmolytes:

i) Glycinebetaine determination

Glycinebetaine contents in fresh leaf tissues were determined by following Grieve and Grattan (1983). Fresh leaf tissue 0.5 g was finely grinded with distilled water and volume was made upto 10 ml. The homogenate was then filtered with Whatman No. 2 filter paper. To 1 ml of above filtrate added 1 ml of 2 N H₂SO₄. Then to the 0.5 ml of above mixture added 0.2 ml K-I₃ solution in an ice bath and mixture was cooled for 90 minutes at 0-4°C. After that 2.8 ml of chilled distilled water and 6 ml of 1-2-dichloromethane was added to the sample mixture. Two distinct layers were formed and absorbance of the colored solution was read at 365 nm using a spectrophotometer (IRMECO U2020).

ii) Leaf free proline

Free proline contents were determined from the leaf tissues according to the method of Bates et al. (1973). Fresh leaf (0.5 g) was properly homogenized in 10 ml of sulphosalicylic acid (w/v) solution and obtained filtrate using Whatman No. 2 filter paper. To the 2 ml filtrate added 2 ml each of acid ninhydrin and glacial acetic acid and heated at 100°C in a water bath for 1 h. After that reaction mixture was placed in an ice bath to terminate the reaction. Then to the reaction mixture added 4 ml toluene and extracted after vigorous vortexing for 15 sec. The free proline was aspirated from the chromophore layer, kept at room temperature and
absorbance was read at 520 nm on a spectrophotometer (IRMEOC U2020). Blanks were run with the same method using 2 ml 3% sulphosalicylic acid solutions. A standard curve was made by running the proline standards. The free proline contents in the leaf tissues were calculated by using the following formula:

$$\mu \text{mole proline g}^{-1} \text{fresh weight} = \frac{\mu \text{g proline ml}^{-1} \times \text{ml of toluene}}{115.5} \frac{\text{ml}}{\text{g of sample}}$$

k) **Total free amino acids**

They were determined according to the method of Moore and Stein (1957). Fresh leaves (0.5 g) were homogenized in 10 ml of citrate buffer (pH 5.0). Then mixture was centrifuged at 15000 x g for 10 minutes. The extraction samples were further processed with ninhydrin solution that was made by dissolving 2 g ninhydrin in 100 ml of distilled water and optical density of the solution was read at 570 nm using a spectrophotometer (IRMEOCO U2020).

l) **Total phenolics**

Total phenolic contents were determined by following Julkenen-Titto (1985). Fresh leaves 100 mg were homogenized in 2 ml of 80% acetone, centrifuged at 10,000 x g for 15 minutes, collected supernatant in a microfuge tube and stored at 20°C. Diluted 100 μl of the extract with 2.0 ml of distilled water in a test tube, added 0.5 ml of Folin–Ciocalteau’s phenol reagent and shacked vigorously. Then to the above mixture added 2.5 ml of 20% Na₂CO₃ solution, final volume was made upto 5 ml using distilled H₂O, vortexed for 5-10 second and left samples for 20 minutes. Then absorbance of samples was measured at 750 nm using a spectrophotometer (IRMEOCO U2020).

**Statistical analysis:**

For the present completely randomized designed experiment analysis of variance (ANOVA) for all parameters was made by using MSTAT computer program (MSTAT Development Team, 1989).
CHAPTER 4

RESULTS

4.1 Exogenous application of triacontanol as foliar-spray (Experiment 1)

In order to investigate whether exogenous application of triacontanol (TRIA) as foliar spray could mitigate the adverse effects of salt stress on wheat (*Triticum aestivum* L.), two experiments were carried out in the net house of Botanical Garden University of Agriculture, Faisalabad under natural climatic conditions. First experiment was conducted during November, 2009 to April, 2010 and second experiment was held during November, 2010 to April, 2011. Data of various growth and yield attributes were subjected to analysis of variance using MSTAT computer package and observed that both experiments showed similar results of foliar-applied TRIA on wheat plants under normal and salt stress conditions. The pooled data of both experiments was then subjected to four-way ANOVA (MSTAT computer program).

Salt (NaCl) stress significantly decreased the shoot fresh weight of both wheat cultivars (Table 1; Fig. 4.1a). Cultivars differed markedly in terms of shoot fresh weight as cultivar S-24 was superior in shoot fresh weight than cv. MH-97 under both stressed and non-stressed conditions. Foliar application of TRIA significantly increased the shoot fresh weight of both wheat cultivars when applied at vegetative, boot and veg + boot stages. However, effect of various TRIA levels was variable as under non-saline conditions both TRIA levels 10 and 20 μM were effective in increasing shoot fresh weight, while under salt stress conditions 10 μM TRIA level seemed to be slightly effective at vegetative and boot stages in cv. S-24 only (Table 1; Fig. 4.1a). Overall, comparison of the effect of TRIA application was more prominent at veg. + boot stages as compared to other growth stages particularly in cv. S-24 under non-saline conditions (Table 1; Fig. 4.1b).

Rooting medium salinity of 150 mM (NaCl) significantly decreased the shoot dry weight of both cultivars. Reduction in shoot dry weight was sharper in cultivar MH-97 than cv. S-24 under saline conditions. Foliar application of TRIA significantly increased shoot dry weight of both wheat cultivars at all growth stages under both salt stressed and non-stressed conditions (Table 1; Fig. 4.2a). The most effective TRIA level for increasing shoot dry
Table 1. Mean squares from analysis of variance of data for growth and yield attributes of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with triacontanol at various growth stages under non-stressed and salt-stressed conditions.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot f. wt.</th>
<th>Shoot dry wt.</th>
<th>Root f. wt.</th>
<th>Root dry wt.</th>
<th>Total leaf area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>57.74*</td>
<td>11.60***</td>
<td>2.901***</td>
<td>0.142***</td>
<td>1603.5ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>12960.7***</td>
<td>328.14***</td>
<td>11.00***</td>
<td>0.488***</td>
<td>592031***</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>5.550ns</td>
<td>3.135ns</td>
<td>0.500***</td>
<td>0.004ns</td>
<td>18213.1ns</td>
</tr>
<tr>
<td>Growth stages (Gs)</td>
<td>2</td>
<td>0.962ns</td>
<td>2.244ns</td>
<td>0.037ns</td>
<td>0.0060ns</td>
<td>958.2ns</td>
</tr>
<tr>
<td>Cvs x Gs</td>
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<td>9.755ns</td>
<td>0.408ns</td>
<td>0.035ns</td>
<td>0.004*</td>
<td>19989.1ns</td>
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<tr>
<td>S x Gs</td>
<td>2</td>
<td>0.941ns</td>
<td>0.233ns</td>
<td>0.025ns</td>
<td>0.0001ns</td>
<td>30203.8*</td>
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<tr>
<td>Cvs x S x Gs</td>
<td>2</td>
<td>3.733ns</td>
<td>0.816ns</td>
<td>0.001ns</td>
<td>0.0008ns</td>
<td>1479.6ns</td>
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<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>56.69**</td>
<td>3.782*</td>
<td>0.140***</td>
<td>0.005*</td>
<td>41817.1*</td>
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<td>Cvs x TRIA</td>
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<td>3.620ns</td>
<td>1.125ns</td>
<td>0.011ns</td>
<td>0.0002ns</td>
<td>1264.2ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>35.86*</td>
<td>0.835ns</td>
<td>0.006ns</td>
<td>0.0002ns</td>
<td>22243.7ns</td>
</tr>
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<td>Cvs x S x TRIA</td>
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<td>0.402ns</td>
<td>0.523ns</td>
<td>0.006ns</td>
<td>0.0007ns</td>
<td>6219.5ns</td>
</tr>
<tr>
<td>Gs x TRIA</td>
<td>4</td>
<td>5.518ns</td>
<td>0.303ns</td>
<td>0.037ns</td>
<td>0.0003ns</td>
<td>6146.4ns</td>
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<tr>
<td>Cvs x Gs x TRIA</td>
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<td>15.51ns</td>
<td>0.640ns</td>
<td>0.006ns</td>
<td>0.0004ns</td>
<td>3227.5ns</td>
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<td>S x Gs x TRIA</td>
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<td>7.920ns</td>
<td>0.278ns</td>
<td>0.019ns</td>
<td>0.001ns</td>
<td>3349.4ns</td>
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<td>Cvs x S x Gs x TRIA</td>
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<td>Error</td>
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<td>9.268</td>
<td>0.819</td>
<td>0.017</td>
<td>0.001</td>
<td>9621.1</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Grain yield/plant</th>
<th>Number of grains/plant</th>
<th>100-grain wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>974.5***</td>
<td>17.36**</td>
<td>0.094ns</td>
<td>95.96ns</td>
<td>0.25ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>13236.3***</td>
<td>230.0***</td>
<td>148.5***</td>
<td>65978.2***</td>
<td>12.25***</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>22.26ns</td>
<td>14.69*</td>
<td>0.606ns</td>
<td>857.54ns</td>
<td>0.022ns</td>
</tr>
<tr>
<td>Growth stages (Gs)</td>
<td>2</td>
<td>13.17ns</td>
<td>0.835ns</td>
<td>2.036*</td>
<td>1312.9*</td>
<td>0.469*</td>
</tr>
<tr>
<td>Cvs x Gs</td>
<td>2</td>
<td>23.74ns</td>
<td>0.166ns</td>
<td>0.446ns</td>
<td>118.5ns</td>
<td>0.080ns</td>
</tr>
<tr>
<td>S x Gs</td>
<td>2</td>
<td>11.35ns</td>
<td>2.189ns</td>
<td>0.050ns</td>
<td>26.46ns</td>
<td>0.048ns</td>
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<td>Cvs x S x Gs</td>
<td>2</td>
<td>56.88ns</td>
<td>1.543ns</td>
<td>0.133ns</td>
<td>216.9ns</td>
<td>0.092ns</td>
</tr>
<tr>
<td>TRIA</td>
<td>2</td>
<td>271.2***</td>
<td>11.49**</td>
<td>7.016***</td>
<td>2990.2***</td>
<td>0.876**</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>20.02ns</td>
<td>0.647ns</td>
<td>2.654*</td>
<td>929.1ns</td>
<td>0.258ns</td>
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<td>S x TRIA</td>
<td>2</td>
<td>95.54*</td>
<td>1.783ns</td>
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<td>429.2ns</td>
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<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>10.36ns</td>
<td>1.668ns</td>
<td>0.274ns</td>
<td>219.4ns</td>
<td>0.267ns</td>
</tr>
<tr>
<td>Gs x TRIA</td>
<td>4</td>
<td>31.77ns</td>
<td>1.845ns</td>
<td>0.644ns</td>
<td>536.9ns</td>
<td>0.343ns</td>
</tr>
<tr>
<td>Cvs x Gs x TRIA</td>
<td>4</td>
<td>28.88ns</td>
<td>0.142ns</td>
<td>1.052ns</td>
<td>516.6ns</td>
<td>0.319ns</td>
</tr>
<tr>
<td>S x Gs x TRIA</td>
<td>4</td>
<td>48.80ns</td>
<td>2.726ns</td>
<td>0.889ns</td>
<td>558.6ns</td>
<td>0.237ns</td>
</tr>
<tr>
<td>Cvs x S x Gs x TRIA</td>
<td>4</td>
<td>50.76ns</td>
<td>0.658ns</td>
<td>0.192ns</td>
<td>205.4ns</td>
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</tr>
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<td>Error</td>
<td>108</td>
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<td>2.365</td>
<td>0.648</td>
<td>405.5</td>
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</tr>
</tbody>
</table>

*, **, and *** = significant at 0.05, 0.01, and 0.001, respectively

ns = non-significant; df = degrees of freedom
Fig 4.1a. Shoot fresh weight of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.1b. Shoot fresh weight comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.2a. Shoot dry weight of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.2b. Shoot dry weight comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
weight was 20 \( \mu M \) when applied at veg. + boot stages in both cultivars under both salt stressed and non-stressed conditions. Overall, TRIA application at veg. + boot stages was more effective for increasing shoot dry weight particularly in cv. S-24 under non-stress conditions (Fig 4.2b). Salinity stress significantly decreased root fresh weight of both wheat cultivars (Table 1; Fig. 4.3a). Cultivars showed significant difference in root fresh weight as cv. MH-97 was higher in root fresh weight than S-24 under both prevailing conditions. Foliar application of TRIA significantly increased the root fresh weight of two cultivars at all growth stages (Table 1; Fig. 4.3a). However, effectiveness of TRIA levels variable at various growth stages. The most effective TRIA level for increasing root fresh weight was 10 \( \mu M \) TRIA at vegetative stage, while at veg. + boot stage 20 \( \mu M \) TRIA markedly increased this attribute in both cultivars under both normal and saline conditions (Table 1; Fig. 4.3a). Overall, comparison of the TRIA application exhibit that effect of foliar application of TRIA was same at all growth stages (Table 1; Fig. 4.3b).

Root dry weight of both wheat cultivars significantly decreased under salt stress (Table 1; Fig. 4.4a). Cultivar MH-97 markedly had higher root dry weight as compared to cv. S-24 under both non-saline and saline conditions. Foliar application of TRIA markedly increased the root dry weight of both wheat cultivars under both saline and non-saline conditions at all developmental growth stages (Table 1; Fig. 4.4a). Overall, 10 \( \mu M \) TRIA was more effective at all growth stages except for cv. MH-97 when applied at veg + boot stage where 20 \( \mu M \) was more effective (Table 1; Fig. 4.4a). Comparison of the TRIA application at different growth stages showed that increase in root dry weight was more at veg. + boot stage in cv. S-24, while this increase was observed at vegetative or boot stage in cv. MH-97 (Table 1; Fig. 4.4b).

Total leaf area per plant markedly decreased under saline conditions in both wheat cultivars. Cultivars did not differ significantly in this attribute (Table 1; Fig. 4.5a). Foliar applied TRIA significantly enhanced total leaf area per plant when applied at various growth stages. Foliar-applied 20 \( \mu M \) TRIA was more effective at veg. + boot stage for both wheat cultivars, while 10 \( \mu M \) TRIA was more prominent in enhancing total leaf area at vegetative and boot stages. Overall, TRIA application at boot and veg. + boot stage was more effective in increasing total leaf area per plant in both wheat cultivars particularly under non-saline conditions (Table 1; Fig. 4.5b).
Fig 4.3a. Root fresh weight of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.3b. Root fresh weight comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.4a. Root dry weight of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.4b. Root dry weight comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.5a. Total leaf area/plant of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.5b. Total leaf area/plant comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Shoot length of both wheat cultivars significantly decreased under NaCl induced stress of 150 mM (Table 1; Fig. 4.6a). Cultivars also showed significant difference in above mentioned attribute. Of both cultivars, S-24 was slightly higher and MH-97 lower in shoot length. Foliar application of triacontanol (TRIA) significantly increased the shoot length at all growth stages. However, TRIA induced increase in shoot length was prominent under non-stress conditions as compared to those of salt stress. Effect of various levels of foliar-applied TRIA was not consistent for both wheat cultivars at various growth stages. Low level of TRIA (10 µM) was more effective when applied at boot stage and 20 µM when applied at veg. and veg + boot stages (Fig. 4.6a). Overall, application of TRIA at different growth stages exerted similar effect on shoot length of the two cultivars under non-stressed and salt-stressed conditions (Table 1; Fig. 4.6b).

Root length significantly decreased in both wheat cultivars under salinity stress (150 mM NaCl) (Table 1; Fig. 4.7a). Cultivars significantly differed in root length as cv. S-24 was higher in root length than cv. MH-97. Exogenous foliar application of TRIA significantly increased the root length of both wheat cultivars at all growth stages. The most effective TRIA level for improving root length was 10 µM at vegetative and boot stages, while at veg. + boot stages root length increased with the increasing level of TRIA particularly under non-saline conditions. Overall, foliar application of TRIA at veg. + boot stages was more effective for increasing root length under non-stressed condition particularly in cv. S-24 (Table 1; Fig. 4.7b).

Yield attribute, i.e., grain yield and number of grains per plant significantly decreased in both wheat cultivars under salinity stress (Table 1; Fig. 4.8a and 4.9a). Cultivars showed no significant difference in these attributes. Foliar application of TRIA significantly increased the grain yield and number of grains per plant in the two cultivars (Table 1; Fig. 4.8a and 4.9a). However, variable response of cultivars was observed to foliar application of TRIA. For example, in cv. S-24 there was a consistent increase in grain yield with the increase in TRIA level at all growth stages except at boot stage, while in MH-97 this consistent increase under both saline and normal conditions was only observed at vegetative stage and 10 µM TRIA seemed more effective for cv. MH-97 (Table 1; Fig. 4.8a and 4.9a). Overall, TRIA application at veg. + boot stages was proved to be more effective for enhancing yield.
Fig 4.6a. Shoot length of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.6b. Shoot length comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.7a. Root length of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.7b. Root length comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.8a. Grain yield per plant of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.8b. Grain yield per plant comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.9a. Number of grains per plant of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.9b. Number of grains per plant comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
attributes as compared to other growth stages in both cultivars (Fig 4.8b and 4.9b). Hundred grains weight of both wheat cultivars significantly decreased under saline conditions (Table 1; Fig. 4.10a). Cultivars did not showed difference in this attribute under both non-stressed and salt-stressed conditions. Foliar application of TRIA at different growth stages significantly increased hundred seed weight under stressed and non-stressed conditions in both cultivars (Table 1; Fig. 4.10a). However, comparison of the effectiveness of TRIA application at various growth stages was more prominent at veg. + boot stages in both wheat cultivars under stressed and non-stressed conditions (Table 1; Fig. 4.10b).

Salinity stress significantly decreased number of fertile tillers per plant in both wheat cultivars (Table 2; Fig. 4.11a). Cultivars did not differ significantly in this attribute. Exogenous application of TRIA as foliar spray did not alter number of fertile tillers in both cultivars under both non-stressed and stressed conditions (Table 1; Fig. 4.11a).

Salinity stress significantly decreased the photosynthetic rate \( (A) \) in both wheat cultivars. Cultivars did not differ markedly. Foliar application of TRIA significantly increased the photosynthetic rate \( (A) \) in both wheat cultivars when applied at all growth stages under both saline and non-saline conditions (Table 2; Fig. 4.12a). However, the 10 \( \mu M \) TRIA level was more effective at all growth stages except boot stage where 20 \( \mu M \) TRIA was proved to be more effective in enhancing the photosynthetic rate of both cultivars. Comparison of the effectiveness of TRIA at different growth stages indicated that the more prominent effect of TRIA was at veg. + boot stages in both cultivars under both stressed and non-stressed conditions (Table 2; Fig. 4.12b).

