International Journal of Research
Available at http://internationaljournalofresearch.org/
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 02 Issue 03
April 2015

Effect of salinity on plant water status, solute accumulation and ionic distribution in wheat (*Triticum aestivum* L.) genotypes

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ABSTRACT

The effect of chloride dominated saline irrigation was studied on physiological parameters like osmotic potential, relative water content, fresh and dry weight, lipid peroxidation and biochemicals (total soluble carbohydrates and proline content) and ionic distribution in four wheat genotypes viz. C306, WH1025, WH1080 and WH1081. The saline irrigation were given to different sets of plants after germination and the sampling was done 10 days after treatments. Salinity induced the changes in various physiological and biochemical processes. The osmotic potential (Ψ_s) becomes more negative with progressive increase in the rhizospheric salinity while decrease in relative water content (RWC%) and fresh weight of plants. The malondialdehyde (MDA), proline and total soluble carbohydrate (TSC) content increases under saline irrigation. The Na^+ content increases while K^+ content decreased in leaves with the progressive increase in saline irrigation.

Key words:

Salinity, lipid peroxidation, minerals, proline, water relations

INTRODUCTION

Wheat (*Triticum aestivum*) in the most important widely grown cereal crops of high nutritive value in the world. The grains of wheat, contains large amounts of proteins, carbohydrates in addition to some minerals and vitamins. It is the major food crop for more than one third of the world population and is the main staple food of Asia (Shirazi *et al*., 2001). It provides food to 36% of the global population contributing 20% of the food calories to it. In India it was grown on 29.24 mha with annual production 85.92 million tonnes of food grains, in the year 2011 (Annonymous, 2011). The loss of farmiable land due to salinization, which may touch figure of 50% by the year 2050 (Wang *et al*., 2003) is directly in conflict with need of World population, which is projected to increase by 1.5 billion over the next 20 years, and the challenge of maintaining the world food supply.In wheat growing area of India, the combination of salt affected soils and irrigation by poor quality water severely limit the productivity. Soil salinity is the serious problem in arid and semi-arid tracts of the world. It is widespread and a complex environmental stress in natural and agricultural ecosystem that affects growth and productivity of various important crops. Salinity affects the plants

morphological, physiological and biochemical processes (Grewal 2010). Salt tolerance has been associated with the ability to prevent the uptake and /or translocation of saline ions from the root to shoot. The stability of wheat production depends on its ability to adapt to salinity stress. In the world geographical area, 6% of the total and 20% of irrigate land are salt affected. In Haryana, 0.45 mha of land is estimated to be affected by salinity. The salinity tolerance of the plant changes through the life cycle of the plants; the sensitivity being greatest during germination and seedling growth. The salinization is the scourge of intensive agriculture and high concentration of salts have detrimental effects on germination of seeds and plant growth (Rahman *et al.*, 2000). Several mechanical and chemical methods have been devised to reclaim the saline soil, these are expensive and not readily feasible, an alternative approach for optimal utilization of these saline soils is to have crop species/genotypes that tolerate high level of salt in their rhizosphere. The need of salt tolerant crop around the world increases each year as the growing population seeks to feed itself on ever decreasing soil resources and the dwindling fresh water supplies.

Materials and Methods

The experiment were conducted on a four genotype of wheat (*Triticum aestivum* L.) namely C 306, WH 1025, WH 1080 and WH 1081 in the Department of Botany, Maharshi Dayanand University, Rohtak. The seeds were procured from the Wheat section, Department of Plant Breeding, CCS Haryana Agriculture University, Hissar. The crops were raised in earthen pots filled with dune sand. Some of the physico-chemical characteristics of experimental dune sand were as follows:

