CHAPTER-5

Discussion

Soil salinity is one of the most significant problems affecting crop productivity in the world. The problem is rapidly increasing on a global scale and currently affects more than 10% of the arable land which drastically decreased the average yields of major crops greater than 50% (Wang *et al*. 2009). Seedlings are the most vulnerable stage in the life cycle of plants and germination determines when and where seedling growth begins (Baskin and Baskin, 2014; Gioria *et al*., 2016). Salinity, in general, has inhibitory effects on germination of seeds (Zhang *et al*., 2010; Kaveh *et al*., 2011) due to hyper-osmotic stress and hyper-ionic toxicity (Hasegawa *et al*., 2000). Increased salinity caused a significant reduction in germination percentage, germination rate, root and shoot length and fresh weight of root and shoot (El-Shaieny, 2015). Under salt stress, the plant increase the external osmotic pressure and as soon it increase, the shoot growth rate and new buds emergence significantly decline as well as the shoot dry weight is reduced (Dadkhah, 2011).

Seed Germination

Germination is one of the most important phases in the life cycle of plant and is highly responsive to existing environment. The soluble salt in the root, beyond a critical limit, adversely influenced germination. Salinity causes osmotic stress (Nandawal *et al.*, 2000; Daneiela *et al.*, 2004) or specific ion effects, which delay, reduces or completely inhibit seed germination (Munns, 2002; Hanselin & Eggen, 2005). Present results indicate that the percentage germination reduced significantly with increase the saline concentration in oat cultivars. Variety UPO-212 showed highest percentage germination while UPO-94 revealed least under different salinity levels. It indicates that it is not essential that the variety that shows maximum percentage germination has to be highly tolerant to salinity. Tejovathi *et al*. (1988) reported that the ability of seed germination and emergence under salt stress indicates its genetic potential for salt tolerance. Kumar (2010) also reported the inhibition of germination in some cultivars of oat. Similar results with different crops were recorded by many workers (Dantas *et al*., 2007), in bean; (Zhang *et al*., 2010), in barley; (Akbarimoghaddam *et al*., 2011) in wheat; (Kumar *et al*., 2014) in oat and (Benlioglu and Ozkan, 2016) in oat. Studies on salt stress in germinating eeds showed that during this stage, the seeds are particularly sensitive to the saline environment (Bewely and Black, 1994).

Salt and osmotic stresses are responsible for both inhibition or delayed seed germination and seedling establishment. Seed germination, seedling emergence and early survival are particularly sensitive to substrate salinity (Nasri *et al*., 2015). Salinity inhibition of plant growth is the results of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns, 2002). Higher level of salt stress inhibits the germination of seeds while lower level of salinity induces a state of dormancy (Khan and Weber, 2008). Begum *et al*. (2010) stated that the germination of seed depends on the utilization of reserved food material of the seed. Salinity interferes with the process of water absorption by the the seeds. This subsequently inhibits the hydrolysis of seed reserves which ultimately delays and decreased seed germination. Sayar *et al*. (2010) reported that high salt concentration inhibits the mobilization of the seed reserves and the growth of embryonic axis. Salinity affects the seedling growth of plants by slow and less mobilization of reserve foods (Tezara *et al*., 2003).

Salt induced inhibition in seed germination could be attributed to osmotic stress or ion toxicity (Huang and Redmann, 1995). Increasing NaCl concentration, germination in the cultivars delayed and decreased ((Akbarimoghaddam *et al*., 2011). Increasing salinity concentration often causes osmotic and/or specific toxicity which may reduce germination percentage (Saboora and Kiarostami, 2006). Salinity (NaCl) may also affect germination by facilitating the intake of toxic ions, which may change certain enzymatic or hormonal activities of the seed (Smith and Comb, 1991; Kaveh *et al*., 2011). These physicochemical effects upon the seed seem to result in a slower and/or lower rate of germination or emergence. Both osmotic and toxic effects of salt have been implicated in inhibition of seed germination (El-Hendawy *et al*., 2005). Many scientists had reported the inhibitory effect of salinity on seed germination of various crops like *Glycine max* (Essa, 2002), *Vigna* spp. (Jabeen *et al*., 2003), *Brassica* spp. (Ulfat *et al*., 2007), *Triticum aestivum* (Akbarimoghaddam *et al*., 2011), *Zea mays* (Khodarahmpour *et al*., 2012), *Pisum sativum* (Tsegay and Gebreslassie, 2014).

Shoot and Root Length

Our results indicated that under salt stress, shoot and root lengths were decreased. This reduction with increasing salinity may be due to limited supply of metabolites to young growing tissues because metabolic production is significantly perturbed at high salt stress, probably due to the toxic

effects of NaCl (Yousofinia *et al*., 2012). The present findings indicate that length and dry weight of shoot and root significantly reduced at higher salinity levels. Variety NDO-2 showed lowest reduction while UPO-94 greatest reduction due to salinity. It is clear from the finding that shoot length was more affected than root length at higher salinity level whichcoincide with Turan *et al*. (2010). Gupta and Srivastava (1989) also reported that roots were less affected than shoots in wheat as salinity of the medium increased. Similar observations have been reported by Mauro-micale and Licandro (2002), in globe artichoke; Ratanakar and Rai(2013), in *Trigonella foenum-graecum*and El Goumi *et al*. (2014), in barley.