Rooting medium salinity significantly decreased transpiration rate \( (E) \) in both wheat cultivars (Table 2; Fig. 4.13a). Foliar application of TRIA did not show significant effect on transpiration rate \( (E) \), however, interactions of foliar-applied TRIA with cultivars and salt stress were observed significant (Table 2; Fig. 4.13a). As under both normal and salt stress conditions foliar application of TRIA enhanced transpiration rate at all growth stages only in cultivar MH-97, while in cv. S-24 slight increase was only observed under saline conditions particularly at vegetative stage (Table 2; Fig. 4.13a). Overall, transpiration rate showed uniform behavior to TRIA application at various growth stages (Table 2; Fig. 4.13b).
Table 4.2: Mean squares from analysis of variance of data for number of tillers per plant, leaf gas exchange parameters and chlorophyll contents of wheat (*Triticum aestivum* L.) when plants were foliarily sprayed with triacontanol at various growth stages under non-stressed and salt-stressed conditions.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Number of tillers plant⁻¹</th>
<th>A</th>
<th>E</th>
<th>gs</th>
<th>Ci</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.020ns</td>
<td>0.132ns</td>
<td>0.152ns</td>
<td>336.1ns</td>
<td>129.6ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>92.83***</td>
<td>7.058***</td>
<td>1.651***</td>
<td>69.44ns</td>
<td>529.7ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.005ns</td>
<td>0.565ns</td>
<td>0.0003ns</td>
<td>1344.4*</td>
<td>148.0ns</td>
</tr>
<tr>
<td>Growth stages (Gs)</td>
<td>2</td>
<td>0.024ns</td>
<td>0.247ns</td>
<td>0.002ns</td>
<td>34.03ns</td>
<td>34.6ns</td>
</tr>
<tr>
<td>Cvs x Gs</td>
<td>2</td>
<td>0.078ns</td>
<td>0.581ns</td>
<td>0.102ns</td>
<td>63.19ns</td>
<td>470.0ns</td>
</tr>
<tr>
<td>S x Gs</td>
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<td>0.368ns</td>
<td>0.069ns</td>
<td>0.002ns</td>
<td>88.19ns</td>
<td>21.86ns</td>
</tr>
<tr>
<td>Cvs x S x Gs</td>
<td>2</td>
<td>0.117ns</td>
<td>0.460ns</td>
<td>0.025ns</td>
<td>567.4ns</td>
<td>777.4ns</td>
</tr>
<tr>
<td>TRIA</td>
<td>2</td>
<td>0.667ns</td>
<td>2.209***</td>
<td>0.050ns</td>
<td>786.1*</td>
<td>295.4ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>0.191ns</td>
<td>0.233ns</td>
<td>0.556***</td>
<td>1702.8***</td>
<td>97.34ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>0.179ns</td>
<td>0.129ns</td>
<td>0.302**</td>
<td>1202.8**</td>
<td>661.8ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.017ns</td>
<td>0.169ns</td>
<td>0.156ns</td>
<td>552.8ns</td>
<td>561.8ns</td>
</tr>
<tr>
<td>Gs x TRIA</td>
<td>4</td>
<td>0.089ns</td>
<td>0.689ns</td>
<td>0.071ns</td>
<td>25.69ns</td>
<td>280.0ns</td>
</tr>
<tr>
<td>Cvs x Gs x TRIA</td>
<td>4</td>
<td>0.057ns</td>
<td>0.163ns</td>
<td>0.094ns</td>
<td>223.6ns</td>
<td>927.2ns</td>
</tr>
<tr>
<td>S x Gs x TRIA</td>
<td>4</td>
<td>0.098ns</td>
<td>0.199ns</td>
<td>0.029ns</td>
<td>96.52ns</td>
<td>356.0ns</td>
</tr>
<tr>
<td>Cvs x S x Gs x TRIA</td>
<td>4</td>
<td>0.122ns</td>
<td>0.175ns</td>
<td>0.037ns</td>
<td>169.4ns</td>
<td>580.3ns</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>0.1524</td>
<td>0.297</td>
<td>0.051</td>
<td>207.9</td>
<td>440.7</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Ci/Ca</th>
<th>WUE (A/E)</th>
<th>Chl. a</th>
<th>Chl. b</th>
<th>Chl. a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.001ns</td>
<td>1.509ns</td>
<td>0.056ns</td>
<td>0.256***</td>
<td>2.076***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>0.004ns</td>
<td>0.481ns</td>
<td>0.710***</td>
<td>0.917***</td>
<td>6.214***</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.001ns</td>
<td>0.961ns</td>
<td>0.116**</td>
<td>0.332***</td>
<td>2.243***</td>
</tr>
<tr>
<td>Growth stages (Gs)</td>
<td>2</td>
<td>0.003ns</td>
<td>0.385ns</td>
<td>0.002ns</td>
<td>0.003ns</td>
<td>0.016ns</td>
</tr>
<tr>
<td>Cvs x Gs</td>
<td>2</td>
<td>0.004ns</td>
<td>0.338ns</td>
<td>0.034ns</td>
<td>0.007ns</td>
<td>0.053ns</td>
</tr>
<tr>
<td>S x Gs</td>
<td>2</td>
<td>0.0008ns</td>
<td>0.640ns</td>
<td>0.005ns</td>
<td>0.001ns</td>
<td>0.103ns</td>
</tr>
<tr>
<td>Cvs x S x Gs</td>
<td>2</td>
<td>0.006ns</td>
<td>0.214ns</td>
<td>0.002ns</td>
<td>0.005ns</td>
<td>0.063ns</td>
</tr>
<tr>
<td>TRIA</td>
<td>2</td>
<td>0.002ns</td>
<td>0.460ns</td>
<td>0.121***</td>
<td>0.056*</td>
<td>0.084ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>0.0008ns</td>
<td>1.638ns</td>
<td>0.008ns</td>
<td>0.005ns</td>
<td>0.378ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>0.005ns</td>
<td>3.453**</td>
<td>0.016ns</td>
<td>0.009ns</td>
<td>0.093ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.004ns</td>
<td>0.688ns</td>
<td>0.026ns</td>
<td>0.004ns</td>
<td>0.009ns</td>
</tr>
<tr>
<td>Gs x TRIA</td>
<td>4</td>
<td>0.002ns</td>
<td>0.139ns</td>
<td>0.080ns</td>
<td>0.021ns</td>
<td>0.124ns</td>
</tr>
<tr>
<td>Cvs x Gs x TRIA</td>
<td>4</td>
<td>0.007ns</td>
<td>0.893ns</td>
<td>0.007ns</td>
<td>0.009ns</td>
<td>0.088ns</td>
</tr>
<tr>
<td>S x Gs x TRIA</td>
<td>4</td>
<td>0.003ns</td>
<td>0.405ns</td>
<td>0.056ns</td>
<td>0.010ns</td>
<td>0.132ns</td>
</tr>
<tr>
<td>Cvs x S x Gs x TRIA</td>
<td>4</td>
<td>0.005ns</td>
<td>0.562ns</td>
<td>0.005ns</td>
<td>0.004ns</td>
<td>0.103ns</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>0.004</td>
<td>0.637</td>
<td>0.015</td>
<td>0.014</td>
<td>0.146</td>
</tr>
</tbody>
</table>

*, **, and ***= significant at 0.05, 0.01, and 0.001, respectively.

ns = non-significant; df = degrees of freedom

A = Net CO₂ assimilation rate; E = Transpiration rate

gs = Stomatal conductance; Ci = Sub-stomatal CO₂ conc.

Ci/Ca = Relative internal CO₂ conc.; WUE (A/E) = Water use efficiency

Chl. a = Chlorophyll a; Chl. b = Chlorophyll b; Chl a/b = Chlorophyll a/b ratio
Fig 4.10a. 100-grain weight of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.10b. 100-grain weight comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.11a. Number of fertile tillers per plant of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.11b. Number of fertile tillers per plant comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.12a. Net CO$_2$ assimilation rate of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.12b. Net CO$_2$ assimilation rate comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.13a. Transpiration rate of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.13b. Transpiration rate comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.14a. Stomatal conductance of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.14b. Stomatal conductance comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Data for stomatal conductance ($g_s$) showed a significant reduction in both wheat cultivars under saline conditions. Cultivar S-24 was higher in stomatal conductance ($g_s$) than MH-97 under saline conditions at vegetative and veg. + boot stages of TRIA application (Table 2; Fig. 4.14a). Foliar application of TRIA exerted a prominent effect on stomatal conductance ($g_s$) in cv. MH-97 at all growth stages, while in cv. S-24 only at vegetative stage under saline conditions. Under non-saline conditions stomatal conductance decreased in both wheat cultivars except at boot stage in cv. MH-97 due to foliar application of TRIA (Table 2; Fig. 4.14a). Overall, TRIA application proved to be effective in increasing $g_s$ value of only cultivar MH-97 under saline conditions at all growth stages (Table 2; Fig. 4.14b).

Sub-stomatal internal CO$_2$ concentration ($Ci$) and $Ci/Ca$ ratio did not alter significantly in both wheat cultivars under saline conditions. Cultivars did not differ significantly in both attribute. Application of TRIA as foliar spray was also proved to be non-effective when applied at various growth stages (Table 3; Fig. 4.15a and 4.16a).

Salt stress did not alter water use efficiency (WUE) significantly in both wheat cultivars. However, foliar application of TRIA significantly increased the WUE in cv. S-24 under non-saline conditions at all growth stages and under saline conditions at boot and veg. + boot stages, while in MH-97 the response was variable under both non-saline and saline conditions. Cultivars did not differ markedly in this attribute (Table 3; Fig. 4.17a). Overall, WUE of cv. S-24 increased under non-stressed conditions when TRIA was applied at vegetative growth stage (Table 3; Fig. 4.17b).

Salinity stress significantly decreased chlorophyll a (chl. a) contents in both wheat cultivars (Table 2; Fig. 4.18a). Cultivars did not differ significantly in this character. However, under non-saline conditions, cultivars showed significant difference as cv. MH-97 was higher in chl. a contents than S-24. TRIA at 10 µM level was more effective for enhancing chl. a contents in both cultivars at all growth stages, however, at veg. + boot stages 20 µM TRIA had greater effect to increase chl. a contents in cv. MH-97 under non-saline conditions. Overall, TRIA application at veg. + boot stages was more effective in increasing chl. a contents in cv. MH-97 under non-saline conditions (Fig. 4.18b).
Fig 4.15a. Sub-stomatal CO$_2$ of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.15b. Sub-stomatal CO$_2$ comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.16a. $\frac{C}{C_a}$ ratio of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.16b. $\frac{C}{C_a}$ ratio comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.17a. Water use efficiency of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.17b. Water use efficiency comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.18a. Chlorophyll $a$ of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.18b. Chlorophyll $a$ comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.19a. Chlorophyll $b$ of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.19b. Chlorophyll $b$ comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Chlorophyll b (chl. b) contents significantly decreased in both wheat cultivars under saline conditions (Table 2; Fig. 4.19a). Cultivars differ significantly in this character as cv. MH-97 was higher in chl. b contents than cv. S-24 under non-saline conditions. Foliar application of TRIA markedly increased chl. b in both cultivars at all growth stages (Table 2; Fig. 4.19a). TRIA at 10 μM concentration had increased chl. b contents more effectively in both cultivars at all growth stages except at veg. + boot stages where 20 μM TRIA was more effective in increasing chl. b content in MH-97 only under non-stressed conditions (Table 2; Fig. 4.19a). Overall, TRIA application at all growth stages increased chl. b contents, however, at veg. + boot stages increase in chl. b contents were high in cv. MH-97 under non-stressed conditions (Table 2; Fig. 4.19b). Chlorophyll a/b ratio significantly decreased in both wheat cultivars under salt stress. Cultivars markedly differ in this character as cv. S-24 was higher in chl. a/b ratios than MH-97 under both non-stressed and salt-stressed conditions (Table 2; Fig. 4.20a). Foliar application of TRIA did not significantly alter the chl. a/b ratio in both cultivars. Overall, chl. a/b ratio was uniform at all growth stages and did not change by foliar application of TRIA (Fig. 4.20b).

Salinity stress did not significantly affect efficiency of photosystem II (Fv/Fm) in both wheat cultivars. Cultivars difference was also non-significant in this attribute. TRIA application at boot stage slightly increased Fv/Fm in cv. S-24 only under non-stressed conditions, while under salt-stressed conditions a minute increase was observed when 10 μM TRIA was applied at vegetative growth stage (Table 3; Fig 4.21a). Overall, TRIA effect was variable with respect to cultivars, salinity stress and growth stages (Table 3; Fig. 4.21b).

Salinity stress slightly decreased electron transport rate (ETR) in both wheat cultivars. Cultivars also differed prominently in ETR. Cultivar S-24 was superior, while MH-97 inferior in ETR (Table 3; Fig. 22a). Foliar application of TRIA significantly increased the ETR values in cv. MH-97 at all growth stages except boot stage. However, in cultivar S-24 electron transport rate was observed to be consistent under both non-stress and salt stress conditions at various growth stages (Table 3; Fig. 22a). Overall, TRIA effect on ETR was seemed to be similar with respect to cultivars, salt stress and stages of application (Table 3; Fig. 22b).
Table 4.3: Mean squares from analysis of variance of data for chlorophyll fluorescence, leaf water relations and membrane permeability (%) of wheat (Triticum aestivum L.) when plants were foliarly sprayed with triacontanol at various growth stages under non-stressed and salt-stressed conditions.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>$Fv/Fm$</th>
<th>$ETR$</th>
<th>$qP$</th>
<th>$qN$</th>
<th>$NPQ$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.006ns</td>
<td>26.87***</td>
<td>0.002ns</td>
<td>0.190***</td>
<td>0.397***</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>0.004ns</td>
<td>18.63**</td>
<td>0.004ns</td>
<td>0.011*</td>
<td>0.002ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.006*</td>
<td>7.111ns</td>
<td>0.0004ns</td>
<td>0.014*</td>
<td>0.0000ns</td>
</tr>
<tr>
<td>Growth stages (Gs)</td>
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<td>0.498ns</td>
<td>0.0003ns</td>
<td>0.003ns</td>
<td>0.021ns</td>
</tr>
<tr>
<td>Cvs x Gs</td>
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<td>0.001ns</td>
<td>3.445ns</td>
<td>0.003ns</td>
<td>0.0006ns</td>
<td>0.010ns</td>
</tr>
<tr>
<td>S x Gs</td>
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<td>0.001ns</td>
<td>2.189ns</td>
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<td>0.004ns</td>
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<th>Turgor potential</th>
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<th>MP (%)</th>
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<td>0.040*</td>
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<tr>
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<td>0.021ns</td>
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<td>3.78ns</td>
</tr>
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<td>0.007</td>
<td>0.015</td>
<td>20.51</td>
<td>14.74</td>
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</table>

*, **, and ***= significant at 0.05, 0.01, and 0.001, respectively

ns= non-significant; df= degrees of freedom

$Fv/Fm$ = Efficiency of photosystem II; $ETR$ = Electron transport rate

$qP$ = Photochemical quenching efficiency; $qN$ = Co-efficient of non-photochemical quenching

$NPQ$ = Non-photochemical quenching; RWC (%) = Relative water content in percentage

MP (%) = Percentage membrane permeability
Fig 4.20a. Chl. a/b ratio of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.20b. Chl. a/b ratio comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.21a. Efficiency of photosystem II of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.21b. Efficiency of photosystem II comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.22a. Electron transport rate of PS II of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.22b. Electron transport rate of PS II comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.23a. Photochemical quenching of PS II of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.23b. Photochemical quenching of PS II comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Photochemical quenching \( (qP) \) did not alter under salinity stress (Table 3; Fig. 23a). However, cultivars showed variable behavior when TRIA was applied as foliar spray at different growth stages. For example, under salt stress photochemical quenching increased consistently with increase in level of TRIA at boot stage in both cultivars under saline conditions and at vegetative stage in MH-97 only. However, under non-saline conditions, the effect of different TRIA levels were not consistent at all growth stages as in cv. MH-97 10 \( \mu M \) increased the \( qP \) value at vegetative stage, while decreased in cv. S-24. Similarly, at boot and vegetative + boot stages 10 \( \mu M \) TRIA level was more effective in cv. S-24, while in cv. MH-97 20 \( \mu M \) TRIA level enhanced the photochemical quenching under non-saline conditions (Table 3; Fig. 23a). Overall, \( qP \) value was high at boot stage of TRIA application in cv. MH-97 under saline conditions (Table 3; Fig. 23b).

Coefficient of non-photochemical quenching \( (qN) \) significantly increased in cv. S-24 under salt stress, while in cv. MH-97 did not show any increase. Cultivars showed significant difference as cv. MH-97 was higher and S-24 lower in \( qN \) values. Foliar application of TRIA did not markedly affect \( qN \) values, however, cultivars showed a prominent response to foliar-applied TRIA. At vegetative growth stage, TRIA application increased coefficient of non-photochemical quenching \( (qN) \) values in cv. S-24, while decreased in MH-97 under both saline and non-saline conditions. The most prominent effect of TRIA was at vegetative + boot stages where \( qN \) values decreased successively with increasing TRIA level in cv. MH-97, while reverse was true for cv. S-24 under saline conditions (Table 3; Fig. 24a). Overall, effect of TRIA application at all growth stages was non-prominent and uniform (Table 3; Fig. 24b).

Non-photochemical quenching exciton \( (NPQ) \) did not alter in both wheat cultivars under NaCl stress. However, cultivars differed significantly in this character as cultivar MH-97 was higher in \( NPQ \) value than cv. S-24 under both non-stressed and salt-stressed conditions. Foliar application of TRIA particularly at 20 \( \mu M \) level significantly decreased the non-photochemical quenching exciton \( (NPQ) \) value in both cultivars at all growth stages (Table 3; Fig. 25a). Overall, non-photochemical quenching exciton value had shown similar behavior at all growth stages of TRIA application under both salt-stressed and non-stressed conditions (Table 3; Fig. 25b).
Fig 4.24a. Co-efficient of non-photochemical quenching of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.24b. Co-efficient of non-photochemical quenching comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.25a. Non-photochemical quenching of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.25b. Non-photochemical quenching comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.26a. Leaf water potential of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.26b. Leaf water potential comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.27a. Leaf osmotic potential of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.27b. Leaf osmotic potential comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Leaf water potential significantly decreased in both wheat cultivars under NaCl induced stress. Cultivars significantly differed in this character i.e. leaf water potential was more negative in cv. MH-97 than in cv. S-24 under both saline and non-saline conditions (Table 3; Fig. 4.26a). Foliar application of TRIA significantly increased leaf water potential in both cultivars under both non-saline and saline conditions; however, response of cultivars to foliar-applied TRIA was different at different growth stages as at vegetative growth stage leaf water potential was high in cv. S-24, while low in MH-97 under both salt-stressed and non-stressed conditions. Overall, leaf water potential was similar at all growth stages of TRIA application (Table 3; Fig. 4.26b). Leaf osmotic potential significantly decreased (more negative) in both wheat cultivars under salt stress. Cultivars also significantly differed in this attribute (Table 3; Fig. 4.27a). Foliar application of TRIA slightly decreased leaf osmotic potential at vegetative stage, while increased at boot stage in both cultivars under salt stress conditions (Table 3; Fig. 4.27a). Overall, leaf osmotic potential at all growth stages was not variable to TRIA application (Table 3; Fig. 4.27b). Root-medium applied salinity stress significantly decreased leaf turgor potential in both wheat cultivars. Of both wheat cultivars, cv. S-24 excel cv. MH-97 in leaf turgor potential under both saline and non-saline conditions (Table 3; Fig. 4.28a). Response of cultivars S-24 to TRIA application was more positive than cv. MH-97 under both non-saline and saline conditions (Table 3; Fig. 4.28a). Increase in leaf turgor potential with increase in TRIA levels was consistent in cv. S-24 under both non-stress and stress conditions at all growth stages, while not for cv. MH-97 (Table 3; Fig. 4.28a). Overall, leaf turgor potential of cv. S-24 was high at vegetative stage of TRIA application under both salt-stressed and non-stressed conditions (Table 3; Fig. 4.28b).