Sand (93.3%), silt (3.0%), clay (3.7%), saturation capacity (25%) and organic carbon (0.06%) Before sowing, the seeds of uniform size were surface sterilized with 0.1% mercuric chloride solution for 5 minutes and washed with distilled water. The pots were irrigated with desired level of salinity (0, 2.5, 5.0 and 7.5 dSm^{-1}) levels. The sterilized seeds were grown in pots with drainage holes at bottom surface and plugged with glass wool. After germination, equal number of seedlings have comparable height was maintained in each pot. The plants were irrigated with nutrient solution whenever required. The dune sand was artificially prepared for chloride dominated salinity by using mixture of different salts like NaCl, $MgCl_2$, $MgSO_4$, and $CaCl_2$. The pots were saturated with desired salinity solution (after germination) to each pot so as to maintain four levels, i.e. 0, 2.5, 5.0 and 7.5 dSm^{-1} of chloride dominated salinity. Data were analyzed using completely randomized design (CRD) for the factor. Treatments were compared using critical difference at the 5 % level of significance.

Osmotic Potential (Ψs)

Osmotic potential was determined using a psychometric technique (Model 5100-B Vapor Pressure Osmometer, Wescor Inc. Logan, Utah, USA). The fully expanded leaves were stored in air tight microcentrifuge tubes. The leaves were frozen at -20^{o}C and crushed at room temperature. A filter paper disc was

immediately dipped in the sap and placed in the concave depression of sample holders, avoiding the touching of wet disc on the outer surface of the sample holder. Then pushed the sample slide gently into the chamber of minutes a 'beep' tone was sounded. The osmotic potential reading (mOs kg^{-1}) displayed on the digital meter recorded. The osmometer was calibrated by using Osmolality Reference Standards of Sodium Chloride (Wescor Inc, USA).

Relative Water Content (RWC%) The plants were sampled and the fully expanded leaves from the top were excised from the shoots. Sand was removed with the help of a soft camel hair brush. Then the leaves were separated and weighed immediately to take their fresh weight. Then the leaves were kept separately in petridishes filled with distilled water for 3 hrs. After that the leaves (fully turgid) were weighed again and then kept in oven at 85° C for 72 hrs. or until a constant dry weight. These weights were used to calculate relative water content of leaves according to the formula given by Weatherley (1950).

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

Fresh Weight: The fully expanded leaves were excised from the shoots. Sand was removed with the help of soft brush. The leaves were separated, weighted and kept in the oven at 85°C to obtain the constant weight. The values expressed as mg weight of the tissue.

Lipid Peroxidation: The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) present in the tissues (leaves). The MDA is a product of lipid peroxidation and was measured by thiobarbituric acid (TBA) reaction with minor modification of the method of Heath and Packer (1968). One hundred mg of fresh leaves were homogenized separately with 5ml of 0.1% TCA. The homogenate was centrifuged at 8000 xg for 15 min. The supernatant was collected and then directly used for the assay. One ml of the supernatant was taken in a test tube and precipitated by 4 ml of 20% TCA containing TBA. The mixture was heated in a water bath shaker at 95°C for 30 minutes and quickly cooled in an ice-bath. After centrifugation at 8000 xg for 10 minutes the absorbance of the supernatant was read at 532 nm and 600 nm, the value for non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using its extinction coefficient of 155 nM^{-1} cm^{-1}.

Proline Content: Proline content was estimated by using the method of Bates *et al*., (1973). One hundred mg of fresh leaves were separately homogenized in 5 ml of 3% sulphosalicylic acid and then centrifuged at 5000 rpm for 15 minutes and supernatant was collected. The 2 ml of supernatant was taken in a test tube and 2.0 ml reagent acid ninhydrin was added. This mixture was then kept in boiling water bath for 1 hr at 100°C and thereafter reaction was terminated by keeping tubes in ice-bath. Then 4.0 ml of toluene was added. After vigorous shaking, the upper coloured organic phase was separated and

absorbance was recorded at 520 nm by using toluene as blank.