Growth processes are especially sensitive to the effects of salt, so that growth rates and biomass production provide reliable criteria for assessing the degree of salt stress and the ability of a plant to withstand it as reported by Amor *et al*. (2005). The root and shoot lengths are the most important parameters for salt stress because roots are in direct contact with soil and absorb water from the soil and shoot supplies it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plant to salt stress (Jamil and Rha, 2004). According to Jamil*et al*. (2006), shoots are more sensitive and get hampered with salinity in the environment. Salt stress reduced the ability of plants to absorb water which leads to reduction in growth (Nawaz *et al*., 2010).

Heidari *et al*. (2011) however, suggested that reduction in plant growth of *Helianthus annuus*was due to decreasing turgor pressure in the cells under saline environment. The reduction in shoot and root lengths is due to decreased physiological activities resulting from water and nutrients stress occurring under high salinity stress. Salinity leads to disturbances in plant metabolism, which consequently led to reduction of plant growth and productivity (Shafi *et al*., 2009). Jaleel *et al*. (2008) also reported a decrease in root length in *Catharanthus roseus* under salinity. Such a decrease in root length and shoot length may be due to NaCl toxicity and disproportion in nutrient absorption by the seedling as suggested by Bybordi and Tabatabaei (2009).

Bijeh Keshavarzi *et al*. (2011) reported that salinity leads to reduced water uptake which interferes with cell division and differentiation, thereby affecting the root length and shoot length. In *Trigonella foenum-graecum* the root lengths as well as shoot lengths were adversey affected with

salinity, however, the shoots were found to be more affected as compared to roots. This was consistent with the finding Munns and Termaat (1986) who state that the growth of a plant is generally reduced by salinity. The result showed that the NaCl salt solutions reduced the water content of the test crop. Water content of the seedling decreased with increasing concentration. Osmotic effects of salt on plants are the result of lowering of the soil water potential due to increase in solute concentration in the root zone.

Fresh Weight of Shoots and Roots

Fresh weight (FW) of shoot and root were strongly inhibited at high salinity levels. The effects of salt application on the fresh weight of shoot and root were found to be statistically significant at 25 to 100 mM NaCl level. However, roots were more sensitive to salinity as compared to shoots. The results are similar to those reported by researchers (Akbarimoghaddam *et al*., 2011; Agarwal *et al*., 2015). Giaveno *et al*. (2007) reported that the fresh weight of shoot and root was either unaffected or got slightly decreased at higher salt concentrations. Lyra *et al*. (1992) also reported a similar trend in the fresh weight of *Trigonella* and *Sesamum* seedling. Reduction in fresh biomass at higher concentration might be due to poor absorption of water from the growth medium due to physiological drought (Orak and Ates, 2005; Nedjimi *et al*., 2006). Fresh weights were decreased with an increase in the level sodium chloride as a result of reduced photosynthesis and membrane stability (Mozafariyan *et al*., 2013).

Sharifi *et al*. (2006) attributed the reduced weight to many different factors such as reduced photosynthesis, the destruction of cell membranes, reduced available water in the plant and the accumulation of sodium ions in the self. Also, the study made by Nedjimi *et al*. (2006), on (*Atriplex halimus* L.) where they report an increase in the fresh and dry weight for root and shoot systems of the plants with (50 mM) concentrations of NaCl. In spite of the fact that many studies have pointed to the positive effect of sodium chloride on fresh and dry weight, there are contrary results, as well, pointing to the negative effect of salt stress on fresh and dry weights (Jamil *et al*., 2007; Rui *et al*., 2009 and Memon *et al*., 2010). The increase in fresh weight of the shoot may be due to the ability of the plant to increase the size of its sap vacuoles, which allows for the collection of a lot of water, and this in turn dissolves salt ions that have accumulated and leads to the subsequent increase in fresh weight (Munns, 2002).

Dry Weight of Shoot and Root

Dry weights of shoot and root were strongly inhibited at all salinity treatments. Athigher level of salinity, variety UPO-94 and Kent showed 65.22-73.91 % and 62.50-7.50% reduction in shoot dry weight and 50.44-64.44% and 40.60-68.12% reduction in roots dry weightat 75 to 100 mM salinity levels respectively. These results are similar to those reported by Akbarimoghaddam *et al*.(2011); Zhani *et al*.(2012) and Agarwal *et al*.(2015). Present study indicates that dry weights of root and shoot were significantly reduced at higher salinity levels (100 mM). These findingare also similar to the reports of Maiti *et al*. (1994); Jamil and Rha (2007); Bakht *et al*. (2007) and Anubumalarmathi and Mehta (2013).The decrease of shoot and root dry weight probably may be due to some reasons such as (i) salt stress reduced photosynthesis per unit leaf area which turned into limited supply of carbohydrate needed for shoot length, (ii) reduced turgor resulting in lower water potential. In addition, salinity affected final cell size as well as rate of cell production and thereby resulting in reduced shoot and root dry weight. The results are in agreement with the findings of Alam *et al*. (2004) and Mahmood *et al*. (2009). Raptan *et al*. (2001) reported that salinity decrease dry biomass and decreased root and shoot weight in green gram.

Total Dry Weight of Seedling

It is evident that varietyUPO-94 proved most sensitive and while NDO-2 highly tolerant and UPO-212 moderately tolerant to salinity in the term of total seedling dry matter. The results are similar to those reported by Rastegar and Kandi (2011) in soybean and Hoque *et al*. (2014) in maize. Results showed that the inhibitory effects of salinity on dry weight of seedling where dry weight of seedling reduced significantly in all the cultivars except in NDO-2 and UPO-212 in which non-significant reductions were recorded at 25 to 50 mM while UPO-94 OL-125 and OL-9 were significantly affected at all salinity (25 to 100 mM) levels. Dadkhah and Grrifiths (2006) attributed such a decrease in dry weight to greater reduction in uptake and utilization of mineral nutrients by plants under salt stress. In general, there is a decrease in dry weight of plants under saline conditions which can be attributed to reduced rate of photosynthesis, as suggested by Jafari *et al*. (2009).