Salinity stress significantly decreased relative water content (%) in both wheat cultivars. Cultivars did not differ significantly in this attribute (Table 3; Fig. 4.29a). Foliar application of TRIA significantly increased the relative water content (%) in both wheat cultivars under both non-saline and saline conditions (Table 3; Fig. 4.29a). Relative water contents (%) were more at vegetative stage of TRIA application than other two stages and cv. MH-97 was higher in this attribute than cv. S-24 (Table 3; Fig. 4.29a). Overall, relative water contents (%) were high at boot stage of TRIA application in both wheat cultivars under non-saline conditions as compared to other growth stages (Table 3; Fig. 4.29b).
Fig 4.28a. Leaf turgor potential of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.28b. Leaf turgor potential comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.29a. Relative water content (%) of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.29b. Relative water content (%) comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Shoot Na\(^+\) contents increased significantly in both wheat cultivars under saline conditions (Table 4.4; Fig. 4.30a). The two cultivars did not differ prominently in terms of shoot Na\(^+\) content. Foliar-applied TRIA did not alter shoot Na\(^+\) in both cultivars under non-saline conditions, while slightly reduced shoot Na\(^+\) in both cultivars under saline conditions, when applied at various growth stages (Table 4.4; Fig. 4.30a). Overall, 20 µM TRIA was proved to be more effective in reducing shoot Na\(^+\) in cv. S-24 under saline conditions at all growth stages, while in cv. MH-97 10 µM TRIA was more effective in reducing shoot Na\(^+\) at all growth stages except vegetative growth stage (Table 4.4; Fig. 4.30a). Comparison of TRIA application at various growth stages showed that shoot Na\(^+\) contents were more sharply decreased when TRIA was foliarly applied at boot stage under salt stress in both wheat cultivars (Table 4.4; Fig. 4.30b). Exogenous application of TRIA did not affect root Na\(^+\) content whereas root Na\(^+\) content significantly increased in both wheat cultivars under salt stress (Table 4.4; Fig. 4.31a). The two cultivars did not differed significantly with respect to root Na\(^+\) (Table 4.4; Fig. 4.31a). Comparison of TRIA application at different growth stages indicated that TRIA had non-significant effect on root Na\(^+\) when applied at various growth stages (Table 4.4; Fig. 4.31b).

Shoot K\(^+\) ions decreased prominently in both wheat cultivars under salinity stress (Table 4.4; Fig. 4.32a). Both cultivars differed significantly. Cultivar S-24 was higher in shoot K\(^+\) content than cv. MH-97 under both saline and non-saline conditions (Table 4.4; Fig. 4.32a). The exogenous application of TRIA as foliar spray increased the shoot K\(^+\) contents in both cultivars at all growth stages (Table 4.4; Fig. 4.32a). The effect of foliar-applied TRIA on shoot K\(^+\) contents was uniform at all growth stages in both wheat cultivars, except on S-24 at boot stage under non-saline conditions (Table 4.4; Fig. 4.32a). Overall, TRIA application at various growth stages did not show tremendous difference in modulation of shoot K\(^+\) in both cultivars under non-stressed and salt stressed conditions (Table 4.4; Fig. 32b).

Root K\(^+\) contents decreased significantly in both wheat cultivars under salinity stress (Table 4.4; Fig. 4.33a). Cultivars also showed variable response to rooting medium saline conditions. Cultivar S-24 excel cv. MH-97 in root K\(^+\) contents at all growth stages under non-saline conditions, while difference was not prominent under saline conditions. Foliar application of TRIA did not modulated root K\(^+\) content significantly (Table 4.4; Fig. 4.33a).
Table 4.4: Mean squares from analysis of variance of data for shoots and roots mineral nutrients of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with triacontanol at various growth stages under non-stressed and salt-stressed conditions.

<table>
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<th>Source of variation</th>
<th>df</th>
<th>Shoot Na+</th>
<th>Root Na+</th>
<th>Shoot K+</th>
<th>Root K+</th>
<th>Shoot Ca^2+</th>
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<td>4.223ns</td>
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<th>Root Cl-</th>
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<td>22.56ns</td>
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<td>8.53ns</td>
<td>101.4ns</td>
<td>0.027ns</td>
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</tr>
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<td>Cvs x TRIA</td>
<td>2</td>
<td>0.262ns</td>
<td>613.8*</td>
<td>10.65ns</td>
<td>41.71ns</td>
<td>0.261*</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>0.484ns</td>
<td>2401.9***</td>
<td>8.491ns</td>
<td>135.4ns</td>
<td>0.126ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.429ns</td>
<td>35.16ns</td>
<td>26.80ns</td>
<td>42.70ns</td>
<td>0.156ns</td>
</tr>
<tr>
<td>Gs x TRIA</td>
<td>4</td>
<td>0.132ns</td>
<td>125.7ns</td>
<td>1.706ns</td>
<td>49.28ns</td>
<td>0.053ns</td>
</tr>
<tr>
<td>Cvs x Gs x TRIA</td>
<td>4</td>
<td>0.080ns</td>
<td>59.39ns</td>
<td>7.338ns</td>
<td>45.30ns</td>
<td>0.018ns</td>
</tr>
<tr>
<td>S x Gs x TRIA</td>
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<td>0.505ns</td>
<td>172.9ns</td>
<td>53.66ns</td>
<td>47.16ns</td>
<td>0.065ns</td>
</tr>
<tr>
<td>Cvs x S x Gs x TRIA</td>
<td>4</td>
<td>0.220ns</td>
<td>4.130ns</td>
<td>16.46ns</td>
<td>47.77ns</td>
<td>0.024ns</td>
</tr>
<tr>
<td>Error</td>
<td>108</td>
<td>0.728</td>
<td>152.2</td>
<td>31.86</td>
<td>64.39</td>
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</table>

*, **, and ***= significant at 0.05, 0.01, and 0.001, respectively.

ns= non-significant; df= degrees of freedom
Fig 4.30a. Shoot sodium content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.30b. Shoot sodium content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.31a. Root sodium content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.31b. Root sodium content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.32a. Shoot potassium content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.32b. Shoot potassium content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.33a. Root potassium content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.33b. Root potassium content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Overall, root K$^+$ contents were uniform when TRIA applied at various growth stages under both saline and non-saline conditions (Table 4.4; Fig. 4.33b).

Root-medium applied salinity stress decreased shoot and root Ca$^{2+}$ contents prominently in both wheat cultivars (Table 4.4; Fig. 4.34a and 4.35a). Of two cultivars, cv. S-24 was higher in shoot and root Ca$^{2+}$ content than that of cv. MH-97 (Table 4.4; Fig. 4.34a and 35a). The exogenous application of TRIA as foliar spray markedly increased the shoot and root Ca$^{2+}$ contents in both cultivars under both non-saline and saline conditions. The effect of foliar-applied TRIA was almost uniform at all growth stages. However, under normal conditions 10 µM TRIA was more effective in enhancing shoot and root Ca$^{2+}$ contents in cv. S-24 at all growth stages, while a consistent increase in shoot Ca$^{2+}$ contents was observed at vegetative and veg. + boot stages in cv. S-24 under salt stress and under non-saline conditions in cv. MH-97 (Table 4.4; Fig. 4.34a and 35a). Overall, effect of foliar application of TRIA was slightly high in cv. S-24 at vegetative and veg. + boot growth stages (Table 4.4; Fig. 4.34b and 35b).

Salinity stress caused a remarkable increase in shoot and root Cl$^-$ contents in both wheat cultivars (Table 4.4; Fig. 36a and 37a). The response of two wheat cultivars in terms of shoot and root Cl$^-$ contents was markedly variable. Cultivar S-24 accumulated more shoot and root Cl$^-$ contents than MH-97 under both salt-stressed and non-stressed conditions (Table 4.4; Fig. 36a and 37a). Cultivars response to foliar application of TRIA in terms of shoot Cl$^-$ contents was also different under saline or non saline conditions as shoot Cl$^-$ content slightly increased with increasing TRIA level under non-saline conditions, while decreased under saline conditions at all growth stages in both cultivars except at boot stage in cv. MH-97 (Table 4.4; Fig. 36a and 37a). Overall, effect of foliar-applied TRIA was consistent at all growth stages on shoot and root Cl$^-$ contents in both wheat cultivars (Table 4.4; Fig. 36b, 37b).

Shoot K$^+$/Na$^+$ ratios decreased significantly in both wheat cultivars under saline conditions (Table 4.4; Fig. 4.38a). However, neither cultivar showed significant difference nor foliar-applied TRIA at various growth stages under both non-stressed and salt stressed conditions (Table 4.4; Fig. 4.38a).
Fig 4.34a. Shoot calcium content of wheat (Triticum aestivum L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.34b. Shoot calcium content comparison of wheat (Triticum aestivum L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.35a. Root calcium content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.35b. Root calcium content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.36a. Shoot chloride content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.36b. Shoot chloride content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.37a. Root chloride content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.37b. Root chloride content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.38a. Shoot $K^+$/Na$^+$ ratio of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.38b. Shoot $K^+$/Na$^+$ ratio comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Salinity stress significantly decreased root K+/Na+ ratios in both wheat cultivars (Table 4.4; Fig. 4.39a). The two wheat cultivars showed significant difference in terms of root K+/Na+ ratios, as cultivar S-24 was higher in root K+/Na+ ratios than MH-97 only under non saline conditions (Table 4.4; Fig. 4.39a). The foliar application of TRIA markedly enhanced root K+/Na+ ratios in cv. S-24 under non-saline conditions, while TRIA application was non-significant in both cultivars under saline conditions (Table 4.4). Overall, TRIA application increased root K+/Na+ ratio in only cv. S-24 under non-saline conditions (Table 4.4; Fig. 4.39b).

Membrane permeability (%) markedly enhanced in both wheat cultivars under saline conditions. Cultivars showed variable response in terms of membrane permeability cultivar MH-97 was superior and cv. S-24 inferior under both saline and non-saline conditions (Table 4.3; Fig.4.40a). Foliar application of TRIA significantly decreased the membrane permeability (%) of both wheat cultivars at all growth stages (Table 4.3; Fig. 4.40a). A consistent decrease in membrane permeability with the increasing levels of foliar-applied TRIA was observed in cv. S-24 under both saline and non-saline conditions at all growth stages, while in cv. MH-97 only at boot stage under saline conditions. Overall, TRIA application showed non-variable effect at all growth stages (Table 4.3; Fig. 4.40b).

Hydrogen peroxide (H₂O₂) significantly increased in both wheat cultivars under salt stress. Cultivar MH-97 accumulated more H₂O₂ contents as compared to cv. S-24 under saline conditions (Table 4.5; Fig. 4.41a). Foliar application of TRIA reduced H₂O₂ contents of both wheat cultivars when applied at various growth stages under both salt stress and non-stress conditions. Furthermore, H₂O₂ contents decreased successively with the increasing level of TRIA at vegetative and boot stages in cv. MH-97 under saline conditions (Table 4.5; Fig. 4.41a). Overall, various growth stages did not showed marked difference in H₂O₂ contents (Table 4.5; Fig. 4.41b).

Wheat cultivars showed non-significant difference in malonedialdehyde (MDA) contents, while these contents significantly increased in both wheat cultivars under saline conditions (Table 4.5; Fig. 4.42a). Foliar application of TRIA significantly decreased the MDA contents of both wheat cultivars and the 10 µM TRIA was more effective in decreasing MDA contents under both non-saline and saline conditions at all growth stages (Table 4.5; Fig. 4.42a).
Fig 4.39a. Root K⁺/Na⁺ ratio of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.39b. Root K⁺/Na⁺ ratio comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.40a. Membrane permeability (%) of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.40b. Membrane permeability (%) comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Table 4.5: Mean squares from analysis of variance of data for hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), malondialdehyde (MDA), activities of superoxide dismutase (SOD), peroxidae (POD) and catalase (CAT), soluble proteins, total free amino acids, free proline, glycinebetaine and total phenolics of wheat (\textit{Triticum aestivum} L.) when plants were foliarly sprayed with triacontanol at various growth stages under non-stressed and salt-stressed conditions.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>H\textsubscript{2}O\textsubscript{2}</th>
<th>MDA</th>
<th>SOD</th>
<th>POD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
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<td>225.8*</td>
<td>0.844ns</td>
<td>10.35***</td>
<td>12.35***</td>
<td>55.27ns</td>
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<td>953.2***</td>
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<tr>
<td>Cvs x S</td>
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<td>169.7ns</td>
<td>30.86*</td>
<td>20.65***</td>
<td>16.52***</td>
<td>137.4*</td>
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<tr>
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<td>0.043ns</td>
<td>8.666ns</td>
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<td>0.463ns</td>
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</tr>
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<tr>
<td>TRIA</td>
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<td>25.92**</td>
<td>0.276ns</td>
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<tr>
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<td>0.502ns</td>
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<tr>
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<td>1.916ns</td>
<td>0.675*</td>
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<tr>
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<th>Soluble proteins</th>
<th>Free amino acids</th>
<th>Proline</th>
<th>Glycinebetaine</th>
<th>Phenolics</th>
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<td>Cultivars (Cvs)</td>
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<td>35.48***</td>
<td>33.85***</td>
<td>201.9***</td>
<td>44.58ns</td>
<td>8.219*</td>
</tr>
<tr>
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<td>616.12***</td>
<td>3209.0***</td>
<td>1160.4***</td>
<td>18.42***</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>37.15***</td>
<td>4.539ns</td>
<td>19.05ns</td>
<td>9.653ns</td>
<td>30.96***</td>
</tr>
<tr>
<td>Growth stages (Gs)</td>
<td>2</td>
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<td>7.265ns</td>
<td>0.030ns</td>
<td>39.82ns</td>
<td>4.652*</td>
</tr>
<tr>
<td>Cvs x Gs</td>
<td>2</td>
<td>5.670ns</td>
<td>0.931ns</td>
<td>1.058ns</td>
<td>64.58ns</td>
<td>1.551ns</td>
</tr>
<tr>
<td>S x Gs</td>
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<td>2.884ns</td>
<td>6.583ns</td>
<td>6.422ns</td>
<td>36.76ns</td>
<td>2.044ns</td>
</tr>
<tr>
<td>Cvs x S x Gs</td>
<td>2</td>
<td>3.758ns</td>
<td>1.161ns</td>
<td>5.509ns</td>
<td>30.09ns</td>
<td>0.907ns</td>
</tr>
<tr>
<td>TRIA</td>
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<td>8.635ns</td>
<td>2.247ns</td>
<td>28.07ns</td>
<td>3.785*</td>
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<td>80.35ns</td>
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<td>67.53ns</td>
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<tr>
<td>Gs x TRIA</td>
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<td>1.372ns</td>
<td>2.845ns</td>
<td>5.789ns</td>
<td>61.10ns</td>
<td>1.020ns</td>
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<td>Cvs x Gs x TRIA</td>
<td>4</td>
<td>1.202ns</td>
<td>4.049ns</td>
<td>3.558ns</td>
<td>40.59ns</td>
<td>0.464ns</td>
</tr>
<tr>
<td>S x Gs x TRIA</td>
<td>4</td>
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<td>3.186ns</td>
<td>3.819ns</td>
<td>33.07ns</td>
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</tr>
<tr>
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*, **, and ***= significant at 0.05, 0.01, and 0.001, respectively.

ns= non-significant; df= degrees of freedom
Fig 4.41a. Hydrogen peroxide content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.41b. Hydrogen peroxide content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.42a. Malondialdehyde content of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.42b. Malondialdehyde content comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.43a. Activity of superoxide dismutase enzyme of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.43b. Activity of superoxide dismutase enzyme comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.44a. Activity of peroxidase (POD) enzyme of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.44b. Activity of peroxidase (POD) enzyme comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Overall, MDA contents were low when TRIA was applied at vegetative growth stage (Table 4.5; Fig. 4.42b).

Activity of superoxide dismutase (SOD) enzyme of both wheat cultivars showed a prominent decrease under NaCl stress (Table 4.5; Fig. 4.43a). Behavior of both wheat cultivars was significantly different under both saline and non-saline conditions. Cultivar MH-97 was higher in SOD activity than cv. S-24 at all growth stages under non-saline conditions while reverse was true under saline conditions (Table 4.5; Fig. 4.43a). Exogenous application of TRIA as foliar spray did not alter the SOD activity under both non-saline and saline conditions (Table 4.5; Fig. 4.43a).

The activity of peroxidase (POD) did not influenced under salt stress in both wheat cultivars. However, cultivars differed significantly in POD enzyme activity under saline and non-conditions. Cultivar S-24 showed high POD activity under saline conditions, while reverse was true for MH-97 under non-saline conditions (Table 4.5; Fig. 4.44a). Foliar application of TRIA significantly increased the POD activity in both wheat cultivars (Table 4.5; Fig. 4.44a). A consistent increase in the POD activity with the increasing level of TRIA was observed at vegetative stage in both wheat cultivars under both saline and non-saline conditions (Table 4.5; Fig. 4.44a). At boot and veg. + boot stages POD activity decreased in cv. MH-97 under salt stress. Overall, effect of foliar-applied TRIA was more prominent in cv. MH-97 at veg. + boot stages as it increased POD activity under non-saline conditions, while decreased under saline conditions (Table 4.5; Fig. 4.44b).

Salinity stress significantly increased the activity of catalase (CAT) enzyme in both wheat cultivars. Cultivars showed non-significant difference in CAT activity (Table 4.5; Fig. 4.45a). Effect of foliar-applied TRIA on CAT activity was also non-significant except the differential behavior of both wheat cultivars when applied at veg. + boot stages where CAT activity was decreased in cv. S-24 and increased in MH-97 under non-saline conditions (Table 4.5; Fig. 4.45a). Overall, TRIA application did not showed a prominent effect in enhancing CAT activity when applied at various growth stages (Table 4.5; Fig. 4.45b).

Data for soluble proteins showed a significant increase under saline conditions (Table 4.5; Fig. 4.46a). A marked variation among two wheat cultivars was also observed cultivar S-24 excel cv. MH-97 in soluble proteins. Difference was more prominent under non-saline
Fig 4.45a. Activity of catalase enzyme of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.45b. Activity of catalase enzyme comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.46a. Total soluble proteins of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.46b. Total soluble proteins comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.47a. Total amino acids of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.47b. Total amino acids comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.48a. Free proline contents of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.48b. Free proline contents comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.49a. Glycinebetaine content of wheat (*Triticum aestivum* L.) when plants were foliarily sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.49b. Glycinebetaine content comparison of wheat (*Triticum aestivum* L.) when plants were foliarily sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
Fig 4.50a. Total phenolics of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.

Fig 4.50b. Total phenolics comparison of wheat (*Triticum aestivum* L.) when plants were foliarly sprayed with TRIA at various growth stages under non-stressed and salt-stressed conditions.
conditions as compared to saline conditions (Table 4.5; Fig. 4.46a). Exogenous application of TRIA did not affect soluble proteins (Table 4.5; Fig. 4.46b). Total free amino acids and proline contents significantly increased under NaCl stress in both wheat cultivars. Cultivars differed significantly in both total free amino acids and proline contents as cv. S-24 was higher and cv. MH-97 lower in both attributes (Table 4.5; Fig. 4.47a and 4.48a). The effect of foliar application of TRIA was statistically non-significant when applied at various growth stages (Table 4.5; Fig. 4.47a and 4.48a). Overall, effect of TRIA on total free amino acids and proline contents were uniform at all growth stages in both wheat cultivars (Table 4.5; Fig. 4.47b and 4.48b).