Total Soluble Carbohydrates (TSC): Total soluble carbohydrates were determined with the method of Yemm and Willis (1954) using anthrone reagent. One hundred mg fresh samples of leaves were homogenized separately in 80% ethanol using acid washed sand as an abrasive. The homogenate was refluxed volume made to 80% ethanol. The extract so obtained was used for estimation of TSC.

RESULTS

Osmotic potential (Ψ_s) of leaf

Data presented in table 1 indicates that salinity causes the decline in osmotic potential (Ψ_s) of leaves of all the wheat genotypes. Osmotic potential becomes more negative with progressive increase in the rhizosphere salinity level in all the genotypes at all the stage. The Ψ_s of leaf was -0.94, -0.88, 0.80 and -0.92 MPa in the genotypes C306, WH1080, WH1081 and WH1025, respectively under non-saline conditions. Results presented in the Table 1 clearly evince that osmotic (Ψ_s) potential of leaf decline considerably in response to salinity in all the four genotypes studied and also at all the stages 10, 15 and 20 DAT. At 7.5 dSm^{-1} salinity, the values of Ψ_s were -1.19, 1.18, 1.08 and 1.08 mPa in the genotypes C306, WH1080, WH1081 and WH1025 respectively at 10 DAT. The genotype C306 shows more decline in Ψ_s (-1.19MPa) with progressive at salinity level 7.5 dSm^{-1}.

Relative Water Content (RWC %) **R**relative water content of leaf did not differ much among the tested genotypes. At salinity 7.5 dSm^{-1}, the decrease in RWC (%) of leaf in all the genotype studied and decrease being minimum in genotype WH1025 (87.14) while maximum decrease was observed in genotype C306 (85.11). A decreasing trends of RWC (%) was observed, the decrease was more in WH1081 (77.24) (Table 1)

Fresh Weight of leaf

Data presented in table 1 evinces leaf fresh weight of WH1025 was higher than three genotypes at control condition. The fresh weight of the leaves decreased in all the genotypes with progressive increase in the level of saline irrigation over their controls. The decrease in leaf fresh weight was upto the 26.57%, 25.75%, 26.74% and 26.51% at salinity level 7.5 dSm^{-1} in the genotypes C306, WH1080, WH1081, WH1025 over their respective control.

Lipid peroxidation (MDA)

The lipid peroxidation of leaves under saline irrigation was measured in term of MDA in m moles gm^{-1} fresh weight. MDA content of leaf was highest in C306 followed by WH1080, WH1081 and lowest in the genotype WH1025 under non-saline condition (Fig.1) and salinity increased MDA content in the leaves of all the four genotypes at all the stages, the increase being highest in genotypes WH1080 (48.00%) over its control followed by WH1025 (41.15%), C306 (28.34%) and least in the genotypes WH1081 (26.14%).

Proline Content

Increase in proline content in leaves under saline irrigation in all the genotypes of wheat. The increase in proline content of leaf was more in WH1081 (83.86%) at highest level of salinity. The increase was two folds among all the other genotypes at highest level of salinity when compared over their respective control. The genotype WH1025 (35.76%) shows lowest value of proline at 7.5 dSm^{-1} salinity level over the control (Fig.2).

Total soluble carbohydrate (TSC):

The osmolytes increases with progressive increase in salinity levels. The increase in TSC in leaves of wheat genotype under saline irrigation was observed. The maximum increase in levels of TSC was reported in genotype WH1081 (37.97%) followed by WH1080 (31.10%), C306 (28.14%) and least in WH 1025 (27.55%) at the highest level of salinity 7.5 dSm^{-1} (Fig. 3).

Na^+ and K^+ Content in leaves:

The Na^+ was 2.55, 3.00, 2.68 and 4.78 mg g^{-1} under non- saline condition. Sodium increased with progressive increase in saline irrigation in all the genotype. At highest level of treatment, the maximum increase in sodium was observed in genotype C306 (110.78%), followed by WH1080 (80.19%) and least in WH1081 (70.33%) and WH1025 (70.33%). But in contrast to Na^+ , the potassium content decrease under saline irrigation with the progressive increase in saline irrigation in all the genotypes. The decrease was found maximum in WH1025 (12.68%) and minimum in WH1081 (42.18%) at the highest level of salinity (7.5 dSm^{-1}) and is statistically significant (Table 2).