Ashraf (2002) reported that the reduction in seedling fresh and dry weight was due to decreasing water uptake by seedling under salt stress. Reduction in total dry weight may be due to the considerable decrease in plant growth, photosynthesis and canopy structure in *Abelmoschus*

esculentum (Bhatt and Rao, 2005); *Andrographis paniculata* (Talei *et al*., 2012);*Ricinus communis* (Janmohammadi *et al*., 2012) andin *Salvadora persica* (Sharma and Ramawat, 2013)). Mohamedin *et al*. (2006) also reported that salinity induced weter deficit hence the reduced plant growth. Cha-Um and Kirdmanee (2009) reported a decreasing trend in fresh and dry weights in maize seedling under NaCl salinity. According to them, salinity leads to water deficit in plants thereby causing a decrease in fresh and dry weight (Ratnakara and Rai, 2013).

Salt Tolerance Index (STI)

The salt tolerance index can be used as an effective criterion to choose tolerant cultivars.It is cleared from the data that salt tolerance index decreased with the increasing salt stress. Variety NDO-2 and UPO-212 showed highest salt tolerance index (93.75 and 86.63%) while UPO-94 and OL-125 showed the lowest (74.36 and 76.44%) respectively at 25 mM NaCl. The adverse effect of salt tolerance index was strongly affected at high salinity levels (100 mM), which showed the lowest index value in UPO-94 (24.62%). Variety NDO-2 showed a higher Salt Tolerance Index (56.25) followed by UPO-212with the index value (52.56) .Our results indicated that salt tolerance index was negatively affected by salt stress in all cultivars of oat, which coincide with Abbas *et al*. (2013). The tolerant cultivars showed better STI values. Thus, NDO-2 was considered highly tolerant, variety UPO-212 moderately tolerant and variety UPO-94 as most sensitive.

The present study indicates that salt tolerance in early seedling stage was not correlated with seed germination. However the germination of seeds was observed at the highest concentration (100 mM) of salinity but some seeds died up to three days after germination. This revealed that the germination stage is more saline tolerant, which could results from lower absorption of salt components by the seed during this stage. Germination process is also less responsive to high tissue sodium concentration than seedling growth. Similar results were recorded by El-Goumi *et al*. (2014). Mahdavi and Sanavy (2007) ina study of *Schinopsis quebracho* observed that germination stage was relatively tolerant to salinity than that of the later stage of the development. The difference in response to salinity shown by the two species at early stage of development may be related to their morphological variation (Tsegay and Gebreslassie, 2014). Results by Munns *et al*. (2006) showed that crop plants could be salt tolerant at germination but turned salt sensitive during vegetative development.

Seed Germination (SG)

Seed germination and early seedling growth under saline condition are considered as major factor limiting the establishment of crops (Kitajima and Fenner, 2000). According to (Zhang *et al*., 2010), in barley; (Akbarimoghaddam *et al*., 2011), in wheat and (Zhang *et al*., 2013), in oat species reported that the salinity, in general has inhibitory effect on germination of seeds. Our results indicate that germination percentage and germination rate of oats seeds would decrease by increasing salinity levels (with and without GA_3). Selected tolerant variety NDO-2 revealed higher germination percentage while selected sensitive variety UPO-94 showed lower under different salinity levels (with & without GA_3). It is cleared from the findings that gibberellic acid (GA_3) improved germination percentage in both varieties of oat and reduced the adverse effect of the salinity. These results areagreement with the reports of Ghodrat and Rousta (2012) and Patel and Mankad (2014) who suggested that high salt concentration could be alleviated by the presence of gibberellic acid (GA_3).

The seeds pretreated with results GA_3 also exhibited highest germination percentage in salinity treatments than untreated seeds. The results coincidet with the finding of Bahrani and Pourreza (2012) in sesame and Samad and Karmoker (2012) in triticale. GA_3 alleviated the effect of salinity on germination were also reported by several researchers (Khan and Gul, 2006; Egamberdieva, 2009; Samad and Karmoker, 2012). Exogenous application of plant hormone (GA_3) through foliar or presoaking seed is good option to alleviate the adverse effect of salinity stress on crops (Ashraf *et al*., 2008). The increased germination percentage in GA_3 treated seeds might be attributed to fact that the GA_3 helps in breaking the seed dormancy which results in early and enhanced seed germination due to the diffusion of endogenous auxin and gibberellins like substances (Gurung *et al*., 2014). GA_3 enhanced seed germination, because it might have antagonized the effect of inhibitors present in caonla seeds (Kumari *et al*., 2007; Sundeep *et al*., 2016). On the contrary, the decrease in germination rate particularly under drought and salt stress conditions may be due to the fact that seeds seemingly develop an osmotically enforced “dormancy” under water stress conditions. This may be an adaptive strategy of seeds to prevent germination under stressful environment thus ensuring proper establishment of the seedlings (Gill *et al*., 2003).