Salt stress markedly increased the glycinebetaine contents in both wheat cultivars. Cultivars did not differ significantly in glycinebetaine contents (Table 4.5; Fig. 4.49a). The effect of foliar application of TRIA at various growth stages was also non-significant (Table 4.5; Fig. 4.49a). Salt stress significantly increased the total phenolic contents in cv. S-24, while reverse was true for cv. MH-97. Cultivars differed prominently in this character as salt stress increased total phenolics in cv. S-24 at all growth stages (Table 4.5; Fig. 4.50a). Furthermore, under salt stress total phenolics contents were high when TRIA was applied at vegetative growth stage. Foliar application of TRIA significantly decreased total phenolic contents when applied at boot and veg. + boot stages in both wheat cultivars under saline conditions (Table 4.5; Fig. 4.50a). Foliar-applied 10 µM TRIA was more effective in decreasing total phenolics under saline conditions. However, this reduction in total phenolic contents was more in cv. MH-97 than cv. S-24 particularly at boot and veg. + boot stages under saline conditions. Overall, effect of foliar-applied TRIA in decreasing total phenolics was more at boot and veg. + boot stages particularly under saline conditions in both wheat cultivars (Table 4.5; Fig. 4.50b).

### 4.2 Exogenous application of triacontanol as seed-priming (Experiment 2)

Salinity stress significantly decreased shoot and root fresh weights of both wheat cultivars (Table 4.6; Fig. 4.51). Cultivars differed only in shoot dry weight that was high in cv. S-24. Effect of pre-sowing seed treatment with TRIA was non-significant. However, pre-sowing
seed treatment with TRIA slightly increased shoot fresh and dry weight of MH-97 under saline conditions, while in cv. S-24 under non saline conditions (Table 4.6; Fig. 4.51).

Root fresh and dry weights decreased in both wheat cultivars under salinity stress. Seed treatment with TRIA significantly decreased root fresh weight of both wheat cultivars under both salt stress and non stress conditions, while root dry weight remained unaffected (Table 4.6; Fig. 4.51). In the present study, application of TRIA as pre-soaking seed treatment decreased root fresh weight of both salt tolerant and salt sensitive wheat cultivars under both stress and non stress conditions (Table 4.6; Fig. 4.51).

Total leaf area significantly decreased under salinity stress in both wheat cultivars (Table 4.6; Fig. 4.51). Pre-soaking seed treatment with TRIA increased total leaf area of cultivar S-24 under non-stress conditions (Table 4.6; Fig. 4.51). Salinity stress significantly decreased shoot and root length of both wheat cultivars. Cultivars differed significantly as cv. S-24 was higher in shoot and root length than cv. MH-97 (Table 4.6; Fig. 4.51). Pre-sowing seed treatment with TRIA slightly increased shoot length in both wheat cultivars under non saline conditions. Root length significantly increased under non-saline conditions, while decreased under saline conditions in both wheat cultivars (Table 4.6; Fig. 4.51).

Salinity stress significantly decreased all yield attributes, i.e., grain yield and number of grains per plant, 100-grain weight and number of fertile tillers per plant decreased significantly in both cultivars (Table 4.6; Fig. 4.51 and 4.52). Seed soaking with TRIA exerted no significant effect on yield characteristics although it slightly increased grain yield per plant and 100-seed weight in cv. S-24 under salt stress, while in cv. MH-97 under non stress conditions. Exogenous application of TRIA as seed treatment increased number of tillers per plant in both wheat cultivars but only under non saline conditions (Table 4.6; Fig. 4.51 and 4.52). Net CO₂ assimilation rate (A), transpiration rate (E) and stomatal conductance (gs) significantly decreased, while sub-stomatal CO₂ concentration, Ci/Ca ratio and water use efficiency remained unaffected under salinity stress in both wheat cultivars. Pre-sowing seed treatment with TRIA did not modulated the gas exchange characteristics significantly except that it increased the stomatal conductance under non-saline conditions, while under saline conditions the response of both cultivars was inconsistent in terms of gs with respect to TRIA.
Table 4.6. Growth, yield, gas exchange characteristics, chlorophyll pigments of salt-stressed and non-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol for 12 h.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Shoot f. wt.</th>
<th>Shoot dry wt.</th>
<th>Root f. wt.</th>
<th>Root dry wt.</th>
<th>Total leaf area plant⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>1.644ns</td>
<td>0.183*</td>
<td>0.522***</td>
<td>0.004**</td>
<td>110.78ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>360.7***</td>
<td>9.001***</td>
<td>1.611***</td>
<td>0.107***</td>
<td>224486***</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.487ns</td>
<td>0.033ns</td>
<td>0.072***</td>
<td>0.001ns</td>
<td>653.28 ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.003ns</td>
<td>0.014ns</td>
<td>0.256***</td>
<td>0.002*</td>
<td>216.46ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>1.314ns</td>
<td>0.078ns</td>
<td>0.017ns</td>
<td>0.003***</td>
<td>1773.79ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>1.480ns</td>
<td>0.089ns</td>
<td>0.055**</td>
<td>0.0005ns</td>
<td>3133.43*</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.339ns</td>
<td>0.030ns</td>
<td>0.018ns</td>
<td>0.004**</td>
<td>1040.74ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.709</td>
<td>0.027</td>
<td>0.006</td>
<td>0.0004</td>
<td>893.97</td>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Shoot length</th>
<th>Root length</th>
<th>Grain yield plant⁻¹</th>
<th>Number of grains plant⁻¹</th>
<th>100-grain wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>608.27***</td>
<td>29.34***</td>
<td>9.068ns</td>
<td>4276.2ns</td>
<td>2.778ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>1241.38***</td>
<td>108.51***</td>
<td>40.10**</td>
<td>16348.3**</td>
<td>3.800***</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.2986ns</td>
<td>1.590ns</td>
<td>0.207ns</td>
<td>54.066 ns</td>
<td>0.173ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>21.78**</td>
<td>9.507**</td>
<td>1.008ns</td>
<td>76.377ns</td>
<td>0.234ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>13.69**</td>
<td>5.090**</td>
<td>2.643ns</td>
<td>1666.9ns</td>
<td>0.048ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>2.146ns</td>
<td>7.132***</td>
<td>1.521ns</td>
<td>701.7ns</td>
<td>0.013ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>6.424ns</td>
<td>4.882**</td>
<td>1.923ns</td>
<td>89.92ns</td>
<td>0.481ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>2.014</td>
<td>2.014</td>
<td>3.780</td>
<td>1375.8</td>
<td>0.162</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>Number of tillers plant⁻¹</th>
<th>A</th>
<th>E</th>
<th>gᵣ</th>
<th>Cᵢ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>7.260**</td>
<td>0.970ns</td>
<td>0.049ns</td>
<td>1600***</td>
<td>1915.5ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>5.575**</td>
<td>9.191**</td>
<td>1.747*</td>
<td>8100***</td>
<td>572.8ns</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.385ns</td>
<td>0.966ns</td>
<td>0.229ns</td>
<td>325**</td>
<td>302.99ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.340ns</td>
<td>3.033ns</td>
<td>0.483ns</td>
<td>625***</td>
<td>11.33ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>0.802ns</td>
<td>0.077ns</td>
<td>0.056ns</td>
<td>1300***</td>
<td>398.6ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>2.478*</td>
<td>0.701ns</td>
<td>0.063ns</td>
<td>975***</td>
<td>1291.4ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.271ns</td>
<td>1.202ns</td>
<td>0.035ns</td>
<td>175*</td>
<td>82.97ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.561</td>
<td>0.774</td>
<td>0.352</td>
<td>43.75</td>
<td>901.24ns</td>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Df</th>
<th>C/Cᵥa</th>
<th>A/E(WUE)</th>
<th>Chl. a</th>
<th>Chl. b</th>
<th>Chl. a/b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.015ns</td>
<td>2.609ns</td>
<td>0.004ns</td>
<td>0.002ns</td>
<td>0.18ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>0.005ns</td>
<td>10.38ns</td>
<td>0.0004ns</td>
<td>0.067ns</td>
<td>0.195ns</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.002ns</td>
<td>0.885ns</td>
<td>0.001ns</td>
<td>0.002ns</td>
<td>0.002ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.0009ns</td>
<td>0.027ns</td>
<td>0.002ns</td>
<td>0.063ns</td>
<td>0.123ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>0.003ns</td>
<td>2.609ns</td>
<td>0.0006ns</td>
<td>0.027ns</td>
<td>0.075ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>0.010ns</td>
<td>0.031ns</td>
<td>0.002ns</td>
<td>0.042ns</td>
<td>0.074ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.0007ns</td>
<td>7.205ns</td>
<td>0.0006ns</td>
<td>0.001ns</td>
<td>0.007ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.007</td>
<td>3.397</td>
<td>0.001</td>
<td>0.0170636</td>
<td>0.049</td>
</tr>
</tbody>
</table>

*, ** and *** = significant at 0.05, 0.01, and 0.001 levels, respectively

ns = non-significant; df = degrees of freedom; A = net CO₂ assimilation rate; E = transpiration rate; gᵣ = stomatal conductance; Cᵢ = sub-stomatal CO₂ concentration; C/Cᵥa = relative sub-stomatal CO₂ concentration; WUE = water use efficiency
Fig. 4.51 Growth and yield attributes of wheat (Triticum aestivum L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).
Fig. 4.52 Yield and gas exchange characteristics of wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).
application. Performance of both wheat cultivars was similar in gas characteristics except that decrease in stomatal conductance ($g_s$) was low in salt tolerant cultivar S-24 as compared to moderately salt sensitive cv. MH-97 under salt stress (Table 4.6; Fig. 4.52 and 4.53). Salinity stress did not significantly alter chlorophyll $a$, $b$ and $a/b$ ratio in both wheat cultivars. Pre-sowing seed treatment with TRIA also exerted no significant effect on chlorophyll pigments, however, a slight increase in chlorophyll $b$ contents due to pre-sowing TRIA treatment was observed in both wheat cultivars under salt stress (Table 4.6; Fig. 4.53).

Salt stress exerted no significant effect on efficiency of PSII ($F_v/F_m$) of both wheat cultivars. Effect of pre-sowing seed treatment with TRIA was also statistically non significant, however, at 10 \( \mu M \) TRIA $F_v/F_m$ slightly increased in cv. S-24, while decreased in cv. MH-97 under saline conditions (Table 4.7; Fig. 4.53). Electron transport rate ($ETR$) decreased under salinity stress in both wheat cultivars, while pre-sowing seed treatment with TRIA proved statistically non significant in both wheat cultivars under both saline and non saline conditions (Table 4.7; Fig. 4.53). Photochemical quenching ($q_P$) remained unaffected under root medium salinity and pre-sowing treatment with TRIA also exerted no significant effect, however, under salt stress photochemical quenching ($q_P$) increased in both wheat cultivars particularly at 10 \( \mu M \) TRIA (Table 4.7; Fig. 4.53). Coefficient of non-photochemical quenching ($q_N$) and non photochemical quenching exiton ($NPQ$) increased under salt stress in both wheat cultivars.

Although effect of pre-sowing seed treatment with TRIA was statistically non significant, however, under salt stress both $q_N$ and $NPQ$ values decreased by seed treatment with TRIA (Table 4.7; Fig. 4.53 and 4.54). Salinity stress significantly decreased leaf water potential and osmotic potential but did not altered leaf turgor potential and relative water content (%) in both wheat cultivars. Salt-tolerant cultivar S-24 was superior in all leaf water relation attributes except leaf osmotic potential that was more in cv. MH-97 under both non-stress and salt-stress conditions (Table 4.7; Fig. 4.54). Pre-soaking seed treatment with TRIA significantly decreased the leaf water potential but did not affect the leaf osmotic potential, turgor potential and relative water content in both cultivars; however, the leaf turgor potential was slightly increased in cv. MH-97 at 10 \( \mu M \) level (Table 4.7; Fig. 4.54).
Table 4.7. Chlorophyll fluorescence, water relations, H$_2$O$_2$, MDA, free proline, glycinebetaine, soluble proteins, free amino acids, total phenolics and activities of antioxidant enzymes of non-stressed and salt-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol for 12 h.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>$F_{v}/F_{m}$</th>
<th>ETR</th>
<th>$q_P$</th>
<th>$q_N$</th>
<th>NPQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.0003ns</td>
<td>0.321ns</td>
<td>0.0003ns</td>
<td>0.004ns</td>
<td>0.0008ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>0.006ns</td>
<td>15.47*</td>
<td>0.0008ns</td>
<td>0.010**</td>
<td>0.0140**</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.005ns</td>
<td>1.228ns</td>
<td>0.0003ns</td>
<td>0.004ns</td>
<td>0.0005ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.002ns</td>
<td>0.64ns</td>
<td>0.0004ns</td>
<td>0.0007ns</td>
<td>0.000003ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>0.003ns</td>
<td>2.018ns</td>
<td>0.0004ns</td>
<td>0.0005ns</td>
<td>0.0006ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>2.604ns</td>
<td>0.0003ns</td>
<td>0.001ns</td>
<td>0.0023ns</td>
<td></td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.015*</td>
<td>0.122ns</td>
<td>0.000003ns</td>
<td>0.0010</td>
<td>0.00004ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.003</td>
<td>0.390</td>
<td>0.0007</td>
<td>0.0013</td>
<td>0.00104</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Water potential</th>
<th>Osmotic potential</th>
<th>Turgor potential</th>
<th>RWC (%)</th>
<th>MP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.481***</td>
<td>0.086***</td>
<td>0.160**</td>
<td>72.74*</td>
<td>68.555*</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>0.368***</td>
<td>0.256***</td>
<td>0.010ns</td>
<td>38.73ns</td>
<td>257.498***</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.040*</td>
<td>0.009ns</td>
<td>0.036ns</td>
<td>39.08ns</td>
<td>12.037ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.022ns</td>
<td>7.640ns</td>
<td>0.030ns</td>
<td>2.451ns</td>
<td>4.674ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>0.006ns</td>
<td>0.036*</td>
<td>0.055ns</td>
<td>2.387ns</td>
<td>1.379ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>0.003ns</td>
<td>0.010ns</td>
<td>0.021ns</td>
<td>8.812ns</td>
<td>5.393ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.007ns</td>
<td>0.001ns</td>
<td>0.008ns</td>
<td>2.220ns</td>
<td>21.528ns</td>
</tr>
<tr>
<td>Error</td>
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<td>0.008</td>
<td>0.009</td>
<td>0.017</td>
<td>16.13</td>
<td>11.5576</td>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>H$_2$O$_2$</th>
<th>MDA</th>
<th>Proline</th>
<th>GB</th>
<th>Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>437.9*</td>
<td>3.456ns</td>
<td>0.015*</td>
<td>0.0002ns</td>
<td>0.427ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>633.9*</td>
<td>136.6**</td>
<td>0.070***</td>
<td>0.936***</td>
<td>6.600**</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>78.41ns</td>
<td>2.906ns</td>
<td>0.007ns</td>
<td>0.051ns</td>
<td>5.105ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>9.514ns</td>
<td>39.73**</td>
<td>0.008ns</td>
<td>0.006ns</td>
<td>0.049ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>52.408ns</td>
<td>0.544ns</td>
<td>0.003ns</td>
<td>0.010ns</td>
<td>4.453ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>96.24ns</td>
<td>3.666ns</td>
<td>0.0004ns</td>
<td>0.008ns</td>
<td>0.117ns</td>
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<td>0.018ns</td>
<td>0.522ns</td>
</tr>
<tr>
<td>Error</td>
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<td>58.60</td>
<td>4.478</td>
<td>0.003</td>
<td>0.023</td>
<td>1.922</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Amino acids</th>
<th>Total phenolics</th>
<th>SOD</th>
<th>POD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.288ns</td>
<td>0.622ns</td>
<td>0.010ns</td>
<td>0.338ns</td>
<td>6.673ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>36.98*</td>
<td>5.530ns</td>
<td>20.90**</td>
<td>0.156ns</td>
<td>372.5*</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>2.459ns</td>
<td>0.359ns</td>
<td>4.472ns</td>
<td>2.203*</td>
<td>24.67ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>1.708ns</td>
<td>3.644ns</td>
<td>0.150ns</td>
<td>0.715ns</td>
<td>80.97ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>4.292ns</td>
<td>2.729ns</td>
<td>1.287ns</td>
<td>0.085ns</td>
<td>32.56ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>3.175ns</td>
<td>0.492ns</td>
<td>2.471ns</td>
<td>2.507*</td>
<td>41.98ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>3.401ns</td>
<td>4.513ns</td>
<td>0.187ns</td>
<td>0.064ns</td>
<td>19.64ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>8.629</td>
<td>1.441</td>
<td>1.600</td>
<td>0.605</td>
<td>52.72</td>
</tr>
</tbody>
</table>

*, ** and *** = significant at 0.05, 0.01, and 0.001 levels, respectively; ns = non-significant; df = degrees of freedom; $F_{v}/F_{m}$ = efficiency of PS II; ETR = electron transport rate; $q_P$ = photochemical quenching; $q_N$ = coefficient of non-photochemical quenching; NPQ = non photochemical quenching exiton; SOD = superoxide dismutase; POD = peroxide dismutase, CAT = catalase; GB = glycinebetaine; MP = membrane permeability; H$_2$O$_2$ = hydrogen peroxide; MDA: malondialdehyde; RWC = relative water content
Fig. 4.53 Water use efficiency, chlorophyll pigments and chlorophyll fluorescence of wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).
Fig. 4.54 NPQ, water relations, membrane permeability (%), MDA and H$_2$O$_2$ contents of wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).
Root medium salinity significantly increased membrane permeability (%) in both wheat cultivars. Cultivar MH-97 was higher in membrane permeability than cv. S-24 under either saline or non-saline conditions (Table 4.7; Fig. 4.54). Seed-soaking treatment with TRIA did not alter membrane permeability significantly in both cultivars under salt-stress and non-stress conditions (Table 4.7; Fig. 4.54).

Malondialdehyde and hydrogen peroxide contents significantly increased in both cultivars under root medium salinity (Table 4.7; Fig. 4.54). Cultivar differed significantly as cv. MH-97 showed more H₂O₂ accumulation than S-24 under both saline and non-saline conditions, while MDA contents were more in cv. S-24 only under non-saline conditions. Seed treatment with TRIA did not significantly alter H₂O₂ MDA and H₂O₂ contents in both cultivars under both prevailing conditions (Table 4.7; Fig. 4.54).

Proline and glycinebetaine contents significantly increased in both wheat cultivars under salinity stress (Table 4.7; Fig. 4.55). Cultivar S-24 accumulated more proline contents than cv. MH-97 particularly under saline conditions, while in glycinebetaine contents both wheat cultivars were similar under both saline and non-saline conditions (Table 4.7; Fig. 4.55). Seed treatment with TRIA did not alter these attributes in both cultivars under salt-stress or non-stress conditions (Table 4.7; Fig. 4.55). Soluble protein contents did not altered, while total free amino acids increased in both wheat cultivars under salinity stress (Table 4.7; Fig. 4.55). Pre-sowing seed treatment with TRIA did not change these biochemical attributes in both cultivars under prevailing conditions (Table 4.7; Fig. 4.55). Total phenolic contents did not altered in both wheat cultivars under salt stress (Table 4.7; Fig. 4.55). Both cultivars did not differ significantly in this attribute under saline or non-saline conditions. Seed treatment with TRIA also not exerted any significant effect on total phenolic contents in both wheat cultivars under stressed or non-stressed conditions (Table 4.7; Fig. 4.55). Root medium salinity significantly decreased superoxide dismutase activity in both wheat cultivars (Table 4.7; Fig. 4.55). The two cultivars did not differ significantly in this attribute under both saline and non-saline conditions. The pre-sowing seed treatment with TRIA did not module the SOD activity in both cultivars under stressed or non-stressed conditions (Table 4.7; Fig. 4.55). Peroxidase (POD) activity did not changed in both cultivars under salt stress; however, seed treatment with TRIA at 20 µM level increased the peroxidase activity in both
Fig. 4.55 Free proline, glycinebetaine, total soluble proteins, free amino acids, total phenolics and antioxidant activities of wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).
cultivars but only under non-stress conditions (Table 4.7; Fig. 4.55). Salinity stress significantly increased the activity of catalase enzyme in both cultivars. Both cultivars did not differ significantly; however, cv. S-24 was slightly higher in CAT activity than MH-97 under salt stress (Table 4.7; Fig. 4.55). The exogenous application of TRIA as seed treatment did not modulate CAT activity significantly (Table 4.7; Fig. 4.55).