DISCUSSION

Wheat is one of the most important staple food crops and is a common source of energy and proteins for the world population. Salinity is the key constraint to wheat production in irrigated agriculture in many parts of the world. Attempt has been made to study the salinity mediated physiological and biochemical responses of wheat genotypes differing in their relative tolerance to salinity.

Osmotic Potential

In the present investigation salinity cause the decline in osmotic potential of leaves of all the wheat genotypes. Osmotic potential becomes negative with increase in salinity level irrespective of genotypes; however tolerant genotype responds lower than sensitive genotype. This differential response of tolerant genotypes to salinity stress is due to the reduced transpiration rate and closure of stomatal openings during stress period, with no significant change in chlorophyll content (Senguttuvel *et al*., 2014). Plants cope up salinity by lowering their osmotic potential through accumulation of ions and compatible organic solutes. This is accompanied by a decline in ψ_s and degree of decline in ψ_s depends upon the salinity resistance status of crop genotypes and accordingly these are able to absorb water from the rhizosphere. Under identical situation, changes in Ψ_s of leaf can be used in

screening of wheat genotypes for difference in osmotic adjustment. More negative value of Ψ_s of leaf are indicative of no significant change in the leaf turgor pressure and thus better water status as also proposed by Lauter and Munns (1987). Similar decline in leaf osmotic potential were reported significantly with increase in salinity level in wheat (Mandhania *et al*., 2006), chickpea (Kukreja *et al*., 2010). Our results confirms the earlier finding in wheat (Khatkar and Kuhad, 2000, Shirazi, 2001).

Relative water content

Present study vividly evinces that salinity significantly lowered the RWC (%) of leaf with the increase in salinity levels in all the four genotypes of wheat at all the samplings stages. In confirmation with our results, similar reduction in relative water content (Nandwal *et al*., 2007, Kukreja *et al*., 2010, Singh, 2010) has been reported in various plant parts. Similar decrease in RWC of was observed in wheat (Ghogdi *et al*., 2012). Salt sensitive cultivars show lowest RWC in all the level of salinity while salt tolerant cultivars show highest RWC at all the salinity levels.

Fresh weight

Salinity decreases the plant growth which consequently declines in the availability of assimilates to growing tissues and organs (Munns, 2007). Salt sensitive genotypes had greater decrease in plant dry biomass than salt tolerant genotypes. The variation in response of the tested genotypes could be largely related to plant genetics. It has been reported that wheat genotypes having greater plant biomass at the seedling stage show better salt tolerance at maturity (Ahmadi and Ardekani, 2006). In the present investigation it was found that fresh weight of leaves decreased in all the genotypes with the progressive increase in the level of saline irrigation. Similar findings reported (Kandil *et al*., 2012) that is the decrease in fresh weight of leaves with the increase in level of salinity. Rahman *et al*. (2008) reported the decrease in length of wheat genotype which ultimately causes the decline in fresh weight of leaves.

Proline

In present investigation proline content increased with increase in salinity level. It observed that when wheat plants irrigated with saline water causes the increase in proline content as compared with control plants. Similar increase in proline content with the increase in salinity level reported in wheat (Mandhania *et al*., 2010). Datta *et al*. (2009) observed the enhanced level of proline accumulation in plants under NaCl treatments from 25mM to 100 mM. Similar increase in proline content has been reported several workers in wheat (Greenway *et al*., 1980). Similar results and suggested that this rapid proline accumulation appeared to play a positive role rather than to be a cause of growth failure. Our results are in accordance with the results shown in mungbean (Zayed and Zeid, 1997-98 and Nandwal *et al*., 2000 a,b). The enhanced level of proline in the leaves of the salt treated plants may be due *de novo* synthesis or from breakdown of proline rich proteins during stress. Besides this, proline has been shown to protect

plants against free radical induced damage by quenching of singlet oxygen.