Shoot and Root Length

Present results indicated that shoot and root length in different oat cultivars reduced significantly with increasing the salinity levels from 25 to 100 mM. Tolerant cultivars NDO-2 revealed highest shoot and root length while sensitive cultivars UPO-94 noticed minimum shoot and root length under different salinity levels. These results are similar with the finding of Akbarimoghaddam *et al*. (2011) in two wheat cultivars. The inhibitory effect of salinity on growth rate and development of seedling as found earlier by Bhatt and Rao (2005) in okra; Mehrabi *et al*., (2007) inwheat and Agarwal *et al*. (2008) in*Brassica*. Salinity reduces growth and finally causes death through osmotic, ionic and nutritional imbalances (Afifi *et al*., 2010). Sensitive varieties loss vigour quickly by losing water from the stress shocks. But resistant genotypes can tolerant well and survive in severely saline soils (Blaylock, 1994).

GA_3 treated oat plants showed an increase in tolerance to salt treatment. This increase in salt tolerance was reflected in the measured growth criteria. Length of shoot and root were increased comparing with non-treated plants. The effect of gibberellic acid was also consistent over the salinity levels. Our findings are also similar with Jasmine and John, (2012) for okra. Gibberellic acidalleviated the harmful effects of salinity and enhanced plant height (Hisamatsu *et al*., 2000; Sastry and Shekhawa, 2001;Afjal *et al*., 2005; Ashraf *et al*., 2008; Javid *et al.,* 2011; Shaddad *et al*., 2013; Afrigan *et al*., 2013).

The increase in plant height may be due to the effect of GA_3 on the cell devision and enlargement, and also GA_3 stimulated the growth and expension of cell through increasing the wall plasticity of cells (Saleh, 1990). Bejaoui (1985) has concluded that the effects of exogenously applied GA_3 in alleviation of salt stress may be caused by activation of special enzymes which participate in RNA and protein synthesis. The shoot and root lengths were higher in NDO-2 and lower UPO-94 across salinity levels and GA_3. That could be predicated due to genetic potentials of varieties. Such variations for salt tolerance in rice and other species were already reported (Maghsoudi Moud, 2008). Cicek and Cakirlar (2002) reported that salinity results in a decline in metabolic activity of plant cells, which should be inevitably reflected in inhibition of their growth. The reason for reduced shoot and root development may be due to toxic effects of the NaCl used as well as resultant unbalanced nutrient uptake by the seedling due to increased salinity. NaCl inhibits growth

by reducing noth cell division and cell enlargement (Sobhanian *et al*., 2010); Mohammed *et al*., 2012) and the observed increased seedling growth of salinity stressed plant seeds with GA_3 treatment could be attributed to the positive effect of gibberellic acid, which encourages cell division and cell elongation (Moore *et al*., 2011).

At high salinity levels (100 mM), shoot and root length were highly reduced in all cultivars of oat. Similar results were argued by Gain *et al*. (2004). In our present study, all cultivars were more responsive to gibberellic acid and to increase the length of shoot and root. Same results were claimed by Misratia *et al.* (2013). Iqbal *et al*. (2008) found that plant height of *Cicer arietinum* was increased with GA_3 treatment under salinity. Root length was also observed to increase by GA_3 treatment by Sidiras and Karsioti (1996), in *Lupinus albus*. In our study, the plant growth regulator gibberellin did reverse the growth inhibiting the effect of salt stress to a certain extent in both shoot and root. The selected three varieties were more responsive to GA_3 and to increase the height. Similar results were claimed by Watanabe and Saigusa (2004) and Bahrani and Pourreza (2012) that height increased significantly due to GA_3 over salinity. The results of this study are also in agreement with Suge (1985) who found that gibberellicacid enhanced growth through forming new cells in the intercalary meristem.

Fresh Weight of Shoot and Root

The effect of salt application on the shoot and root fresh weight of NDO-2, UPO-212 and UPO-94 cultivars were found to be statistically significant at 25 to 100 mM. The results are similar to those reported by Akbarimoghaddam *et al*. (2011), in wheat and Agarwal *et al*.(2015), in soybean. Giaveno *et al*. (2007) suggested that the salt treatment affected root and shoot fresh weight. However, plant species differ in their tolerance or sensitivity to salt stress (Ashraf and Harris, 2004). Interestingly in the present study, under salinity stress, root length increased at lower concentration (25 mM) but decreased at higher salt concentration (100 mM NaCl). In *Avena sativa* water-deficit and NaCl treatments also caused reduction in seedling fresh weight which is in agreement with earlier reports (Ramezani *et al*., 2011; Agarwal and Panday, 2004). This inhibitory effect may be attributed to the effects of salinity on several facets of plant activities such as enzyme activity (Seckin e*t al*., 2009), mitosis (Tabur and Demir, 2010) and DNA, RNA, protein synthesis (Anuradha and Rao, 2001).

Exogenous application of GA_3 (100 ppm) had generallypromoted the growth criteria (fresh weight of shoot and root) of the oat plants and thus alleviated to some extent the suppressive effect of salinity. This observed increase in fresh matter of salt stressed plants after hormonal treatment (GA) may indicate that the GA_3 application increased the plant efficiency of water uptake and conservation. The results are similar to those reported by Javid *et al*. (2011). Abd El-Samad and Shaddad (2014) reported that the concentration of gibberellic acid and Kinetin (200 ppm) were increased, generally the fresh and dry matter yield of the five crop plants and alleviated the salinity effect. Salinity stress significantly decreased savory growth so that root and shoot weight (either fresh weight or dry weight) decreased with increasing salinity level.