Salinity stress significantly increased shoot and root Na$^+$ contents in both wheat cultivars (Table 4.8; Fig. 4.56). Cultivars differed only in accumulation of shoot Na$^+$ ion, while in root Na$^+$ contents remained unaffected under salt stress and cv. S-24 accumulated more shoot Na$^+$ than cv. MH-97 (Table 4.8; Fig. 4.56). Seed-priming treatment with TRIA increased the shoot Na$^+$ contents in both cultivars particularly at 10 µM TRIA under salt stress. Root Na$^+$ ion accumulation although remained statistically non-significant but slightly increased by pre-sowing treatment with TRIA in both cultivars under salt stress (Table 4.8; Fig. 4.56).

Salinity stress significantly decreased shoot and root K$^+$ contents in both cultivars. Pre-sowing seed treatment with TRIA further decreased shoot K$^+$ contents in both cultivars under non-stress conditions, while under salt-stressed conditions only in cv. MH-97. Although effect of seed treatment with TRIA was statistically non-significant, however, it decreased root K$^+$ contents of both wheat cultivars under non-saline in cv. S-24 and saline conditions in cv. MH-97 successively with the increasing level of TRIA (Table 4.8; Fig. 4.56).

Under saline conditions shoot Ca$^{2+}$ did not affected, however, root Ca$^{2+}$ significantly decreased in both cultivars (Table 4.8; Fig. 4.56). Cultivars did not show any significant difference in shoot or root Ca$^{2+}$ contents (Table 4.8; Fig. 4.56). Shoot and root Ca$^{2+}$ contents remained unaffected by pre-sowing seed treatment with TRIA in the two wheat cultivars under saline or non-saline conditions (Table 4.8; Fig. 4.56). However, shoot and root Ca$^{2+}$ slightly decreased in both wheat cultivars under both saline and non-saline conditions except in cv. MH-97 under saline conditions (Table 4.8; Fig. 4.56). Salinity stress significantly increased shoot and root contents of Cl- ions in both cultivars (Table 4.8; Fig. 4.56). Cultivars did not show significant difference in this character. Shoot and root Cl- ions accumulation did not significantly altered by seed treatment with TRIA in both cultivars under saline or non-
Table 4.8. Shoot and root mineral nutrients and K+/Na+ ratios of non-stressed and salt-stressed wheat (*Triticum aestivum* L.) plants raised from seed treated with triacontanol (TRIA) for 12 h.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Shoot Na⁺</th>
<th>Root Na⁺</th>
<th>Shoot K⁺</th>
<th>Root K⁺</th>
<th>Shoot Ca²⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>8.507***</td>
<td>0.562ns</td>
<td>103.4***</td>
<td>10.028*</td>
<td>0.0001ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>706.7***</td>
<td>232.6***</td>
<td>992.2***</td>
<td>1863.4***</td>
<td>0.694ns</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>3.938*</td>
<td>1.507ns</td>
<td>36.19***</td>
<td>4.75ns</td>
<td>0.771ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>7.562***</td>
<td>0.840ns</td>
<td>124.69***</td>
<td>21.778**</td>
<td>0.444ns</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>2.174ns</td>
<td>0.562ns</td>
<td>0.465ns</td>
<td>0.361ns</td>
<td>0.021ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>3.840*</td>
<td>1.938ns</td>
<td>86.646***</td>
<td>0.361ns</td>
<td>0.049ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>1.938ns</td>
<td>0.299ns</td>
<td>1.174ns</td>
<td>2.528ns</td>
<td>0.132ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.924</td>
<td>1.062</td>
<td>1.729</td>
<td>1.930</td>
<td>0.257</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Root Ca²⁺</th>
<th>Shoot Cl⁻</th>
<th>Root Cl⁻</th>
<th>Shoot K⁺/Na⁺ ratio</th>
<th>Root K⁺/Na⁺ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivars (Cvs)</td>
<td>1</td>
<td>0.062ns</td>
<td>629.17ns</td>
<td>121.0ns</td>
<td>122.79ns</td>
<td>1.318ns</td>
</tr>
<tr>
<td>Salinity (S)</td>
<td>1</td>
<td>3.674*</td>
<td>52022***</td>
<td>23307***</td>
<td>14188***</td>
<td>223.6***</td>
</tr>
<tr>
<td>Triacontanol (TRIA)</td>
<td>2</td>
<td>0.340ns</td>
<td>185.14ns</td>
<td>71.256ns</td>
<td>123.52ns</td>
<td>0.236ns</td>
</tr>
<tr>
<td>Cvs x S</td>
<td>1</td>
<td>0.840ns</td>
<td>269.51ns</td>
<td>3.757ns</td>
<td>57.54ns</td>
<td>2.089*</td>
</tr>
<tr>
<td>Cvs x TRIA</td>
<td>2</td>
<td>0.271ns</td>
<td>215.26ns</td>
<td>2.896ns</td>
<td>88.016ns</td>
<td>0.605ns</td>
</tr>
<tr>
<td>S x TRIA</td>
<td>2</td>
<td>0.340ns</td>
<td>287.59ns</td>
<td>163.59*</td>
<td>114.9ns</td>
<td>0.053ns</td>
</tr>
<tr>
<td>Cvs x S x TRIA</td>
<td>2</td>
<td>0.299ns</td>
<td>817.01ns</td>
<td>17.312ns</td>
<td>85.64ns</td>
<td>0.793ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>0.562</td>
<td>564.62</td>
<td>38.326</td>
<td>172.7</td>
<td>0.361</td>
</tr>
</tbody>
</table>

*, **, and *** = significant at 0.05, 0.01 and 0.001, respectively

ns = non-significant; df = degrees of freedom
Fig. 4.56 Shoot and root mineral nutrients of wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).
saline conditions. However, pre-sowing TRIA treatment decreased shoot and root Cl⁻ contents particularly in cv. MH-97 under salt stress (Table 4.8; Fig. 4.56).

Salt stress markedly decreased shoot and root K⁺/Na⁺ ratios in the two cultivars (Table 4.8; Fig. 4.57). Cultivars differed only in root K⁺/Na⁺ ratio under salt stress as cv. MH-97 was higher in K⁺/Na⁺ ratios than cv. S-24 (Table 4.8; Fig. 4.57). Pre-sowing seed treatment with TRIA did not significantly change the shoot and root K⁺/Na⁺ ratios in both wheat cultivars under saline or non-saline conditions, however it slightly decreased shoot and root K⁺/Na⁺ ratios in both wheat cultivars under both stressed and non-stressed conditions (Table 4.8; Fig. 4.57).
Fig. 4.57 Shoot and root K⁺/Na⁺ ratios of wheat (*Triticum aestivum* L.) when 24-day old plants subjected for 21 days to saline or non-saline conditions (seed priming for 12 h).
CHAPTER 5
DISCUSSION

5.1 Exogenous application of triacontanol as foliar-spray (Experiment 1)

Plant hormones are known to play key role in plants growth and development (Lucas et al., 2001). Salinity stress has been reported to decrease the synthesis or causes degradation of plant growth regulators (PGRs) (Kuiper et al., 1988; Debez et al., 2001). However, exogenous application of plant growth regulators as foliar spray or through seed priming can improve their deficiency (Ashraf et al., 2008). Salinity induced reduction or degradation of plant hormones (Kuiper et al., 1988; Debez et al., 2001) could be overcome by their exogenous application (Ashraf et al., 2008). Triacontanol regulate different growth processes under various environmental stresses in different crop species as reported in various studies (Muthuchelian et al., 1996, 1997, 2001, 2003; Borowski and Blamowski, 2009). However, optimal concentration of triacontanol and plant age is among important factors that control growth and final yield of various plant species (Sagaral et al., 1978). Under both normal and stress conditions TRIA had been extensively studied to enhance various growth and yield attributes (Menon and Srivastava, 1984; Patil and Bangal, 1985; Hashim and Lundergan, 1985; Krishnan and Kumari, 2008, Singh et al., 2011).

Crops sensitivity to salinity stress differs at various growth stages (Bybordi, 2010; Flowers et al., 2010). Genetic variation with reference to salt stress is existed in wheat (Eskandari and Kazemi, 2011). Salt tolerant wheat genotypes had high yield capacity than salt sensitive genotypes under NaCl stress (Zheng et al., 2009). Genotypes that are better under non-saline condition also do well under saline conditions (Flowers et al., 2010). Wheat has been known to show differential sensitivity to salt stress at various growth stages e.g., it is more salt-sensitive at early vegetative stage (Ayers and Hayward, 1948; Emam and Ranjbar, 2001; Shalhevet, 1994), while less salt-sensitive at grain filling stages (Emam and Ranjbar, 2001).

Reduced growth is the typical morphological symptom of salinity stress (Jaleel et al., 2008). In this study, salt stress of 150 mM NaCl significantly decreased all growth and yield
attributes including shoot and root fresh and dry weights, total leaf area per plant, shoot and root length, grain yield and number of grains/plant, hundred-grain weight and number of fertile tillers/plant of wheat (Table 1; Fig. 4.1-4.11) similar to many other studies (Akbarimoghaddam et al., 2011; Bagdi et al., 2011; Eleiwa et al., 2011; El-Hendawy et al., 2011; Fercha, 2011; Perveen et al., 2010, 2011). Salinity stress decreased plant growth because plants are unable to take up water in the presence of excess salts in the soil solution resulting in reduced growth and other associated various metabolic processes (Munns and Tester, 2008). However, salt stress induced negative effects on plant growth and yield in bread wheat had been reported to be ameliorated by foliar application of various plant growth regulators (Eleiwa et al., 2011; Bagdi et al., 2011). As a foliar spray TRIA had been reported to enhance crop growth and final yield under stressful conditions in a number of studies (Chatterjee, 1999; Muthuchelian et al., 1996, 1997, 2001, 2003; Krishnan and Kumari, 2008; Kilic et al., 2010). It is effective at very low concentrations to different crop species. Different concentrations of TRIA have been reported in different plant species that can enhance various growth and metabolic processes e.g., growth and yield in Artemisia annua L. (@ 1.0 to 1.5 mg L⁻¹) (Shukla et al., 1992), chilli (@ 5 mg L⁻¹) (Chaudhary et al., 2006), tomato (@1 mg L⁻¹) (Khan et al., 2006), chickpea (@ 4 mg L⁻¹) (Singh et al., 1991), okra (@ 2.5 mg L⁻¹) (Nargisjahan et al., 1997), hyacinth bean and ginger respectively (@ 10⁻⁶ M) (Naeem et al., 2009; Singh et al., 2011) and ameliorated various abiotic stresses @ 1 mg L⁻¹ (Muthuchelian et al., 1996, 1997, 2001, 2003).

In the current study foliar application of TRIA significantly increased all growth and yield attributes when applied at three different growth stages under both stressed and non-stressed conditions. Overall, 10 μM TRIA level was better for improving growth and yield of cultivar MH-97 as compared to S-24, however at vegetative + boot stages 20 μM TRIA was more effective in increasing growth and yield parameters in both wheat cultivars particularly cv. S-24 under non-saline conditions. There are reports that showed that two times foliar spray could promote crop yield more effectively than one or three sprays (Pandey et al., 2001; Sarada et al., 2008). For example, foliar application of TRIA at early vegetative and anthesis has been reported to increase growth and yield of most economic crop species including wheat (Ries, 1991). In some other studies three time foliar spray of TRIA at various growth
stages i.e., vegetative, flowering and poding stages had been found to be more effective in enhancing crop growth and yield (Reddy et al., 2002; Singh et al., 2011) as TRIA can exerts its stimulatory effects equally well at different growth stages (Singh et al., 2011). Singh et al. (1991) was of the view that at pre-flowering stage TRIA is more effective to increase seed weight, seed yield and protein contents. It has been suggested that increased growth and final productivity due to TRIA application could be due to increased nutrients uptake, enhanced photosynthetic rate and increased mobilization of reserve materials (source-sinks relationship) (Singh et al., 2011). Triacontanol improve growth that could be due to its effect on certain enzymes that regulate major metabolic pathways (Ries et al., 1990; Singh et al., 2011). TRIA induce formation of second messenger 9-β-L (+) adenosine that is similar in structure to cytokinins and exogenous application of cytokinins had been well known to induce growth, flowering and increased final yield productivity (He and Loh, 2000; Bonhomme et al., 2000). In addition TRIA can interact with other growth hormones such as cytokinins and gibberellic acid to regulate growth, yield and metabolic processes (Pandey et al., 2001; Aftab et al., 2010).

In the present study, salinity stress significantly decreased the photosynthetic rate \( (A) \) in both wheat cultivars similar to other studies (Zheng et al., 2009; Abdeshahian et al., 2010). Foliar application of TRIA significantly increased the photosynthetic rate \( (A) \) in both wheat cultivars when applied at all growth stages particularly at veg. + boot stages in both cultivars under both stressed and non-stressed conditions. Increased growth in the present study might be due to increase in photosynthetic rate due to TRIA spray. Srivastava and Sharma (1990) was of the view that foliar spray of TRIA at different growth stages increased total chlorophyll and \( \text{CO}_2 \) exchange rate. Photosynthetic rate exhibit a positive relationship with growth attribute as had been reported in different plants by foliar application of TRIA under normal and stressed conditions (Haugstad et al., 1983; Houtz et al., 1985a; Muthuchelian et al., 1994, 1996, 2001, 2003; Rajasekaran and Blake, 1999; Borowski and Blamowski, 2009; Krishnan and Kumari, 2008; Naeem et al., 2009; Perveen et al., 2010). Under long term salt-exposure photosynthesis declined due to decreased stomatal conductance (Ouerghi et al., 2000; Kasai et al., 1998). Decreased stomatal conductance due to stomatal closure is thought to be the main reason of decreased photosynthetic rate and consequently growth reduction by
toxic Na\(^+\) and Cl\(^-\) salt ions (Tavakkoli et al., 2011). The decreased stomatal conductance could be due to the fact that intercellular spaces decreased under salt stress (Delfine et al., 1998). Salt tolerant wheat genotypes had high photosynthetic capacity than salt sensitive genotypes under NaCl stress (Zheng et al., 2009). Although, overall, performance of salt tolerant cultivar S-24 was high than MH-97 in net CO\(_2\) assimilation rate, stomatal conductance (\(g_s\)), however, foliar application of TRIA proved to be more effective in enhancing stomatal conductance in cultivar MH-97 than S-24 under salt stress conditions at all growth stages. Stomatal closure might be due to synthesis of ABA as reported by Fricke et al. (2004) or it can be transported to leaves from roots (Wolfe et al., 1990). TRIA has a role in up-regulating photosynthetic genes and down-regulating abscisic acid action in plants (Chen et al., 2002) so, it can be suggested that it play some role in stomatal regulation, so increased photosynthetic rate could be due to TRIA effect on stomatal functioning.

In this study, salinity stress did not alter sub-stomatal internal CO\(_2\) concentration (\(C_i\)), \(C_i/Ca\) ratio and water use efficiency (WUE) significantly in both wheat cultivars. Foliar spray of TRIA did not bring any prominent change in \(C_i\) and \(C_i/Ca\) ratios; however, it increased the WUE in cultivar S-24 at various growth stages.

In the present study, salinity stress significantly decreased photosynthetic pigments in the two cultivars. Decrease in chlorophyll content has been reported in wheat genotypes under salt stress (Zheng et al., 2009; Bagdi et al., 2011). Decreased chlorophyll content could be due to increased activity of chlorophyllase a chlorophyll degrading enzyme (Reddy and Vora, 1986). The reduction in photosynthetic pigments might be related to the increased levels of H\(_2\)O\(_2\) (Hossain et al., 2011), photodamage of chlorophyll (Asada, 1999) due to stimulation of H\(_2\)O\(_2\) by salinity (Sumithra et al., 2006). In the present study, although foliar application of TRIA proved quite effective in increasing photosynthetic pigments at all growth stages, however, at vegetative + boot stages this increase was more in salt sensitive cv. MH-97 particularly under non-saline conditions. Salinity stress induced negative effects on chlorophyll pigments and photosynthetic efficiency has been ameliorated by foliar application of plant growth regulators (Eleiwa et al., 2011; Bagdi et al., 2011). Triacontanol has been known to increase cell number through cell division that might be due to increase in chlorophyll contents and CO\(_2\) fixation (Haugstad et al., 1983; Houtz et al., 1985a). In the
present study, TRIA increased chlorophyll pigments and photosynthetic process in both wheat cultivars under both stressed and non-stressed conditions similar to many other studies (Haugstad et al., 1983; Houtz et al., 1985a; Muthuchelian et al., 1994, 1996, 2001, 2003; Rajasekaran and Blake, 1999; Borowski and Blamowski, 2009; Krishnan and Kumari, 2008; Naeem et al., 2009; Perveen et al., 2010). Thus increased photosynthetic rate can be related to TRIA induced increase in photosynthetic pigments (Ivanov and Angelove, 1997). It has also been suggested that increased CO2 assimilation in Calvin cycle might be due to TRIA induced increase in rubisco activity (Erikson, et al., 1981).

Chlorophyll fluorescence attributes PSII e.g. photochemical quenching (qP), non photochemical quenching (NPq) and maximum quantum yield (Fv/Fm) had been reported to be adversely affected by NaCl stress in bread wheat (Abdessahian et al., 2010). Similarly, in this study salinity stress negatively influenced chlorophyll fluorescence attributes. Foliar application of TRIA ameliorated the salt-induced damage by regulating PSII related characteristics in both cultivars to varying levels under salinity stress. TRIA induced increase in photosynthetic rate can also be related to the photochemical properties in the two cultivars under stressed and non stressed conditions. Foliar application of TRIA had been suggested to restore normal metabolic processes under salt stress (Krishnan and Kumari, 2008). So, in this study, adverse effects of salinity stress on photosynthetic machinery ameliorated by TRIA could be due to its regulatory role in leaf stomatal regulation, enhancing photosynthetic pigments, CO2-assimilation, efficiency of PS II (Fv/Fm), quantum yield of PS II, photochemical quenching (qP) and whole chain electron transport (ETR) similar to many other studies (Moorthy and Kathiresan, 1993; Muthuchelian et al., 1994, 1996, 1997, 2001, 2003; Borowski et al., 2000; Borowski and Blamowski, 2009).

Salt stress reduces external water potential of soil solution that is responsible for low uptake of water, while stimulate electrolytes uptake that lead to ion toxicity and growth reduction (Akram et al., 2007; Carpici et al., 2010; Singh et al., 2010). Growth rate of cell depends upon the extensibility of cell wall and effective turgor potential (Lockhart, 1965) which again depends upon the uptake of water which relies on the difference between water potential of enlarging cells and water source (Farouk, 2011). Genotype which possesses high osmotic adjustment can maintain high photosynthetic rate due to high water status that lead to better
growth and biomass production (Farouk, 2011). Generally water relation parameters like water content, relative water content, water potential and osmotic potential decreased, while turgor potential and osmotic adjustment increased under salinity stress (Farouk, 2011).