Total soluble carbohydrate

In the present investigation increase in the content of total soluble carbohydrates was observed. Table of total soluble carbohydrate evinces the increase in total soluble carbohydrate content in leaves of wheat genotype under saline irrigation. The highest level of TSC was observed in genotype WH1081 (37.97%) and least in WH1025 (27.55%) at highest level of salinity 7.5 dSm^{-1}, among all the genotypes over their respective control values. The significant increase in amount of TSC was observed in wheat varieties Kharchia and Ghods with increasing level of salinity (Javed, 2002, Hajihashemi *et al*., 2006, Naureen and Naqui, 2010). TSC were studied under NaCl stress in wheat genotypes LU-26 and Potohas and observed similar trend with increasing level of salinity. Increase in TSC and reducing sugar under salt stress were also reported (Liu and Staden, 2001). Similar result were obtained in wheat (Asha, 2013).

Accumulations of compatible solute like soluble carbohydrate, polyols, amino acid, proline, glycine betaine which helps plant for osmotic adjustment in saline environment. These compounds do not interfere with normal biochemical reaction rather than replace water in biochemical reaction. Carbohydrate such as glucose, fructose, sucrose and starch accumulate under salt stress. Total soluble carbohydrate content increased in leaves of wheat genotype under saline irrigation. At 10 days after treatment, highest level of TSC was observed in genotype WH1081 at least in WH1025 at highest level of salinity 7.5 dSm^{-1}. (Naureen and Naqui, 2010) significant increase in amount of TSC was observed in wheat with increasing level of salinity. TSC content increased under NaCl stress in wheat genotypes (Javed, 2002, Hajihashmi *et al*., 2006).

Minerals content

The present investigation has revealed an accumulation of Na^+ with an increase in salinity level concomitant with a decline in potassium content in all the genotypes. However, the extent of accumulation varied with genotypes. Na^+ content increased more in C306 (110.78%) leaves and minimum in with increase in a salinity level while WH1025 (70.33%) showed least accumulation of Na^+. The transpiration rate and stomatal conductance is positively associated with intake of Na ions into the plant system (Senguttuvel *et al*., 2014). Conversely, K^+ content decreased with the increasing level rhizospheric salinity; the decrease being maximum in the genotypes C306 in leaves and least in WH1081 leaves. The increase Na+ content concomitant with a decline in K^+ content has been reported with the increase a salinity treatment in mungbean (Amarjani, 2010), chickpea (Dhingra *et al*. 1994). Similar findings were reported in leaf Na^+ concentration of all genotypes increased significantly with an increase in soil salinity and K^+ concentration were decreases (Asgari *et al*., 2012). Similar

results were observed in wheat also with the increase in salinity levels in wheat (Sairam *et al*., 2002 and Ghoghdi *et al*., 2012).

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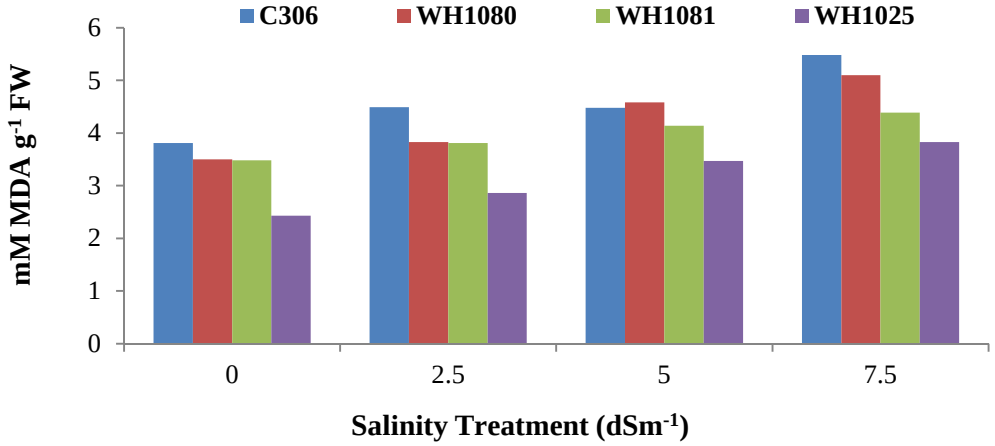

