ALLEVIATION OF SALT STRESS BY K_2SO_4 IN TWO WHEAT (*TRITICUM AESTIVUM* L.) CULTIVARS

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Abstract. Salinity is a major abiotic stress which adversely affects productivity of all crops in the world specifically in cereals. Different strategies are being utilized to enhance the overall plant growth and productivity all over the world. The crop nutrients management is one the best options to increase the plant productivity in saline soils. The present study investigated the influence of potassium sulphate (K_2SO_4) in improving plant productivity and nutrient uptake in wheat grown under saline environment. Two wheat genotypes were subjected to different concentrations i.e., 0, 50, 100, 150 and 200 mM of K_2SO_4 grown at 0, 150 mM sodium chloride stress. The use of K_2SO_4 increased the fresh and dry plant biomass of both wheat genotypes with a maximum increase at 200 mM K_2SO_4 under saline and nonsaline conditions. It was observed that the uptake and accumulation of nutrients like calcium, magnesium, potassium and phosphorus increases in plants subjected with K fertilizer application under saline environments in both wheat genotypes.

Keywords: *potash fertilizer, biomass production, salinity, nutrients uptake*

Introduction

Wheat is the most important among the cereal grain crops in nutrition supply (Kausar and Gull, 2014a) of ever increasing world population. Its role has been increased exclusively in developing countries of the world where it is used as a staple food. The rain fall 30-90cm, 20-30C° temperature and clay loam soils are required to have optimum wheat grain yield (Kausar and Gull, 2014a). Adequate water supply and suitable potassium fertilization are mandatory for optimal shoot and proliferation of root for better uptake of nutrients (Ashraf et al., 2013; 2015).

Soil salinity is the major abiotic stress observed all over the world which causes severe crop productivity losses by affecting nutrient uptake to maintain proper metabolic activities (Ashraf et al., 2012; Kosova et al., 2013; Zafar et al., 2015). Due to

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soil salinity billions of dollars losses in crop productivity have been reported every year (Alam and Naqvi, 2003; Ashraf et al., 2003, 2009, 2011, 2015). Uptake of toxic ions by plants growing on saline soils disturb their metabolic activities and concentration of toxic ions like Na^+ and Cl^- increases inside the tissues consequently water potential decreases that reduces growth and productivity of plants (Akhtar et al., 2013; Kausar and Gull, 2014b). The increased concentrations of toxic ions decreases the absorption of essential nutrients like calcium, megnessium, phosphorus, potassium andiron (Marjan et al., 2012; Han et al., 2014) and as a result plants suffer from nutritional imbalance (Ashraf et al., 2013). The development of plants, mainly depends upon the rate of photosynthesis which is adversely affected by salt stress, especially sensitive genotypes of all crops (Kausar et al., 2012; 2015). Other investigators have also reported that salinity stress adversely affects the growth, physiological and biochemical attributes of plants (Ebrahimi et al., 2012; Ashraf et al., 2013; Hasanuzzaman et al., 2013; Zafar et al., 2015). The decrease in plant growth and yield depend on intensity of salt stress (Kausar and Gull, 2014b).The deficiencies in essential nutrient uptake and reduction in K^+/Na^+ is observed in plants growning on saline soils, however, nutrient management may be effective in achieving economical crop productivity from these soils (Ashraf et al., 2014). The potash fertilizer is best option among others particularly under saline conditions (Ashraf et al., 2013). Potassium is an essential element for plant growth because it plays a key role in regulation of plant metabolic activity as well as physiological and biochemical requirements of plants (Kausar and Gull, 2014a). Its role as macronutrient is very important in many enzymatic reactions and high concentrations are required for the best growth of plants (Ashraf et al., 2013). Activation of many enzymes, opening and closing of stomata, photosynthesis and tropism movements are controlled by potassium (Golldack et al., 2003; Kausar and Gull, 2014a). It also controls in certain cases the osmotic adjustment in plants under various stressed conditions (Kausar et al., 2012). It is well known fact that potassium deficiency closes the stomata openings which in turn reduces the rate of photosynthesis in many crops, so its adequate amount is necessary for better growth and more wheat production (Mesbah, 2009; Ashaf et al., 2015).

Keeping in view the significance of potassium in saline environments, the current study was planned to test hypotheses; if potassium alleviates the negative effects of salt stress in wheat cultivars as well as if application of K_2SO_4 enhances the wheat growth and nutrient uptake in wheat under saline conditions.

Materials and Methods

The study was completed in GC University Faisalabad and GC Women University Faisalabad Pakistan in collaboration with the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan in 2013-2014. The seeds of two cultivars of wheat i.e., MILLAT-11 and NAYAB-11 were obtained from Wheat Section, Ayub Agriculture Research Institute Faisalabad, Pakistan. Pots of 35 cm in diameter were filled with washed river sand (10 kg) and arranged in a completely randomized design (CRD) with four different treatments of K_2SO_4 having four replications. The seeds of two cultivars of wheat were subjected to 150mM NaCl stress in half strength of Hoagland nutrient solution except control. After two weeks of plant sowings, potassium sulphate at 0, 50,100,150 and 200 mM was applied (*Table 1)*. The watering was done as and when required. Physico-chemical characteristics of water used in this study are summarized in *Table 2*.