These results indicate that GA_3 application could improve salinity tolerance in oat plants grown under saline condition. These results are conformity with the finding of Javid *et al*. (2011); Misratia *et al*. (2013); Iqbal *et al*. (2014) reported that gibberellic acid interacts with other hormones to regulate various metabolic processes in the plant. Furthmore, leaf weight and leaf area decreased due to salinity stress while gibberellin application caused an increase in oat growth parameters i.e. root and shoot fresh and dry weight (Nikee, *et al*., 2014) in savory. Exogenous application of GA_3 might increase plant growth by enhancing the content of endogenous gibberellin as that mentioned by Rodriguez *et al*., (2006). Gibberellic acid has been reported to be helpful in enhancing oat growth under saline condition by balancing effect of enzyme activity and antioxidant system (Tuna *et al*., 2008). GA_3-promoted destabilization of DELLA protein is modulated by environmental signals (such as salt and light) and other plant hormone signaling (such as auxin and ethylene), which reveals the mechanisms of this cross-talking at the molecular level (Achard *et al*., 2006)

Dry Weight of Shoot and Root

Data indicated that the alleviating effects of GA_3 on dry weight of shoots and roots were more pronounced in cultivar NDO-2 in which application of GA_3 showed marginal reduction in dry weight of shoot from 10.02 to 32.22% and in root from 16.79 to 37.86% followed by UPO-212 in shoot from 13.95 to 39.81% and in root from 20.90 to 45.16% respectively when levels of salinity raised from 25 to 100 mM as compared to the control.Similar results were also reported by Jasmine and John (2012) in okra; Misratia *et al*. (2013) in rice. Razmjoo *et al.* (2008) attributed reduction in

dry weight under saline condition to inhibition of hydrolysis of reserve foods and their translocation to the grown shoots. Maiti *et al*. (1994) and Bakht *et al*. (2007), stated that shoot and root dry weight decreasedwith an increase in salt concentration. Iqbal. (2008) noted reduction in dry weight of shoot due to salinity. Salinity lowered the growth rate and biomass production (Lin and Kao, 2001). In present finding, NDO-2, UPO-212 and UPO-94 showed reduction in dry weight of shoot and root at different salinity levels without gibberellic acid. Conversely, plants under GA_3 treatment increased the dry weight. These results are conformity with the finding of Ashraf *et al*. (2002) that gibberellic acid in salt stressed plants showed an increased photosynthetic capacity-a vital factor for higher dry matter synthesis. The present results showed that the vegetative growth of oat plant was negatively affected by salinity treatments. The reduction of growth is a common indicator of salt stress because of inadequate water uptake.This results are similar to those reported by Borsani *et al*. (2003); Ali *et al*. (2012).

The plant height, leaf and stem dry weights as well as leaf area were gradually decreased with increasing salinity levels. The vegetative growth reduction occurred as a result of salinity may be due to the reduction of both cell division and enlargement (Yasseen *et al*., 1987).GA_3 treatment of salt stressed wheat plants resulted in an increased photosynthetic capacity, which was discussed as a major factor for greater dry matter production. GA_3 increased dry matter and leaf-area index in mustard plant (Khan, 1996) and photosynthetic rate in leaves of wheat (Ashraf *et al*. 2002). Khan *et al*. (1996) also reported an increase in the activity of carbonic anhydrase (CA) in mustard leaves following the application of GA_3 treatment. GA induces the aleurone cells of barley seeds to produce *a*-amylase which is then transported to the endosperm, where it helps in the production of soluble sugars from starch.

Na^+ ion accumulation

Na^+ ion concentration significantly increased in oat varieties NDO-2, UPO-212 and UPO-94 under salinity stress. In general, the tolerant cultivars showed lesser increase in Na^+ ion concentration as compared to sensitive genotype at all salinity levels. Similar results are reported by Kumar *et al*., (2013) in oat and Hakim *et al*. (2014) in rice.In the present investigation sodium (Na^+) content increased in leaves of *Avena sativa* under salinity stress. Na^+ accumulation in salt stressed plant led to low water potential, change in ion uptake and ionic imbalance, reduced leaf expansion,

photosynthetic rate and limited growth (Kiarostami *et al*., 2010).It is evident that 2.33and 3.06 times higher Na^+ accumulation were noted in UPO-94 at 75 to 100 mM when compared with control and NDO-2 had 2.0and 2.24 times higher Na^+ accumulation at these levels of salinity as compared to control set. Present finding are in consistent with the finding of Haq *et al*. (2003). GA_3 treatment (100 ppm) alleviated the adverse effects of NaCl salinity stress and thus, reduced accumulation ofsodium ions in the three cultivars of oat. Similar results obtained by Samad and Karmoker (2012) in wheat. These finding are in accordance with Iqbal and Ashraf (2013) who found that GA_3 treatment (150 mg L^{-1})decreased sodium concentrations both in the shoots and roots and increased Ca^{2+} and K^+ concentrations in wheat cultivars under salinity condition.Exogenous application of GA_3 improved the water stress tolerance in maize plants by maintaining membrane permeability, enhancing some macro-nutrient concentrations in leaves (Kaya *et al*., 2006).

Exogenous application of GA_3 could reduce salinity stress in all cultivars of oat by reducing Na^+ accumulation and increasing uptake of other essential nutrients. Similar finding were also reported by Misratia *et al*. (2015) who reported that the application of GA_3 is usefull to mitigate salinity stress in rice plant and its effectiveness is higher for salt tolerant varieties. GA_3 treatment increased the mineral nutrient levels of *Vigna unguiculata* roots and shoots (Al-Rumaih *et al*., 2003).Wakeel *et al*. (2011) reported that Na^+ toxicity affects plant growth, thus displacement of K^+ by Na^+ in the plant cell affects the activity of plasma membrane (PM) H^+ -ATP ase.