Fig. 1. Changes in the Lipid peroxidation in leaves of wheat genotypes under saline irrigation after 10 days of treatment

CD at 5% Genotype =N.S. Salinity =0.94 Salinity x Genotype =N.S.

International Journal of Research
Available at http://internationaljournalofresearch.org/
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 02 Issue 03
April 2015

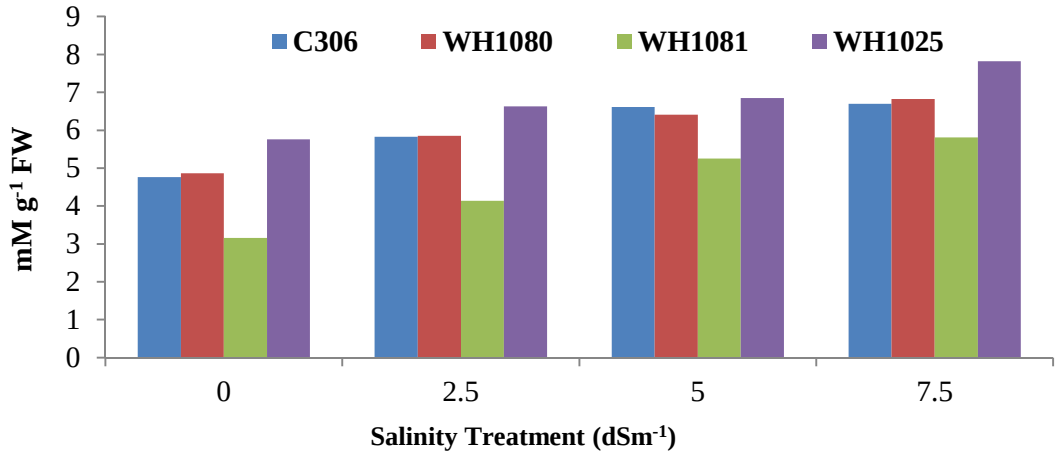

Fig.- 2. Changes in the proline content in leaves of wheat genotypes under saline irrigation after 10 days of treatment.

CD at 5% Genotype = 0.83 Salinity =0.83 Salinity x Genotype = 1.67

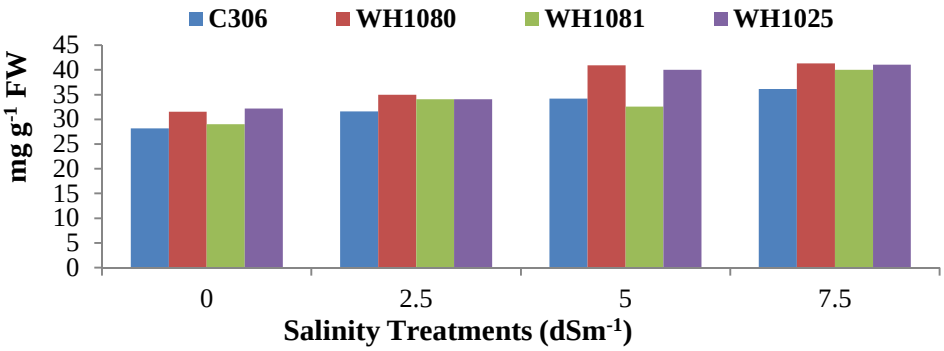

Fig.- 3. Changes in the total soluble carbohydrates (TSC) in leaves of wheat genotypes under saline irrigation after 10 days of treatment

CD at 5% Genotype = 1.56. Salinity = 1.57 Salinity x Genotype = 3.12

International Journal of Research
Available at http://internationaljournalofresearch.org/
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 02 Issue 03
April 2015

Table : 1 Changes in the osmotic potential (-MPa), relative water content(RWC%) and fresh weight content (mg) in leaves of wheat genotypes under saline irrigation after 10 days of treatments.