In the current study, root-medium applied salinity stress significantly decreased leaf water relations in both wheat cultivars. Of the two cultivars, cv. S-24 excel cv. MH-97 in leaf water relations. As increase in water uptake, cell elongation, increased cell division and permeability of membranes is the major role that TRIA plays in the regulation of plant growth (Hangarter et al., 1978). Similarly, in the present study, increased growth and productivity could be due to TRIA-induced increase in leaf water relations and relative water contents that again might be due to increased uptake of water, essential nutrients and synthesis of organic compounds by enhanced photosynthesis under salt stress. Osmotic adjustment that is a net accumulation of inorganic or organic osmolytes/solutes, total free amino acids, total soluble sugars, glycinebetaine, proline, sodium, potassium and chloride (Munss, 2005; Bandeh-hagh et al., 2008) in response to decreased external water potential (Farouk, 2011) and leads to decreased osmotic pressure, while maintaining appropriate turgor potential (Blum et al., 1996). TRIA-induced increase in water status and organic solutes accumulation has already been reported in various studies (Ries, 1991; Misra and Srivastava, 1991; Savithry et al., 1992; Ivanov and Angelove, 1997; Khan et al., 2007; Naeem et al., 2011; Singh et al., 2011).

Under high salinity level non-stomatal factors become more damaging than stomatal factors as increased dehydration of cells, reduced chlorophyll synthesis, ionic imbalance and toxicity lead to decreased net photosynthetic rate, stomatal conductance and an increased intercellular CO₂ in leaves (Qin et al., 2010). Excessive Na⁺ accumulation lead to stomatal closure reduced CO₂ availability and decreased photosynthetic rate (Khan et al., 2010; Ma et al., 2011).

The mechanism of salt tolerance varies not only in closely related species but also within varieties and even organs of same plant species (Azhar and McNeilly, 1989; Ismail, 2003; Tammam et al., 2008). Shoots of wheat plant are more affected by salt stress than roots as Na⁺ contents had been observed higher in shoots than in roots due to increased ion transport.
Various mechanisms of Na\(^+\) and Cl\(^-\) exclusion are possessed by different genotypes due to variation in ion accumulation process under salt stress (Tammam et al., 2008; Tavakkoli et al., 2011). In this study, NaCl induced stress increased shoots and roots Na\(^+\) and Cl\(^-\) contents in both wheat cultivars. The exogenous foliar application of TRIA proved to be successful in decreasing shoot Na\(^+\) and shoots and roots Cl\(^-\) contents in both wheat cultivars under saline conditions.

Foliar application of TRIA stimulate the influx of Ca\(^{2+}\) into the cytoplasm (Ries et al., 1993) that bind to some receptor proteins like calmodulin (Evans et al., 1991; Marme, 1986) and consequently regulate growth processes to certain external stimuli (Ries et al., 1993). In the current study, shoots and roots K\(^+\) and Ca\(^{2+}\) ions decreased under salinity stress in both wheat cultivars, while increased by exogenous foliar application of TRIA at all growth stages. Cultivar S-24 was higher in shoots and roots K\(^+\) and shoot Ca\(^{2+}\) content than MH-97. Uptake of essential nutrients e.g. K\(^+\) and Ca\(^{2+}\) decreased by toxic Na\(^+\) and Cl\(^-\) ions (Tavakkoli et al., 2011). Salt-tolerant genotypes posses higher K\(^+\) uptake due to selective absorption of K\(^+\) rather than Na\(^+\) (Carden et al., 2003; Ashraf et al., 2010). Varietal differences in wheat genotypes most probably are due to properties of cellular compartmentation and ion transport (Munns, 1988). Overall, TRIA application at veg. + boot stages proved more effective in increasing shoot and root K\(^+\) and Ca\(^{2+}\) contents in both wheat cultivars particularly under salt stress conditions. Our findings are in accordance with Srivastava and Sharma (1990), who reported that foliar spray of TRIA at different growth stages increased shoot nutrients contents. Under salt stress K\(^+\) and Ca\(^{2+}\) contents had been reported to be increased by foliar application of TRIA in soyabean plants (Krishnan and Kumari, 2008). Contrarily, Naeem et al. (2009) reported that nutrients level remained unaffected in TRIA treated plants.

Shoot and root K\(^+\)/Na\(^+\) ratios are known as an important criterion for salt tolerance in plants (Ashraf et al., 2010; Benderradji et al., 2011). Salt-tolerant genotypes posses higher K\(^+\) uptake due to selective absorption of K\(^+\) rather than Na\(^+\) and showed high K\(^+\)/Na\(^+\) ratio as compared to sensitive cultivars (Carden et al., 2003; Ashraf et al., 2010). In wheat increased discrimination of K\(^+\)/Na\(^+\) ratio trait reported to be linked with salt tolerance (Tammam et al., 2008). In the current study, NaCl stress decreased shoots and roots K\(^+\)/Na\(^+\) ratios in both
wheat cultivars. Cultivar S-24 showed high values for K⁺/Na⁺ ratios as compared to MH-97. Overall, foliar application of TRIA increased shoot and root K⁺/Na⁺ ratios particularly in cv. S-24 at vegetative stage and at veg. + boot stages in both wheat cultivars. Increased uptake of K⁺ could be due to increased competition at plasmamembrane sites (Epstein, 1966). Since plasmamembrane has been suggested as the initial site of signal transduction pathways in plant growth regulation (Morre et al., 1991; Trewavas and Gilroy, 1991), TRIA-mediated increase in membrane-bound enzyme activities e.g. Ca²⁺/Mg²⁺ ATPases (Lesniak et al., 1986), fluidity of membranes to several solutes by generation of an electrochemical gradient across plasmamembranes and increased uptake of essential nutrients Ca²⁺, Mg²⁺ and K⁺ (Ries, 1991; Ries et al., 1993) could be considered as a possible mechanism of TRIA action in plants.

Salt stress adversely affects structure and chemical composition of plant cell membranes. Changes are quite variable in salt sensitive and tolerant species (Mansour et al., 2005). It had been reported that H₂O₂, MDA and antioxidant defense system play variable roles under short and long term salt stress conditions (Ben Amor et al., 2006; Ellouzi et al., 2011). In this study, NaCl stress significantly increased membrane permeability (%), H₂O₂ and MDA contents in the two cultivars. Salt sensitive cv. MH-97 showed higher membrane permeability (%), H₂O₂ and MDA contents than salt tolerant cv. S-24 under both non-stressed and salt-stressed conditions. The reduction in fresh and dry biomass and photosynthetic pigments might be related to the increased levels of H₂O₂ (Hossain et al., 2011) and photodamage of chlorophyll (Asada, 1999) due to stimulation of H₂O₂ under saline conditions (Sumithra et al., 2006). TRIA had a role to inhibit lipid peroxidation of thylakoid membranes of chloroplast (Ramanarayan et al., 2000). TRIA role is by modulation the composition of membrane lipids and increasing the level of polyunsaturated fatty acids that are involved in the packaging of PS I proteins (Shripath and Swamy, 1994; Shripath et al., 1997; Swamy et al., 2009). So in this study, foliar-applied TRIA induced decrease in membrane permeability (%), H₂O₂ and MDA contents of both wheat cultivars could be due to its putative role to reduce oxidative stress under salt stress by an unknown mechanism. TRIA application is also effective in declining membrane integrity in drought-stressed jack pine
Salinity stress produce H₂O₂ and its elimination from stressed cells depends upon various functionally interrelated enzymes such as catalase and peroxidase (Kim et al., 2005; Rady et al., 2011). To protect themselves from the damaging effects of oxidative stress plants with efficient enzymatic system have good resistance to abiotic stresses (Parida and Das, 2005). The activity of CAT may depend on the growth stage, metabolic state, plant species, intensity and time of exposure of salt stress (Chaparzadeh et al., 2004). Salt tolerance is associated with increased activity of CAT (Sairam et al., 2002; Kim et al., 2004; Ashraf, 2009). However, there are reports which also show alternative behavior of CAT activity under salt stress. For example, salt stress preferentially increases H₂O₂ contents, while decrease the activity of CAT in salt sensitive rice cultivars under salt stress (Lee et al., 2001). However, in mulberry CAT activity was reported to be not related to H₂O₂ contents under high levels of salt stress (Poontariga et al., 2003). Similarly in Arabidopsis antioxidant enzyme system proved to be insufficient to ameliorate the salt-induced oxidative stress and lipids peroxidation (Ellouzi et al., 2011). In wheat flag leaf, salinity decreased the CAT and POD activity, while increased H₂O₂ and MDA contents (Gadalla, 2009), while in rice, NaCl stress exerted no significant effect on the activities of CAT and SOD (Tsai et al., 2004). In view of the results obtained from the current study, it was assessed that salinity stress significantly increased the activity of catalase (CAT) enzyme in both wheat cultivars. Cultivar S-24 was higher in CAT activity than cv. MH-97 under non-saline conditions. Increase in catalase activity had been reported in various studies including wheat under salt stress (Gao et al., 2008, Arora et al., 2008; Zheng et al., 2009). Superoxide dismutase (SOD) activity significantly decreased under NaCl stress of both wheat cultivars in the current study. Decreased SOD activity has been reported in pea plants (Hernandez et al. 1993, 1995) that could be due to direct effect of Na⁺ toxicity that inhibit the catalysis activity of SOD and result in increased accumulation of H₂O₂ under salt stress (Dionisio-Sese and Tobita, 1998). Inhibited enzyme activity might also be due to toxic effect of H₂O₂ or other ROS (Noctor and Foyer, 1998; Djanaguiraman et al., 2005). It has been thought that salt stress tolerance of crops is also associated with high antioxidant enzymes activities (Shalata and Neumann,
2001). However, this is not always true as some transgenic plants which show overexpression of antioxidant enzymes did not always prove to be effective in inducing salt tolerance (Munns and Tester, 2008). There are also some reports which show that CAT and POD enzymes are unable to completely metabolize H₂O₂ under oxidative stress (Shalata and Neumann, 2001; Rady et al., 2011). As efficient antioxidant enzyme system is not always involved in enhancing salt tolerance in plants (Cavalcanti et al., 2004). In this study, foliar application of TRIA did not affect activities of antioxidant enzymes CAT and SOD. However, foliar-applied TRIA at vegetative growth stage markedly increased POD activity in both wheat cultivars, while decreased at boot and veg. + boot stages in cultivar MH-97 under salinity stress. Lesniak and Ries (1983) reported that peroxidase enzyme activity decreased in corn by foliar application of TRIA under normal conditions.

Soluble proteins occur in the form of nitrogen under salt stress and reused when salt stress effect goes away (Singh et al., 1987). Accumulation of total soluble proteins depends upon plant species and cultivars (Tammam et al., 2008). Under salinity stress, soluble proteins markedly increased in the two wheat cultivars in this study. Protein contents increased under salt stress in wheat genotypes also in other studies (Javed, 2002; Zheng et al., 2009; Farouk, 2011). In current study, salt tolerant cultivar S-24 was higher in soluble proteins than salt sensitive cv. MH-97. According to Ashraf and O’Leary (1999) total soluble proteins (stress proteins) accumulated more in salt tolerant wheat cultivar than salt sensitive cultivar. Similarly, soluble proteins had been reported higher in tolerant barley cultivars than sensitive ones (Hurkman et al., 1989). Accumulation of total soluble proteins under salt stress reduces the osmotic potential of plant tissues and consequently play role in osmotic adjustment (Abed El-Baki, 1996; Shaddad et al., 2005; Mohamed and Ismail, 2011). In the current study, effect of foliar-applied TRIA was non-significant on total soluble protein contents. Contrarily, Krishnan and Kumari (2008) reported increased total protein contents by foliar application of TRIA in soybean plants under salt stress. Similarly, foliar spray of TRIA had been reported to enhance soluble proteins in pearl millet under normal conditions (Sivakumar et al., 2006).

Free amino acids reduce osmotic potential and act as osmoprotectants under salt stress (Sadak et al., 2010; Rady et al., 2011). Proline is known as one of the physiological markers used to determine the salinity tolerance in wheat, however, various genotypes of wheat
responded in proline contents differentially at different salt levels (Shafi et al., 2011). In this study, root medium salinity stress markedly increased total free amino acids and free proline contents in both wheat cultivars. Proline acts as a nitrogen reserve and had been reported to accumulate under salinity stress (Sairam and Tyagi, 2004; Khattab, 2007; Cha-um and Kirdmanee, 2009; Sadak et al., 2010; Summart et al., 2010; Malik et al., 2010; Heidari, 2010; Abbas et al., 2010; Akhkha et al., 2011). Increased proline content under NaCl stress might be due to reduced oxidation of proline, activation or induction of proline biosynthesis or increased protein turnover or decrease protein synthesis (Misra and Saxena, 2009; Ma et al., 2011). As salinity stress reduces the uptake of atmospheric CO₂ and its fixation by Calvin cycle (Lawlor and Cornic, 2002), consequently the availability of NADP⁺ decreases during photosynthesis for electron acceptance (Hossain et al., 2011). As a consequence NADP⁺ is regenerated for biosynthesis of proline by using the reducing power of NADPH₂ produced during photosynthesis (Wang et al., 2007). Cultivar S-24 was high in free amino acids and proline contents than MH-97. In the current study, effect of foliar-applied TRIA was non-significant on free amino acid and proline contents in both wheat cultivars under stressed and non-stressed conditions. Contrarily, Borowski and Blamowski (2009) and Krishnan and Kumari (2008) reported decreased free proline contents by foliar application of TRIA under different abiotic stress conditions.

Glycinebetaine is thought to protect plants against various abiotic stresses at all growth stages (Chen and Murata, 2011) and its accumulation differs in different varieties of plant species and also under different abiotic stress conditions including salt stress (Moghaieb et al., 2004). In the current study, salinity stress significantly increased the glycinebetaine contents in both wheat cultivars. Foliar application of TRIA did not changed glycinebetaine contents significantly in the two wheat cultivars under non-stressed or salt stressed conditions.

Total phenols had been reported to mitigate the adverse effects of salinity stress as they can acts as substrates for many antioxidant enzymes (Lewis and Yamamoto, 1990) and considered as main lines of acclimatization against abiotic stresses due to their role in protection from oxidative damage and membrane stability (Burguieres et al., 2006; Rady et al., 2011). In this study, total phenolic contents markedly increased only in cultivar S-24
under salt stress at various growth stages. Foliar-applied TRIA reduced total phenolic contents particularly at boot and veg. + boot stages in both wheat cultivars under saline conditions that means it might have played some role in decreasing salt-induced oxidative damage and improved salt stress tolerance of wheat plants. Furthermore, reduction in total phenolic contents was more in salt sensitive cv. MH-97 than salt tolerant cv. S-24 and 10 µM TRIA proved more effective in decreasing total phenolic contents in both wheat cultivars.

In conclusion, application of triacontanol as foliar spray reduced the negative effects of salinity stress on all growth, yield, photosynthetic rate (A), stomatal conductance (gs), chlorophyll pigments (chl. a and b), leaf water potential and osmotic potential, relative water content (%), electron transport rate (ETR), membrane permeability (%), mineral nutrients (shoot and root K+ and Ca2+ contents) and root K+/Na+ ratio when applied at three different growth stages as foliar spray. Of various growth stages foliar-applied TRIA was comparatively better at vegetative and veg. + boot stages. Of two TRIA levels (i.e. 10 and 20 µM) foliar-applied 10 µM level proved more effective to enhance growth, yield and photosynthetic pigments (chl. a and b) at all growth stages, while reduced total phenolics, H2O2 and MDA contents when applied at veg. + boot stages in both wheat cultivars under both stress and non-stress conditions. However, foliar application TRIA at 20 µM level reduced more shoot Na+ contents at vegetative stage in both wheat cultivars under saline conditions. Although, salt tolerant cultivar S-24 was superior to salt sensitive cv. MH-97 in growth (shoot fresh and dry weights, shoot and root length), leaf water potential, mineral nutrients such as shoot Ca2+ contents, shoot and root K+ and Cl− contents, root K+/Na+ ratio, free amino acids, proline, total soluble proteins, POD and SOD activities (under salt stress). However, cultivar MH-97 showed more positive response to foliar-applied TRIA in terms of transpiration rate (E), stomatal conductance (gs), photochemical quenching (qP), co-efficient of non-photochemical quenching (QN) under both stress and non-stress conditions.

5.2 Exogenous application of triacontanol as seed-priming (Experiment 2)

Seed-soaking with some plant growth regulators (PGRs) has gained considerable importance to enhance the crop resistance against salt stress (Ashraf and Foolad, 2005, 2007; Ashraf et al., 2008; Atia et al., 2009; Gurmani et al., 2009; Iqbal and Ashraf, 2010). In wheat, different
types of growth regulators have been tried as seed priming agents, e.g., cytokinins, polyamines, kinetin, auxins, gibberellic acid, and brassinolide (Iqbal et al., 2006; Iqbal and Ashraf, 2005, 2007, 2010; Shahbaz et al., 2008; Fariduddin et al., 2008). However, different priming agents differed in their effectiveness in various crop species as well as under different stresses (Iqbal and Ashraf, 2010). Growth enhancing property of TRIA has been found to be due to its stimulatory effect on variety of growth processes (Chen et al., 2003, Naeem et al., 2011). TRIA treatment has been studied mostly as a foliar spray on various crop species (Ries, 1991; Malik and Williams, 2005; Naeem et al., 2010, 2011; Singh et al., 2011) etc. However, literature on the application of TRIA as a seed-priming agent is scarce. So the current study was made to assess whether TRIA application as seed priming agent could mitigate the negative effects of salt stress of wheat plants and enhance growth under normal or saline conditions.

In the current study, salinity stress exerted negative effects on all growth and final yield attributes of the two wheat cultivars similar to many other studies (Akram et al., 2011; Shahbaz et al., 2008; Chaabane et al., 2011). Salt induced growth inhibition could be due decrease in net CO₂ assimilation rate that is accompanied by decrease in stomatal conductance and limit diffusion of CO₂ into the chloroplast due to stomatal closure (Degl’Innocenti et al., 2009). TRIA had been reported to posses the capability to promote crop growth and yield in a number of studies when exogenously applied (Naeem et al., 2009; Singh et al., 2011). Cavusoglu et al. (2007, 2008) found that in barley and radish crops presowing seed treatment with TRIA improved growth under NaCl stress. However, in this study, pre-sowing seed treatment with TRIA exerted no significant effect on growth and yield attributes of wheat plants under both stress and non-stress conditions and had been reported (Perveen et al., 2010, 2011). Since roots play important role in absorption and uptake of water and mineral nutrients under stress and non stress conditions so in the present study TRIA induced inhibitory effect on root fresh biomass could be the main reason for unaffected growth and yield of both wheat cultivars. Although in other studies exogenous application of TRIA has been found effective in enhancing root fresh and dry weights in various studies.
Under normal conditions exogenous application of TRIA has been known to increase photosynthetic rate, transpiration rate and stomatal conductance in different crop species such as rice, wheat and maize (Ries, 1991; Chen et al., 2003). The mechanism of TRIA-induced increase in photosynthesis is not well known, however, it has been reported that TRIA could induce the expression of \textit{rbc} genes and increase the activity of rubisco and consequently enhance photosynthetic process (Chen et al., 2002). In the present study, TRIA application as seed treatment increased the net CO$_2$ assimilation rate and transpiration rate under stress conditions and stomatal conductance under non-stress conditions. TRIA application as foliar spray has been reported to increase chlorophyll pigments in different crops (Chen et al., 2002, 2003; Sivakumar et al., 2006; Krishnan and Kumari, 2008). However, in this study seed treatment with TRIA did not change the chlorophyll contents under stress or non-stress conditions.