K^+ ion accumulation

K^+ is other essential macronutrients, taken up by the roots and generally transpoted to the shoot through the xylem and this transport seems to be controlled by the shoot growth (Lidon and Henriques, 1992). Na^+ concentration were higher than K^+ concentration under different salinity levels in all varieties of oat. K^+ content was significantly higher in NDO-2 and UPO-212 while variety UPO-94 showed lower concentration at all salinity levels including control. These results are similar with finding of Demiral *et al*. (2005) in barley; Mohamedin *et al.* (2006) in sunflower; Akbarimoghaddam *et al*. (2011). Caia *et al*. (2014) concluded that salinity treatment enhances the accumulation of leaf Na^+ and Cl^- ions, thereby reducing plant growth rate and hence minimizing the ion uptake by the roots and ion accumulation in the shoots are important mechanisms of salt tolerance. Bagci*et al*. (2003) suggested that there is an inverse relation betweens K^+ and Na^+ ion of

8 different barley cultivars grown under different salinity levels.Kaya *et al*. (2007) studied that K^+ plays an important role in balancing membrane potential and turgor, regulating osmotic pressure, membrane polarization and stoma movement.Oat plants exposed to high levels of salinity had accumulated less K^+ in all cultivars. Similar observation were reported by Bakht *et al*. (2007) in oat and barley; El-Arquan *et al* (2002) for sugar beet. Gopal and Dube (2003) reported that the high concentration of Na^+ and Cl^- ions in soil solution reduced the uptake of K^+ ions which ultimately caused K^+ deficiency in plants.

The interrelationsheep between the effect of salinity and GA_3 on seed germination and the accumulation of Na+ and K^+ in the seedling was discussed. Salt salinity increased the accumulation of Na^+ in shoots while it decreased that of K^+ in all cultivars of oat. This result in confirmed by Akbarimoghaddam *et al*. (2011) in wheat. Data also revealed that GA_3 application enhanced K^+ uptake upto at 100 mM in all cultivars as compared with salt stressed plants However, this enhancement effect of GA_3 is more pronounced in the case of variety NDO-2. These results are in accordance with the finding of Samad and Karmoker (2012) in wheat and Abdel-Hamid *et al*. (2014) in barley. Amirjani (2010) stated that the K^+ ions in seedling growth of soybean significantly decreased with the increase in different salinity levels. Summart *et al*. (2010) also suggested that Na^+ and K^+ significantly influenced by the effect of different levels of salinity in rice plants. Exogenous application of GA_3 increased K^+ accumulation and reduce salinity stress by reducing Na^+ accumulation (Misratia *et al*., 2015).

Na:K

Ionic ratios are very important to determine the relative toxicities that could providerelative biological process rates under specific ionic antagonisms (Rahman *et al*., 2008).Na^+/K^+ sharply increased in the sensitive variety UPO-94 and lower increased in tolerant cv. NDO-2 with increasing salt concentrations. These finding are similar with finding of Hakim *et al*. (2014) in rice and Ansari *et al*. (2003) Salt-sensitive variety UPO-94 expressed more nutritional imbalance while the salt tolerant variety NDO-2 were able to maintain balance among the nutrient in the tissues. Present finding indicates that Na^+/K^+ significantly increase in all cultivars with increasing salinity levels. The tolerant cultivars registered lowest values of Na^+/K^+ than sensitive ones. Na^+/K^+ may serve as an indicator of tolerance to stress as the increase of Na^+ in salt salt tolerance species is

generally associated with a decreased in K^+ (Greenway and Munns, 1980). Similar results are reported by Bell and O' Leary (2003) in *S. virginicus*; Othman *et al*. (2006) in barley and Zhao *et al*. (2007) in oat.

Application of GA_3 reduced the Na^+/K^+ in all cultivars when compared with non treated plants. Cv. NDO-2 showed lower Na^+/K^+ ranged from 0.03 to 0.011 followed by UPO-212 range from 0.06 to 0.36. It was higher (0.11 to 0.94) in UPO-94 in control as well as in all levels of salinity (25 to 100 mM). Similar results are reported by Abdel-Hamid and Mohamed (2014). It is suggested that the K^+ concentration increase with GA_3 and the variety ratio decrease (Samad and Karmokaer, 2012). The present investigation showed the 100 mM salt concentration induced a significant decrease in K^+ uptake, while GA_3application enhanced K^+ by oat plants. However, this enhancement effect of gibberellic acid (GA_3)is more pronounced in the case of NDO-2. Similar results are also obtained by Ashraf *et al*. (2002) reported that gibberellic acid stimulated the accumulation of potassium ($K^{+)}$ in shoots of a salt tolerant genotype of wheat at 100 mM NaCl with GA_3 application. In addition to its conventional osmoprotective role, Na^+ accumulation and Na^+/K^+ ratio in salt stressed plants depend on salt stress treatments, while K^+ accumulation is generally decreased (Cuin and Shabala, 2005 and Sabir and Ashraf, 2007).Pardo *et al*. (2006)reported that the Na^+/K^+ plays a role for growth of plants, since metabolisms is adversely affected by low Na^+/K^+ ratios under salt condition.

Proline accumulation

Proline functions as an osmolyte for the intracellular osmotic adjustment and its accumulation plays a critical role in protecting photosynthetic activity in plants under salt stress (Silva-Ortega *et al*., 2008). Salinity stress stimulated the accumulation of proline in all cultivars of oat. Similar results were reported by some researchers Ghoulam *et al*. (2002) in sugar beet; Eraslan *et al.* (2007) in lettuce; Tuna *et al*. (2008) in maize; Celik and Atak (2012) in tobacco; Ashfaque *et al*. (2014) in wheat and Ahanger *et al*. (2015) in oat. Significantly greater proline accumulation was observed in the tolerant lines with increasing salinity concentration as compared to the sensitive variety. Similar increase in proline was also reported in niger (Sarvesh *et al*., 1996) and wheat (Goudarzi and Pakniyat (2009) in response to increasing salinity levels.