Genotypes	Salinity Levels (dSm^{-1})				
	0	2.5	5.0	7.5	Mean
	Osmotic Potential				
C306	0.94	1.16	1.14	1.19	1.11
WH1080	0.88	1.06	1.12	1.18	1.06
WH1081	0.80	1.00	1.08	1.08	0.99
WH1025	0.92	1.10	1.19	1.08	0.99
Mean	0.88	1.08	1.13	1.17	
CD at 5%	Genotype = 0.03		Salinity = 0.03	Genotype× Salinity = N.S.	
	Relative Water Content				
C306	91.33	91.95	87.69	85.11	89.02
WH1080	97.12	96.87	91.90	86.36	93.06
WH1081	97.68	91.67	89.72	85.99	91.26
WH1025	95.54	95.56	88.67	87.14	91.73
Mean	95.42	94.01	89.49	86.15	
CD at 5%	Genotype = 1.49		Salinity = 1.49	Genotype × Salinity = 2.99	
	Fresh Weight				
C306	59.45	51.16 (-13.94)	46.35 (-22.03)	43.65 (-26.57)	50.15
WH1080	51.95	49.79 (-4.15)	45.64 (-12.14)	38.58 (-25.73)	46.49
WH1081	64.53	49.34 (-23.53)	45.96 (-28.77)	47.27 (-26.74)	51.77
WH1025	68.46	64.04 (-6.45)	63.32 (-7.50)	50.31 (-26.51)	61.53
Mean	61.10	53.58	50.32	44.95	
CD at 5%	Genotype = 1.93		Salinity = 1.93	Genotype× Salinity = 3.87	

Values in the parenthesis indicate the percent decrease over the respective control

Table : 2 Changes in the sodium and potassium content (mg g^{-1} DW) in leaves of wheat

genotypes under saline irrigation after 10 days of treatments.

Genotypes	Salinity Levels (dSm^{-1})				
	0	**2.5**	**5.0**	**7.5**	**Mean**
	Sodium Content				
C306	2.55	4.97 (94.90)	4.82 (89.01)	5.37 (110.78)	4.42
WH1080	3.00	3.55 (18.33)	5.32 (77.20)	5.41 (80.19)	4.32
WH1081	2.68	3.77 (40.67)	4.40 (64.36)	4.56 (70.33)	3.85
WH1025	4.78	5.52 (15.48)	5.26 (64.36)	5.40 (70.33)	5.24
Mean	3.25	4.45	4.95	5.19	
CD at 5%	Genotype = 0.22		Salinity = 0.22	Genotype × Salinity = 0.45	

genotypes under saline irrigation after 10 days of treatments.

International Journal of Research
Available at http://internationaljournalofresearch.org/
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 02 Issue 03
April 2015

Potassium Content					
C306	29.45	24.90 (-15.44)	22.95 (-22.07)	22.50 (-23.59)	24.95
WH1080	23.80	22.65 (-4.83)	21.05 (-11.05)	16.20 (-31.93)	20.90
WH1081	20.15	20.65 (2.48)	17.85 (-11.41)	19.30 (-42.18)	19.48
WH1025	24.05	24.50 (1.87)	24.00 (-207.90)	20.95 (-12.68)	23.37
Mean	24.36	23.17	21.46	19.73	
CD at 5%	Genotype = 1.02		Salinity = 1.02	Genotype × Salinity = 2.04	

Values in the parenthesis indicate the percent changes over the respective control