Furthermore, In the present study, although effect of seed-soaking with TRIA was statistically non significant, however, it slightly proved effective in enhancing efficiency of PSII (in cv. S-24 only) and photochemical quenching ($qP$), while decreasing coefficient of non-photochemical quenching ($qN$) and non-photonic quenching exciton ($NPQ$) values under salt stress. In other studies, TRIA had been found effective in improving characteristics of PS II when exogenously applied as a foliar spray (Borowski et al., 2000; Chen et al., 2003).

Salinity stress exerts adverse effects on water relations due to decreased external osmotic potential of soil solution (Munns, 2005). Decrease in leaf water potential and relative water contents are among early responses to salt stress (Alarcon et al., 1993; Gucci et al., 1997). In the present study, under NaCl stress leaf water potential decreased, leaf osmotic potential increased, while leaf turgor potential and relative water content (%) remained unaffected in both wheat cultivars. TRIA application as seed soaking agent further reduced the leaf water potential of both cultivars under saline and non-saline conditions. It was found by Krishnan and Kumari (2008) that foliar spray of TRIA could improve water status of soybean plants by decreasing leaf osmotic potential and increasing relative water contents.
In wheat, proline has been known as an endogenous osmotic regulator and play role in salt stress tolerance (Munns *et al.*, 2006). Similarly, Ashraf and Foolad (2007) reported that glycinebetaine improve water status and increase abiotic stress tolerance in plants. In this study, total free proline and glycinebetaine contents increased under salt stress and proline accumulation was higher in salt tolerant cultivar S-24 than moderately salt sensitive cv. MH-97. In the present study decreased leaf water potential and increased osmotic potential could be due to increased accumulation of free proline and glycinebetaine contents under salt stress as these osmotica has already been known to play role in osmotic adjustment (Moghaieb *et al.*, 2004; Shao *et al.*, 2006). In previous studies foliar application TRIA has been known to mitigate the adverse effects of chilling stress in *Ocimum basilicum* L. (Borowski and Blamowski, 2009) and salt stress in soybean (Krishnan and Kumari, 2008) by decreasing the proline accumulation. However, in the present study, seed treatment with TRIA did not change the proline and glycinebetaine contents under stress or non-stress conditions. So, it could be suggested that TRIA had no stimulatory or inhibitory effect on solutes (proline and glycinebetaine) accumulation when it was applied as pre-sowing seed treatment.

In the present study, total free amino acids increased, while total soluble protein and total phenolic contents remained unaffected in both wheat cultivars under salinity stress. Under salt stress total free amino acids play role in decreasing the osmotic potential and maintain osmotic balance (Salama *et al.*, 1994) and increased accumulation of amino acids has been reported in wheat under salt stress (Tammam *et al.*, 2008). In the current study, seed-soaking with TRIA did not alter total proteins, phenolics or total free amino acids under control or stress conditions. However, as a foliar spray TRIA has been reported to increase free amino acids, soluble proteins and phenolics in green gram under normal conditions (Kumaravelu *et al.*, 2000), while total protein in soybean under salt stress (Krishnan and Kumari, 2008).

Root medium salinity significantly increased the membrane permeability (%), H₂O₂ and MDA contents in this study. Triacontanol that has been reported to process anti-inflammatory properties and a powerful antioxidizing agent due to its inhibitory effect on peroxidation of membrane lipids (Ramanarayan *et al.*, 2000; Grzegorezyk *et al.*, 2006; Swamy *et al.*, 2009; Khan *et al.*, 2009). However, in this study, seed treatment with TRIA did not exert any significant effect on the contents of hydrogen peroxide, malondialdehyde
and membrane permeability (%) that are the measures of oxidative stress in plants under salt stress.

Oxidative stress to plant tissues due to salinity stress has been reported to overcome by an active antioxidative defense enzyme system such as catalase, peroxidase and superoxide dismutase (Jalali-e-Emam et al., 2011; Joseph and Jini, 2011). In the present study, the activity of catalase increased, superoxide dismutase decreased and peroxidase remained unaltered in both wheat cultivars under stress or non-stress conditions. It has been reported that antioxidant enzymes activities differed not only under different levels of salt stress, in different plant species, but also among different genotypes of same crop species e.g. SOD activity varies under different salt levels in Colza (Brassica napus L.) (Jalali-e-Emam et al., 2011). Similarly, different wheat genotypes show different response in SOD activity that has been reported to be due to differential expression of SOD isozymes under control and stress conditions (Abdel Latef, 2010). Similarly, a decrease in catalase activity in bean (Kant and Turan, 2011) and wheat cultivar Seds 1, while an increase in CAT activity in wheat cultivars Sakha 68 and Banyssoif 1 has been reported under salt stress (Abdel Latef, 2010). In the present study, TRIA application as seed treatment increased the POD activity but only under non-saline conditions, while CAT and SOD activity remained unaffected under both stressed and non-stressed conditions. Increased POD activity under normal conditions has been reported in pea by Henry and Gordon (1980). Similarly, Borowski and Blamowski (2009) reported increased CAT activity by foliar treatment with TRIA in Ocimum basilicum L. under chilling stress. Triacontanol has been reported to influence various enzymes of metabolic processes when applied as a foliar spray (Ries et al., 1977), but in the present study it was used as seed-soaking treatment and has not exerted any stimulatory or inhibitory effect on the CAT and SOD activities that are the major enzymes involved in scavenging of reactive oxygen species produced under salt stress. So, it could be suggested that although TRIA has a potential role in improving growth of various crops when applied as a foliar spray under prevailing conditions, but as a pre-sowing seed treatment it has no any stimulatory or inhibitory effect that means its mode of application is the main factor that regulates growth and other physiological and biochemical processes. As is the case with the present study, pre-sowing seed treatment with TRIA did not improve growth, gas exchange,
chlorophyll pigments, water relations, organic solutes accumulation and antioxidant enzymes activities of wheat plants under both normal and salt-stressed conditions. Since all the above mentioned physiological and biochemical attributes are collectively inter-linked in the growth process so TRIA-induced non-significant effect on growth of wheat plants could be due to its non-significant effect on other studied physiological and biochemical attribute.

Growth enhancing effects of TRIA have been reported to be associated partly with increased mineral nutrients uptake their absorption and translocation from source to sinks (Ramani and Kannan, 1980; Misra and Srivastava, 1991). This TRIA induced increased nutrients uptake (Miniraj and Shanmugavelu, 1987) could lead to increased metabolites synthesis such as soluble proteins, free amino acids, sugars and starch etc., and consequently enhanced crop growth and productivity under prevailing conditions (Kumaravelu et al., 2000; Aftab et al., 2010; Naeem et al., 2011; Singh et al., 2011). Exogenous TRIA application has been found to be effective in changing the nutrients (N, P, K and Ca) uptake and their accumulation in plants under both normal (Naeem et al., 2009) and salt stress conditions (Krishnan and Kumari, 2008). In the present study, pre-sowing treatment with TRIA did not ameliorated the salt induced negative effects on essential minerals, however, it increased shoot Na\(^+\) accumulation in both wheat cultivars under saline conditions. In contrast to many other reports in which TRIA application had been reported to enhance nutrients uptake and their absorption but not in this case where toxic Na\(^+\) ions accumulation in shoots and roots of both wheat cultivars lead to a non-significant positive changes of this plant growth regulator particularly under salt stress. It could be due to the reasons that the effectiveness of various priming agents varies under different stresses as well as in different crop species (Iqbal and Ashraf, 2010). For example, in poppy TRIA application had differential effects on the uptake and distribution of micronutrients (Srivastava and Sharma, 1990), wheras in barley roots plasma membrane-enriched Ca\(^{2+}\) and Mg\(^{2+}\) dependent ATPase activity rapidly increased (Lesniak et al., 1986). Similarly, TRIA induced negative effect on shoot and root essential mineral nutrients like K\(^+\) and Ca\(^{2+}\) accumulation and their K\(^+\)/Na\(^+\) ratios under saline or non-saline conditions could be the main reason for non-significant effect of this plant growth regulator particularly when used as a pre-sowing seed priming agent in this study. Similar to this study, Nandini and Subhendu (2002) reported that essential nutrients like K\(^+\) and Ca\(^{2+}\)
accumulation remained unaffected when mung bean plants were treated with plant hormones such as indole-3 acetic acid, gibberellic acid and kinetin.

Finally, seed-soaking treatment with TRIA could not prove effective to alleviate salt stress induced adverse effects on growth, gas exchange, chlorophyll pigments, efficiency of PS II, water relations, antioxidant enzymes activity, mineral nutrition, membrane permeability (%), H$_2$O$_2$ and MDA contents, free proline, glycinebetaine and total free amino acids were not ameliorated by pre-sowing seed treatment with TRIA (except stomatal conductance, leaf water potential, shoot K$^+$ contents and POD activity) that could be due to lack of its effective penetration through seed coat (Hoagland, 1980).

So from the present study, it could be inferred that triacontanol is more effective when it is applied as foliar spray than pre-sowing seed treatment on both wheat cultivars. Effectiveness was prominent when applied at vegetative and veg. + boot growth stages as compared to application at boot stage.
Growth, chlorophyll pigments, photosynthetic efficiency and grain yield are adversely affected in wheat under salt stress (Eleiwa et al., 2011) and improving salinity tolerance in wheat is the main issue of present day for supporting rapidly growing world population (FAO, 2010). High level of salts in the external soil solution causes dehydration and osmotic stress in plant cells (Greenway and Munns, 1980; Tavakkoli et al., 2010). However, several inter-related morphological, physiological and biochemical processes play an important role in the salt tolerance mechanism (Ashraf, 1993). The growth and yield of wheat crop depends upon its ability to osmotically adjust, stomatal functioning, enzymes activity, photosynthesis, protein synthesis and antioxidant metabolism (Zheng et al., 2008b). Of various approaches to reduce salt stress effects shotgun approaches have gained much success during the last few decades (Ashraf et al., 2008). Plant growth regulators have been known to mitigate the adverse effects of salt stress on various crops when applied exogenously e. g. brassinosteroids in wheat, mustard, pepper etc. (Shahbaz et al., 2008; Eleiwa et al., 2011; Houimli et al., 2008, 2010), salicylic acid in faba bean (Khafaga et al., 2009), 5-aminolevulinic acid in Brassica napus (Naeem et al., 2010), gibberellic acid in green gram (Akbari et al., 2008), and putrescine, ascorbic acid and thiamine in gladiolus plants (Nahed et al., 2009). Triacontanol (TRIA) is a newly discovered plant growth regulator that enhances crops growth and yield when applied exogenously. In order to explore whether exogenous application of TRIA as seed-priming or foliar spray could be effective in amelioration of adverse effects of salinity on wheat crop, the current study was proposed.

The major role TRIA plays in plants is regulation of different metabolic processes such as increase in photosynthetic rate (Eriksen et al., 1981; Houtz et al., 1985a; Haugstad et al., 1983), cell division (Hangarter et al., 1978; Houtz et al., 1985), enzymes activity (Naeem et al., 2009, 2010, 2011) and leaf mesophyll protoplast and chloroplast membranes viscosity and fluidity (Shripathi et al., 1997; Ivanov and Angelov, 1997). Exogenous application of triacontanol as foliar spray proved quite effective in improving growth and yield of wheat plants when applied at various growth stages under saline or non-saline conditions. TRIA can exerts stimulatory effects equally well at different growth stages on all growth attributes,
photosynthetic pigments, leaf nutrients, protein and carbohydrate contents, quality and productivity of plants yield (Singh et al., 2011). In betelvine, that was subjected to water stress total yield and essential oil contents were increased by foliar application TRIA (Chatterjee, 1999).

The major role of TRIA is the regulation of photosynthesis as it increases net CO$_2$ assimilation rate by enhancing the specific activity of rubisco enzyme (Erikson et al., 1981), exerts a positive effect on complexes focusing light in PSI and PSII (Moorthy and Kathiresan, 1993) and up-regulate genes involved in photosynthetic process and down-regulate stress related genes (Chen et al., 2002). Some reports show that foliar spray of TRIA mitigate the negative influences of various abiotic stresses (drought, flooding, water, salt, heavy metal, acidic mist and chilling stress) on chlorophyll pigments, photosynthesis, stomatal conductance, chlorophyll fluorescence (efficiency of PSII), photochemical quenching ($qP$), electron transport rate ($ETR$), O$_2$ evolving complex (thylakoid polypeptide of 33, 23 and 17 kDa), activity of small (15kDa) and large (55 kDa) subunits of rubisco (Rajasekaran and Blake, 1999; Muthuchelian et al., 1994, 1996, 1997, 2001, 2003; Borowski et al., 2000; Borowski and Blamowski, 2009). In wheat foliar spray of TRIA also reduced the negative influences of salinity on $ETR$, $qN$ and $NPQ$ values.

Leaf water relation parameters like water content, relative water content, water potential and osmotic potential decreased, while turgor potential and osmotic adjustment increased that could be due to organic solutes accumulation in wheat under salinity stress (Farouk, 2011). Increase in water uptake, cell elongation and cell number is the major role that TRIA plays in the regulation of plant growth (Hangarter et al., 1978). It also improved leaf water potential in both cultivars under salt stress conditions. Reduced water potential of external soil solution, Na$^+$ and Cl$^-$ ion toxicity and interference in essential nutrients uptake are the potential effects of salinity stress (Carpici et al., 2010). Mineral nutrients of shoot and root i.e. Na$^+$ and Cl$^-$ contents increased, while K$^+$, Ca$^{2+}$ and K$^+$/Na$^+$ ratios decreased in both wheat cultivars under saline conditions. Foliar application of TRIA reduced shoot Na$^+$ and Cl$^-$, while increased K$^+$ and Ca$^{2+}$ contents and K$^+$/Na$^+$ ratios in the two cultivars under salinity stress. Foliar application of TRIA had been reported to increase mineral nutrients absorption and their uptake of many crops under stress and non-stress conditions (Muthuchelian et al.,
Treatment with TRIA increases L(+)adenosine level (Ries and Wert, 1988) and a picomole concentrations of (+)-adenosine can enhance Ca\textsuperscript{2+}, Mg\textsuperscript{2+}, and K\textsuperscript{+} concentrations (Ries et al., 1993). Foliar application of TRIA had been reported to stimulates the influx of Ca\textsuperscript{2+} into the cytoplasm (Ries et al., 1993) that could bind to some receptor proteins like calmodulin (Evans et al., 1991; Marme, 1986), while increased uptake of K\textsuperscript{+} could be due to increased competition at plasmamembrane sites (Epstein, 1966). In the present study cultivar S-24 was superior in K\textsuperscript{+}, Ca\textsuperscript{2+} contents and K\textsuperscript{+}/Na\textsuperscript{+} ratios than MH-97. Tolerant genotypes posses higher K\textsuperscript{+} uptake due to selective absorption of K\textsuperscript{+} rather than Na\textsuperscript{+} (Carden et al., 2003; Ashraf et al., 2010). Since stimulation of photosynthetic processes, CO\textsubscript{2}-fixation, increased uptake of water and nutrients, increased cell division and permeability of membranes are among major contributors to regulate plant growth by TRIA application (Ries, 1991; Savithry et al., 1992; Ivanov and Angelove, 1997; Khan et al., 2007). It could be suggested that increased growth and final productivity of various crop species due to TRIA application could be due to increased absorption and utilization of nutrients, enhanced photosynthetic rate and translocation of photosynthates and metabolites from site of synthesis to site of utilization (source-sink relationship). Some researchers also reinforced these facts on various plant species (Dhall et al., 2004; Naeem et al., 2010, 2011; Singh et al., 2011).

Genotype which possesses high osmotic adjustment could maintain high photosynthetic rate due to high water status that lead to better growth and biomass production (Farouk, 2011). Under severe salinity stress growth decreased due to excess ion accumulation and consequently, ionic effect predominate the osmotic effect (Zhu et al., 2011b). However, plants overcome osmotic effect of salinity by accumulation of inorganic or organic osmolytes/solutes such as sodium, potassium and chloride, free proline, glycinebetaine and free amino acids etc., by a process known as osmotic adjustment (OA) (Munss, 2005; Bandeh-hagh et al., 2008; Zhu et al., 2011a, 2011b) in response to decreased external water potential (Farouk, 2011) and leads to decreased osmotic pressure, while maintain turgor potential (Blum et al., 1996). A positive relationship exists between osmotic adjustment and relative water content under water deficit conditions leads to better association with leaf area.
and consequently crop growth (Subbarao et al., 2000). In this study, salinity stress increased total free amino, free proline, glycinebetaine, soluble proteins and total phenolic contents in both wheat cultivars. Foliar-applied TRIA did not alter total free amino acids, free proline, glycinebetaine and soluble protein contents significantly. However, foliar-applied TRIA decreased total phenolic contents more in salt sensitive wheat crop. Under high salinity level non-stomatal factors become more damaging than stomatal factors as increased dehydration of cells, reduced chlorophyll synthesis, ionic imbalance and toxicity lead to decreased net photosynthetic rate, stomatal conductance and an increased intercellular CO₂ in leaves (Qin et al., 2010).

Foliar spray of TRIA had been reported to inhibit membrane lipids peroxidation (Ramanarayan et al., 2000), involved in changing the chemical composition of membrane by differentially organizing the membrane lipid (Shripathi et al., 1997; Swamy et al., 2009) and down regulating proteinase inhibitors (Ramanarayan et al., 2000).

In the current study, salinity stress increased oxidative stress in both wheat cultivars that was more in salt sensitive cv. MH-97 than salt tolerant cv. S-24. Foliar-applied TRIA also decreased oxidative stress by decreasing the membrane permeability (%), MDA (a product of lipid peroxidation) and H₂O₂ (a most stable ROS in plants) contents of both wheat cultivars.

TRIA has been known to regulate the activities of different enzymes of major metabolic pathways e.g., rubisco, peroxidase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase and Ca²⁺/Mg²⁺ dependent ATPases (Lesniak et al., 1986, 1989; Lesniak and Ries, 1983; Muthuchelian et al., 1994, 1997, 2003). So in the present study, increased POD activity could be for the detoxification of ROS (H₂O₂ in this case) for the mitigation of salt stress effect on wheat plants.
CONCLUSION:

- Rooting medium salinity significantly decreased growth, yield, gas exchange, \( ETR \), leaf water relations, SOD and POD (in MH-97) enzyme activities and shoot and root potassium, calcium and \( K^+/Na^+ \) ratios, while increased \( qN \), \( NPQ \), membrane permeability (\%), \( H_2O_2 \), MDA, total soluble proteins, total amino acids, total phenolics, free proline, glycinebetaine, CAT, POD (in S-24) and shoot and root \( Na^+ \) and \( Cl^- \) ions in the two wheat cultivars.

- Foliar application of TRIA significantly increased growth, yield, photosynthetic rate (\( A \)), photosynthetic pigment (chl. \( a \)), shoot and root \( K^+ \) and \( Ca^{2+} \) contents, root \( K^+/Na^+ \) ratio, POD enzyme activity, total phenolics, while decreased membrane permeability (\%), MDA, \( H_2O_2 \) and shoot \( Na^+ \) contents in two cultivars under saline conditions.

- Seed-soaking (for 12 h) with TRIA only enhanced the stomatal conductance, activity of POD (in control) and shoot \( Na^+ \) contents, while all other studied parameters remained unaffected.