Application of GA_3 might counteract the adverse effect of salinity and increased proline concentration at different salinity levels including control in three oat cultivars. The application of GA_3 promoted proline content and the effect was greater in tolerant variety NDO-2 compared with sensitive variety UPO-94. Our results are conformily by Misratia *et al*., (2015) in rice and Ali *et al.* (2014) in roses and Iqbal *et al*.(2014),who reported that gibberellic acid treatment counteractthe adverse effects of salinity with accumulation of more proline which maintained membrane permeability and plays important role in alleviating salt stress in crops plants. This enhancement in accumulation of proline may represent a major biochemical adaptation in plants osmotic adjustment (Khan *et al*., 2010). Contrary to these reports to these reports, Kaya *et al*. (2006) and Alia and Gahiza (2007) have found that GA_3 treatment reduced the proline accumulation in salinity stressed *Zea mays* and *Anabaena* plants respectively.It has been widely reported that proline may play a role in stress adaptation within the cell. Similar results were reported by Gilbert *et al*. (1998) and Tuna *et al*. (2008).

The proline content highly increased with the concentration of NaCl (75 to 100 mM). Simarly, salt stress (NaCl) caused a marked increase in proline amino acid (Cakrabarti and Mukherji, 2003 and Mohammed, 2007). Maggio *et al*. (2002) suggested that proline may act as a signaling/regulatory molecule able to activate multiple responses that are component of the adaptation process and stabilize the structure of membranes and proteins to minimize the damage of cell under salt stress. The higher accumulation of proline could be due to enhanced activities of ornithine aminotransferse (OAT) and pyrroline-5-carboxylate reductase (P5CR), the enzyme involved in proline biosynthesis in legume root nodules (Kohl *et al*., 1990).

Proline plays a protective function against salinity in plants. Proline synthesis in plants occurs mainly from glutamate. Alternatively, proline can also be synthesized from ornithine, which is transaminated first by ornithine-delta-aminotransferse (OAT) producing GSA and pyrroline-5-carboxylate (P5C) and then converted to proline (Kishor *et al*., 2005 and Verbruggen and Hermans, 2008). In the present results, osmotic adjustment was achieved by increase in concentration of proline and K^+ in tissues when water content decreased with increase in salinity. In addition, the primary role of proline may not be solely as an osmolyte, but it also helps the cells to overcome oxidative stress in salt-stressed plants (Rajendrakumar *et al*., 1994 and Bhatt *et al*., 2008).

Chlorophyll *a*

Chlorophyll is the main colour pigment responsible for photosynthesis. Under adverse circumstances, the chlorophyll level is a good indicator for the photosynthesis function (Xu *et al*., 2008). Salinity stress also affected some physiological parameters such as chlorophyll and proline content. In this study, chlorophyll content was significantly decreased with the increasing of salinity levels in all varieties of oat. Similar results were reported by some researchs (Tuna *et al*., 2008; Khalid and Cai, 2011 and Celik and Atak, 2012). On the other hand, salt stress affected proline content in an opposite manner. The decrease may be due to the formation of proteolytic enzymes such as chlorophyllase, which is responsible for the chlorophyll degradation (Sabater and Rodriguez, 1978) as well as damaging to the photosynthetic apparatus (Yasseen, 1983). Application of GA_3 improved the chlorophyll levels in salinity-stressed plants of oat. GA_3 treated plants exhibited higher values of chlorophyll *a* (0.72 to 0.45, 0.54 to 0.30 and 0.45 to 0.19) in varieties NDO-2, UPO-212 and UPO-94 respectively at all levels of salinity including control.

On the basis of present finding the ability of tolerant variety NDO-2 and UPO-212 to maintain higher level of chlorophyll *a*. Similar results were reported by Datta *et al.* (2009). Considering the chlorophyll *a* as the main pigment (Santos, 2004), reduce in the chlorophyll could probably be one of the vital cause of reduced photosynthesis under salt stress as observed in rice (Moradi and Ismail, 2007). Among them, the role of gibberellic acid (GA_3) in stress tolerance and enhancing growth under saline conditions has been reported (Iqbal *et al*., 2011). Reports concerning the application of gibberellic acid (GA_3) under salinity conditions are scarce. A few studied: however, pinpointed the ability of its foliar pre-treatment to overcome adverse effects of NaCl (Chakraborti and Mukherji, 2003) as it alleviates the pessimistic effects on pigment contents and water use efficiency (Aldesuquy and Ibrahim, 2001).

GA_3 treatment alleviated the negative effects of salinity on the morphological traits and physiological attributes such as chlorophyll content and stomatal conductance (Misratia *et al*., 2013 and Nikee *et al*., 2014). Schutz and Fangmeir (2001) have suggested that the reduction of chlorophyll due to stress is related to the increase of production of free oxygen radical in the cell. These free radicals cause peroxidation, disintegration and reduction of chlorophyll content in plant

under stressfull conditions. Khan *et al*. (2002) reported that the increase in N uptake following GA_3 application was due to increase in shoot growth, which requires more utilization of soil N. GA_3 induced N uptake results in increased photosynthetic efficiency through maintenance of photosynthetic enzymes.