- Effect of TRIA application as foliar spray was more pronounced than seed-priming in reducing the negative influences of salt stress on wheat crop.

- Overall, foliar application of TRIA at vegetative and vegetative + boot stages was better in increasing growth, yield, water relations and gas exchange in both cultivars under both normal and salt stress conditions.

- Salt sensitive cultivar MH-97 showed more positive response in terms of growth and yield to 10 \( \mu M \) TRIA level, while S-24 was better at 20 \( \mu M \) in yield attributes under both control and NaCl stress.

- Salt tolerant cultivar S-24 was high in shoot fresh and dry weights, shoot and root length, chlorophyll \( a/b \) ratios, \( ETR \), leaf water relations, total soluble proteins, free amino acids, proline, POD and SOD activities (under salt stress), shoot and root \( K^+ \)
and Cl\(^-\), shoot Ca\(^{2+}\), and root K\(^+\)/Na\(^+\) ratio, while salt sensitive cultivar MH-97 showed high values of \(qN\), \(NPQ\), leaf osmotic potential, membrane permeability (%), \(H_2O_2\) and total phenolics contents under both normal and salinity stress.

**FUTURE PROSPECTS:**

- Salinity stress imposes negative effects on wheat growth, physiological and metabolic processes. Exogenous application of tracontanol as foliar spray rather than as pre-sowing seed treatment ameliorated the salt-induced negative influences on wheat by improving growth, yield, photosynthetic pigments, gas exchange, water relations, enzyme activity (POD) and mineral nutrition at various growth stages in this study.

- It is imperative to know further the mechanism of TRIA action in plants. Although a signal transduction pathway has been suggested as a mode of TRIA action, the study of molecular aspects of TRIA especially those genes which up- or down-regulate metabolic processes are required to be investigated. Furthermore, their specific role in wheat growth is also needed to be explored.

- Furthermore, endogenous level of TRIA and its interaction with other plant growth regulator (e.g., CKs, GA, ABA etc.,) could also be helpful to draw conclusion about its growth promoting property on plants.

- Exogenous application of TRIA as foliar spray was more effective as compared to pre-sowing seed treatment. Its application through rooting medium under salt stress can also be studied.

- TRIA role in amelioration of other abiotic stresses such as drought, heat and cold stress in wheat and other cereal crops can be studied.

- Mechanism of TRIA effect at different growth stages could also be interesting to see variable behavior at different growth stages.
CHAPTER 7
SUMMARY

Salinity stress exerts negative effects on plant growth and productivity all over the world. However, various strategies had been adopted to mitigate the negative effects salinity stress. Among these strategies shotgun approaches have gained much success during the last few decades. Exogenous application of plant growth regulators have been known to mitigate the adverse effects of salt stress on various crops. Triaccontanol (TRIA) is a newly discovered plant growth regulator that enhances crops yield when applied exogenously. In order to investigate the effect of triaccontanol (TRIA) application as seed priming agent and as foliar spray on two wheat cultivars S-24 and MH-97 under salt (NaCl) stress, two experiments were conducted under natural climatic conditions. In first experiment, three TRIA levels (0, 10 and 20 µM) were foliar sprayed on both wheat cultivars that were grown in Hoagland’s nutrient solution (full strength) at three growth stages i.e. vegetative, boot and veg. + boot stages under control (0 mM NaCl) and salt (150 mM NaCl) stress and 92 day old plants were subjected to data analysis. In second experiment, seeds of both cultivars were soaked with same three TRIA levels for twelve hours and were grown in Hoagland’s nutrient solution (full strength). TRIA treated 24 day old plants were subjected to same two salts levels (0 and 150 mM NaCl) and after 21 days data for various attributes was recorded. Salinity stress of 150 mM NaCl significantly decreased all growth and yield attributes of wheat crop, while exogenous application of triaccontanol as foliar spray proved quite effective in promoting growth and yield of wheat plants when applied at three growth stages i.e. vegetative, vegetative + boot and boot stages. Foliar-applied TRIA i.e. 10 and 20 µM increased growth and yield particularly at vegetative + boot growth stages under stress and non-stress conditions. Salinity stress decreased gas exchange characteristics and photosynthetic pigments in both wheat cultivars. Foliar application of TRIA increased photosynthetic rate ($A$) in two cultivars, however, transpiration rate and stomatal conductance ($g_s$) under saline conditions, while photosynthetic pigments under non-saline conditions only in cv. MH-97 particularly at vegetative + boot stages. However, cv. S-24 was good in stomatal conductance ($g_s$) and chl. $a/b$ ratios than salt sensitive cv. MH-97. Salt stress adversely affected only electron transport rate ($ETR$), while $Fv/Fm$, $q_P$ and $NPQ$ remained unaffected. Salinity stress increased $q_N$ and $NPQ$ values more in salt sensitive cultivar MH-97 than tolerant cv. S-24, while cv. S-24
exceeded in \( ETR \) value than cv. MH-97. Foliar-applied TRIA increased \( ETR \) values under salt stress in both wheat cultivars at all growth stages. Foliar-applied TRIA enhanced \( qP \) value at boot and veg. + boot stages under non-saline conditions, while decreased \( NPQ \) and \( qN \) values under saline conditions. Leaf water relations adversely affected under NaCl stress in both wheat cultivars. Foliar-applied TRIA improved leaf water potential at vegetative and veg. + boot stages in two wheat cultivars under salinity stress. Mineral nutrients of shoot and root i.e. sodium and chloride increased, while \( K^+ \), \( Ca^{2+} \) and \( K^+/Na^+ \) ratios decreased in two wheat cultivars under NaCl stress. The exogenous foliar application of TRIA reduced shoot \( Na^+ \) and \( Cl^- \) contents under saline conditions in both wheat cultivars particularly at vegetative and veg. + boot stages. Exogenous foliar application of TRIA significantly increased \( K^+ \) and \( Ca^{2+} \) contents and \( K^+/Na^+ \) ratios particularly at vegetative and veg. + boot stages in both wheat cultivars in saline medium. Imposition of salt stress increased total free amino, free proline, glycinebetaine, soluble proteins and phenolics in both wheat cultivars. Cultivar S-24 was superior to MH-97 in terms of free amino acids, proline and soluble protein contents. TRIA application as foliar spray could not mitigate salt stress effects in terms of soluble proteins, free amino acids, free proline and glycinebetaine contents significantly. Foliar-applied TRIA decreased total phenolic contents more in salt sensitive cv. MH-97 particularly at boot and veg. + boot stages under saline conditions. Salt stress increased oxidative stress in both wheat cultivars; however salt sensitive cv. MH-97 was higher in membrane permeability (%), MDA and \( H_2O_2 \) than salt tolerant cv. S-24 under both prevailing conditions. Foliar-applied TRIA decreased oxidative stress of both wheat cultivars particularly at veg. + boot stages. NaCl stress significantly reduced the activity of SOD activity, while increased the activity of catalase in the two studied cultivars. Peroxidase activity increased in cultivar S-24, while decreased in MH-97 under saline conditions. CAT activity was higher in cultivar S-24, while that of SOD in MH-97 under non-saline conditions. Only the POD activity increased by foliar-applied TRIA at vegetative stage in two wheat cultivars under both stress and non-stress conditions.

In seed priming set of experiment, salt stress (150 mM NaCl) significantly decreased growth, yield, gas exchange, photosynthetic pigments, SOD activity, mineral \( K^+ \), and \( K^+/Na^+ \) ratios, while increased the shoot and root sodium and chloride ions, membrane permeability (%),
hydrogen peroxide, malondialdehyde, catalase activity, free proline, glycinebetaine and total free amino acids contents in the two cultivars under NaCl stress. Salinity stress exerted no prominent effect on quantum yield of PSII, substomatal CO₂ concentration, water use efficiency (A/E), relative water content, total soluble proteins, peroxidase activity and total phenolics contents in seed priming set of both experiments. Pre-sowing seed treatment with TRIA could not reduce the negative effects of salinity stress on wheat crop except that it increased stomatal conductance, leaf water potential, shoot K⁺ contents and POD activity. Overall, performance of cultivar S-24 (salt tolerant) was better than cultivar MH-97 (moderately salt sensitive) in all growth, physiological and biochemical processes like gas exchange characteristics, photosynthetic pigments, leaf water relations, shoot and root K⁺, and Ca²⁺ contents, enzymes activity, leaf soluble proteins, free amino acids, total phenolics and proline contents under both salt stress non-stress conditions in both sets of experiments.
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TITLE: Effect of exogenous application of triacontanol on wheat (*Triticum aestivum* L.) under salt stress

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Regd. No. 2005-ag-47

ABSTRACT

A pot experiment will be conducted to assess the effect of exogenous application of triacontanol on wheat (*Triticum aestivum* L.) under salt stress. There will be two wheat cultivars (S-24 and MH-97), two salinity levels (0 and 150 mM of NaCl), and three optimized levels of triacontanol, which will be applied exogenously as seed priming and as a foliar spray. Levels of triacontanol will be applied as foliar spray at three growth stages (veg, boot, veg+boot stages). The experiment will be laid out in completely randomized design with four factor-factorial arrangement and four replications. Data for morphological, physiological and biochemical attributes will be collected during the course of study.
UNIVERSITY OF AGRICULTURE, FAISALABAD
DEPARTMENT OF BOTANY
(Synopsis for Ph.D. Botany Degree)

TITLE: Effect of exogenous application of triacontanol on wheat (*Triticum aestivum* L.) under salt stress

Date of Admission : 22-09-2007
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Probable Duration : Two year

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NEED FOR THE PROJECT

Plant hormones protect plants from biotic and abiotic stresses and regulate various types of plant responses which affect growth and development (Staswick and Tiryaki, 2004). The synthesis of plant growth regulators is adversely affected by salt stress (Kuiper *et al*., 1988). However, for improving crops salt tolerance, various natural or synthetic plant growth regulators can be applied exogenously (Ashraf *et al*., 2008).

Triacontanol (TRIA), a long 30-carbon primary alcohol is a naturally occurring plant growth promoter (Ries *et al*., 1977) and is used to enhance crop yields in Asia (Ries, 1991). The most beneficial effect of TRIA in plants is increase in growth, biomass, free amino acids, reducing sugars, increase in photosynthetic activities and soluble proteins (Muthuchelian *et al*., 1995). Moreover, it also increases seed weight, chlorophyll
contents of leaves, enhances photosynthesis, branching and shoot length (Tantos et al., 1999).

In view of many researchers, triacontanol affects several metabolic processes such as photosynthesis, nutrient uptake and enzyme activity (Ries, 1991). It increases the production of ATP in rape (Brassica napus L.) and rice (Oryza sativa L.) along with an improved photosynthesis in rice, maize (Zea mays) and peas (Pisum sativum L.) (Ries, 1991). In addition it inhibits lipid peroxidation (Ramanarayan et al., 2000) and negatively modulates the jasmonic acid stimulated protein inhibitors in tomato (Lycopersicon esculentum) (Ramanarayan and Swamy, 2004).

Salinity stress is a major problem to crop productivity all over the world (Ashraf et al., 2008). It reduces the photosynthesis of plants (Kao et al., 2001) and causes changes in chlorophyll pigments (Parida et al., 2002). It affects plants in two main ways: Osmotic stress when high quantity of salts in the soil solution makes it harder for roots to absorb water and ion toxicity occurs when high concentration of salts accumulated in plants (Munns and Tester, 2008). However, the reduction of plants growth under salt stress varies with salt composition, salt concentration, the physiological stage of the plant and the plant species (Jaleel et al., 2008).

Environmental stress inhibits the growth and photosynthetic abilities of plants due to the production of reactive oxygen species (ROS) such as superoxide radical (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH$^-$) (Sairam and Tyagi, 2004). However, plants are protected from oxidative stress by production of ROS Scavenging enzymes, such as: superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and glutathione reeducates (GR) (Apel and Hirt, 2004). The reduction in sodium uptake or sodium partition in older tissues is another biochemical mechanism to escape salinity stress (Iqbal et al., 2006). In wheat, salt tolerance is associated with low rates of Na$^+$ transport to shoot with high selectivity for K$^+$ over Na$^+$ (Gorham et al., 1990).

Wheat (Triticum aestivum L.) is a moderately salt tolerant crop (Mass and Hoffman, 1997) and cultivated worldwide under different climatic conditions. It is a major food crop for a large part of the world population including Pakistan. In Pakistan it is grown on an area of 8414 thousand hectares with total production of 21749 thousand
tons (Anonymous, 2008). In spite of immense importance of wheat, its yield is low and according to an estimate by CIMMYT about 8-10% of wheat planted area in India, Pakistan, Iran, Egypt, Libya and Mexico is affected by salinity (Majeeb-Kazi and Diaz de Leon, 2002).

In view of the scarcity of available information on the role of triacontanol in ameliorating the adverse effects of salt stress on wheat crop, the present studies have been proposed in line with the following objectives:

- To assess whether triacontanol could ameliorate the adverse effects of salt stress on wheat.
- To examine the pattern of changes under different levels of exogenously applied triacontanol in wheat under salt stress.
- To determine which way of triacontanol application (Foliar spray, seed priming) could be most effective in modulating growth of wheat under salt stress.

**REVIEW OF LITERATURE**

Soil salinity is one of the major problems that reduce crop productivity in arid and semiarid regions (Neumann, 1995). Salinity can be natural (primary salinity) or occur as a result of human interferences (secondary salinity) (Munns, 2007). The extent of primary salinity is high as compared to secondary salinity (Rengasamy, 2006). According to an estimate, over 800 million hectares (Mha) of land throughout the world are salt-affected (FAO, 2008) which is more than 6% of the world's land area. Out of the current 230 Mha of irrigated land, 45 Mha are salt-affected soils (20%) and of the almost 1500 Mha of dryland agriculture, 32 Mha are salt-affected soils (2%) to varying degrees by human-induced processes (FAO, 2008). Rengasamy (2006) has reported that salt-affected area occur in more than 100 countries. Out of 20.2 Mha of cultivated land in Pakistan, 4.8 Mha is affected by salinity (26%) at varying degrees (FAO, 2008).

To fulfill the needs of food for growing world population, it is a challenge for agricultural scientists to increase the level of food production by 38% by 2025 and by 57% by 2050 (Wild, 2003) if food consumption remain continuous at current rate. Thus to minimize soil salinization and to improve salt tolerance of crops are issues of global importance (Munns *et al.*, 2006).
Pre-sowing seed treatments with different plant growth regulators is an alternative approach of plant growth regulation (Fletcher et al., 2000). It can be sub-divided into many types depending upon the priming agents e.g. seed hydration in solution of sugars, polyethylene glycol (PEG), glycerol, sorbitol or mannitol followed by air-drying before sowing is termed osmopriming, osmo conditioning or osmotic conditioning; seed soaking in solution of different inorganic salts is termed as halopriming; soaking seeds with water is called hydropriming, treating seeds with low and high temperature termed as thermopriming; presoaking seed, with optimal concentrations of different plant growth regulators is called hormone-priming (Ashraf et al., 2008).

Singh and Darra (1971) reported that presoaking seeds with optimal concentration of phytohormones is beneficial for the growth and yield of some crop species grown under saline conditions. Many growth regulators can be used for seed priming such as auxins (IAA, IBA, NAA), gibberellins (GA), gibberellin antagonists, kinetin, abscisic acid, polyamines (PAs), ethylenes, brassinolide, salicylic acid (SA), triacontanol and ascorbic acid (Ashraf et al., 2008).

The beneficial effects of seed priming with plant growth regulators include less usage and simplicity of application (Fletcher et al., 2000) under both stress and non-stress conditions (Lee et al., 1998). For example, Balki and Padole (1982) observed that seed germination of wheat improved by soaking seed with IAA, NAA or GA under salt stress. Cavusoglu et al. (2007) observed similar findings with barley seeds presoaked in triacontanol under saline conditions.

Triacontanol (TRIA) is an active growth substance which at nanomolar concentration enhances the growth and yield of crops (Ries, 1991). TRIA is a natural component of the epicuticular waxes of alfalfa (Medicago sativa), discovered in 1933 (Chibnall et al., 1933). TRIA have a growth enhancing activity when supplied exogenously to a number of plants (Chen et al., 2002). It stimulates photosynthesis by increasing the level and activity of ribulose-1, 5-bisphosphate carboxylase (Rubisco) and increases the contents of total chlorophyll, chl. a and chl. b by 25.1%, 26.1% and 22.4% respectively 4h after treatment in rice seedlings (Chen et al., 2003). Furthermore, TRIA increases the photosynthesis, chlorophyll pigments, rubisco and nitrate reductase

**PROCEDURE**

A pot experiment will be conducted in the old botanical garden, University of Agriculture, Faisalabad. Seeds of two wheat (*Triticum aestivum* L.) cultivars (S-24 and MH-97) will be sown in plastic pots containing sand. Plants will be applied with two levels of NaCl (0 mM and 150 mM) and three optimized levels of triacontanol. Selected levels of triacontanol will be applied as seed priming and as a foliar spray. All plants will be irrigated with full strength Hoagland’s nutrient solution till the termination of experiment. Levels of triacontanol will be applied as foliar spray at following growth stages.

- Vegetative stage
- Boot stage
- Both at vegetative and boot stages

Data for following parameters will be recorded during the course of study:

**a- Growth attributes:**

- Shoot and roots fresh weights (g plant\(^{-1}\))
- Shoot and root dry weight (g plant\(^{-1}\))
- Plant height, Root length
- Total leaf area per plant

**b- Yield parameters:**

At maturity, the remaining plants will be harvested and the data for the following yield parameter will be recorded.

- Grain yield (g plant\(^{-1}\))
- Number of grains per plant
- 100-seed weight (g)

**c- Water Relations:**

- Water potential (\(\Psi_w\)) Scholander type pressure chamber (Arimad-2-Japan)
- Osmotic potential (\(\Psi_s\)) Vapor pressure osmometer (Vapro, 5520)
- Turgor pressure (\(\Psi_p\)) \(\Psi_p = (\Psi_w) - (\Psi_s)\)
- Relative water content (RWC) (Jones and Turner, 1978)
d- Photosynthetic pigments:
   Chlorophyll \( a \) and \( b \)
   Chlorophyll \( a/b \) ratio
Chlorophyll pigments will be determined by the method of Arnon (1949).

e- Gas Exchange Characteristics:
   Net CO\(_2\) Assimilation rate (\( A \))
   Transpiration Rate (\( E \))
   Stomatal conductance (\( g_s \))
   Sub-stomatal CO\(_2\) concentration (\( C_i \))
   Water use efficiency (WUE)
   \( C_i/Ca \) ratio
Gas exchange characteristics will be measured by an open system LCA-4 ADC portable infrared gas analyzer (Analytical Development Company, Hoddesdon, England).

f- Mineral Nutrients:
   **Shoots and Roots**
   Sodium (Na\(^+\))
   Potassium (K\(^+\))
   Calcium (Ca\(^{2+}\))
   Chloride (Cl\(^-\))
Analysis of nutrients will be done according to the method of Allen et al. (1986).

g- Antioxidant enzyme Assay:
   Superoxide Dismutase (SOD) Giannopolitis and Ries (1977)
   Catalase (CAT) and Peroxidase (POD) Chance and Maehly (1955)

h- Osmolytes:
   Proline (Bates et al., 1973)
   Glycinebetain (Grieve and Grattan, 1983)

Statistical analysis:
Data recorded will be analyzed statistically using the MSTAT Computer Program (MSTAT Development Team, 1989).

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