Chlorophyll *b*

Chlorophyll *b* progressively decreased with increasing levels of salinity in all cultivars of oat. Chlorophyll *b* was higher in NDO-2 followed by UPO-212 and lower in UPO-94 at all salinity levels. These results are similar with those of Khan (2003) and Jaleel *et al.* (2008). The inhibition become more marked in all cultivars at highest salinity levels (75 to 100 mM). It ranged from 0.13 to 0.12 mg, 0.09 to 0.08 mg and 0.06 to 0.05 mg in varieties NDO-2, UPO-212 & UPO-94 respectively. This result is an agreement with those of Tewari and Singh (1991) in lentil, Beinsan *et al*. (2003) in bean, Iqbal *et al*. (2006) in wheat, Chen and Yu (2007) in *Glycine max* seedling, Moussa (2006) in maize, Molazem *et al*. (2010) in cornand Rahdari (2012) in Purslane. The decrease in chlorophyll content in the stressed plants could be due to increased activity of the chlorophyll-degrading enzyme chlorophyllase (Reddy *et al*., 1986). This decrease may be due to a reduction in the uptake of minerals i.e. Mg needed for chlorophyll biosynthesis (Sheng *et al*., 2008), membrane deterioration (Ashraf and Bhatti, 2000), or the suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute *et al*., 2006).

Reduction of chlorophyll content due to salinity stress is very common in salt-sensitive plant species because of salt toxicity which mostly cause burning of leaves or other succulent parts and degradation of other pigments too (Jaleel *et al*., 2008) in *Catheranthus roseus*; (Akca and Samsunlu, 2012) in walnut. Application of GA_3 promoted the chlorophyll *b* in three oat cultivars of oat when compared with non treated plants. Variety NDO-2 depicted higher values followed by UPO-212and UPO-94 showed lowest values at varying salinity levels (25 to 100 mM) including control. The results are similar to those reported by Maggio *et al. (*2010) and Misratia *et al*. (2013) which may be involved in protecting the photosynthetic apparatus and consequently.It was noted that the concentration of Chlorophyll *a* was higher than chlorophyll *b*. Similar results was given by Hendaway (2015) for wheat.

Chlorophyll (*a*+*b*) and Total Chlorophyll

Chlorophyll *a*+*b* and total chlorophyll were higher (0.79 to 0.45and 0.83 to 0.49) in cv. NDO-2 and lower (0.49 to 0.19 and 0.51 to 0.21) were noted by UPO-94 at all salt concentrations including control.Chlorophyll *a* and *b* are the main photosynthetic pigments and they play an important role in photosynthesis. Similar results were given by Hendaway (2015) for wheat.According to data that Chl *a*+*b* and total chlorophyll were highly reduced at high salinity levels (75 and 100 mM). The greater alleviation was observed by tolerant cultivar in total chlorophyll and chl *a*+*b*. Similarly, leaf chlorophyll and carotenoids content were significantly reduced in salt treated plants (El-Tayeb, 2005; Iqbal *et al.,* 2006 and Shah, 2007). Khan *et al*. (1998) reported that a, b and total chlorophylls content reduce in respond to salinity stress, this may be for forming proteolytic enzymes such as chlorophyllas which responsible to decompose chlorophyll and damaging photosynthetic structures.

GA_3 application enhanced the photosynthetic pigments in the leaves of three oat varieties (NDO-2, UPO-212 and UPO-94) under salinity stress. Similar results were found by Turkyilmaz (2012) in wheat.GA_3 treated plants exhibited higher values of pigment concentration than those of control or salinity treated sample in soybean (Zhao *et al*., 1995); in okra (Mary and Meriana, 2012). Moreover gibberellic acid enhances the formation of chlorophyll pigments. Similar findings were reported by Abbas (2011)and Misratia *et al*.(2013). Gibberellic acid (GA_3) enhances the formation of proteins and new RNA and increase chlorophyll content which increases the process of photosynthesis; all this leads to the increase of shoot dry weight (Devlin and Witham, 1998). The observed chlorophyll depletion may be considered to be a result of the inhibition of chlorophyll biosynthesis (Khan, 2003). However, treatment of the salt stressed plants with GA_3 (Shah, 2007) were found to restore normal chlorophyll levels. The reasons of decreased photosynthetic rate might be attributed to the fall on chlorophyll contents, stomatal closure, transpiration and CO_2 assimilation by leaf tissue and finally plant growth (Misra *et al*., 2002 and Tardieu, 2005).

In the present study, the chlorophyll contents significantly decreased under elevated salt stress, as the chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels might be due to salt stress has been reported by Ashraf *et al*.(2002), in wheat and Anuradha and Rao (2003), in rice. The decrease in chlorophyll content under saline stress may be due to different reasons: (i) the inhibitory effect of the accumulated ions (Ali *et al*., 2004). (ii) Salinity damages the structure

and function of thylakoid membrane, electron transport, gaseous exchange and enzymes (Sudhir and Murthy, 2004). It is adopted the view that osmotically increased water stress enhances the decay of chlorophyll. The inhibitory effect of gibberellins on chlorophyll catabolism might be partly due to the down regulation of the activities of enzymes involved in chlorophyll catabolism and the alleviation of oxidation leading to chlorophyll bleaching (Li *et al*., 2010). Zeid (2011) reported an alleviation of adverse effect of salinity on chlorophyll degradation in barley with gibberellic acid treatment. It may be due to the GA_3 generated sweetening of ultra-structural morphogenesis of plastids coupled with the retention of chlorophyll and delay of senescence caused by GA_3 (Arteca, 1997).