***Table 1**. Levels of K_2SO_4 (Potassium sulphate) and NaCl (150mM) stress*

Treatments	NaCl Concentrations	K_2SO_4 Concentrations (mM)
T_0 (control)	0 mM	0
T_1	150 mM	0
T_2	150 mM	50
T_3	150 mM	100
T_4	150 mM	150
T_5	150 mM	200

***Table 2.** Physiochemical characteristics of water used in the experiments*

Water characteristics	Readings	Water characteristics	Readings
PH	7.66	CO_3 (meq L^{-1})	-
EC(µS/cm)	681	Na^+ (mg Kg^{-1})	60
Mg (meq L^{-1})	5	Cl^- (meq L^{-1})	5-6
Ca(meqL^{-1})	4	K^+ (mg Kg^{-1})	3-4.0
HCO_3 (meq L^{-1})	4-6		

After 120 days, five plants were collected and their height, fresh and dry biomass were recorded and their means were calculated. Then the plants were oven dried at 65ºC and dry biomass was calculated. The dried ground plant material was used for nutrient analysis of potassium (K), sodium (Na), calcium (Ca), nitrogen (N) and phosphorus (P) of both cultivars.

The dried plant material was ground and digested according to the method of Wolf, (1982). Filtered aliquate was used to determine Na, K, Ca, K, N and P. Sodium (Na) and K were determined using Flame-photometer (Jenway PFP 7, UK); while Ca and Mg were determined titrametrically (Jackson et al., 1962). Chloride contents were analysed by chloride meter (920, Corning, UK), total phosphorus (P) was determined spectrophotometrically (Jackson, 1962). In the case of phosphorous Barton's reagent was used sepectrophotometerically (U2800, Hitachi, Japan) as described by Jackson (1962). Total nitrogen was measured by micro–Kjeldhal method (Bremner, 1965). The fresh material was taken from third plant leaf and total soluble proteins were determined by Lowery et al. (1951) method. Similarly total free aminoacids were found by the method of Hamilton and Van slyke (1943).

Data was collected and analysis of variance technique was used to determine the significant variations in treatments, varieties and their interactions. Means were compared using DMRT test at 5% probability level (Steel et al., 1997).

Results

Two cultivars (MILLAT-11 and NAYAB-11) of wheat were used and it has been observed that salt stress has significantly affected growth of both the cultivars (*Fig. 1a*). Maximum plant height in both the cultivars was observed under normal conditions, however, salinity stress significantly reduced this parameter. Application of K_2SO_4 improved plant height of both cultivars of wheat under salt stress and the maximum improvement was noted at 200 mM K_2SO_4 level. Minimum plant height was recorded under NaCl salinity stress (*Fig. 1a*). The cultivar NAYAB-11 exhibited maximum plant height at 200 mM K_2SO_4 under salt stress followed by 150, 100 and 50 mM K_2SO_4. The fertilizer application of 200mM of K_2SO_4 proved better than other applications of potash fertilizer under salt stress conditions. Interaction between cultivar and treatment was also significant. Both wheat cultivars exhibited a decrease in plant height in salt stressed soils as compared to normal environments (*Fig. 1a*).

Wheat cultivar NAYAB-11 had higher fresh biomass than cultivar MILLAT-11 both under non saline and saline conditions. Salt stress negatively affected the fresh biomass production in both the cultivars of wheat, however, it varied subject to application of different levels of potassium (*Fig. 1b*). The maximum plant fresh biomass was observed under 200 mM K_2SO_4 treatment as compared to its other treatments under salt stress conditions. Cultivars and treatments interactions showed highly significant results for both wheat genotypes (*Table 3*).

Table 3. *Mean square values from analysis of variance (ANOVA) of data for plant height, fresh and dry weights and calcium contents of plants at different levels of K_2SO_4 of two cultivars of wheat under 0 and 150 mM of NaCl stress.*

Source of variation	**df**	**Plant length**	**Plant fresh weight**	**Plant dry weight**	**Plant Ca^{2+} contents**
Treament	5	201.27616***	1.0599983*	0.3070601 ns	37.035592***
Cultivars	1	301.659***	2.1364694*	0.2669444 ns	51.612251**
Treamentx Cultivars	5	280.86374***	3.3955494***	0.7095045 ns	96.639862***
Error	24	9.3421417	0.3406444	0.2909989	5.1284646
Total	35				

*** , **, * , ns = Significant at 0.05, 01, .001 %, non-significant respectively

Results indicated that maximum dry weight was attained by plants growing under normal conditions in both cultivars of wheat. Salt stress adversely affected the plant dry weights in both genotypes (*Fig. 1c*), however, MILLAT-11showed more reduction in dry biomass than NAYAB-11. The addition of K_2SO_4 increased plant dry weight under sodium chloride stress (*Fig. 1c*), with maximum increase at 200 mM K_2SO_4. Sodium chloride stress increased sodium and chloride contents in all cultivars of wheat. Plants grown in saline environments showed a noticeable increase in Na^+ uptake however, wheat cultivar MILLAT-11 had higher Na^+ than NIAB-11(*Fig.1d*). Minimum concentrations of Na^+ were present in plants grown under controlled conditions in both cultivars. The plants of both the wheat cultivars under salt stress, also exhibited higher

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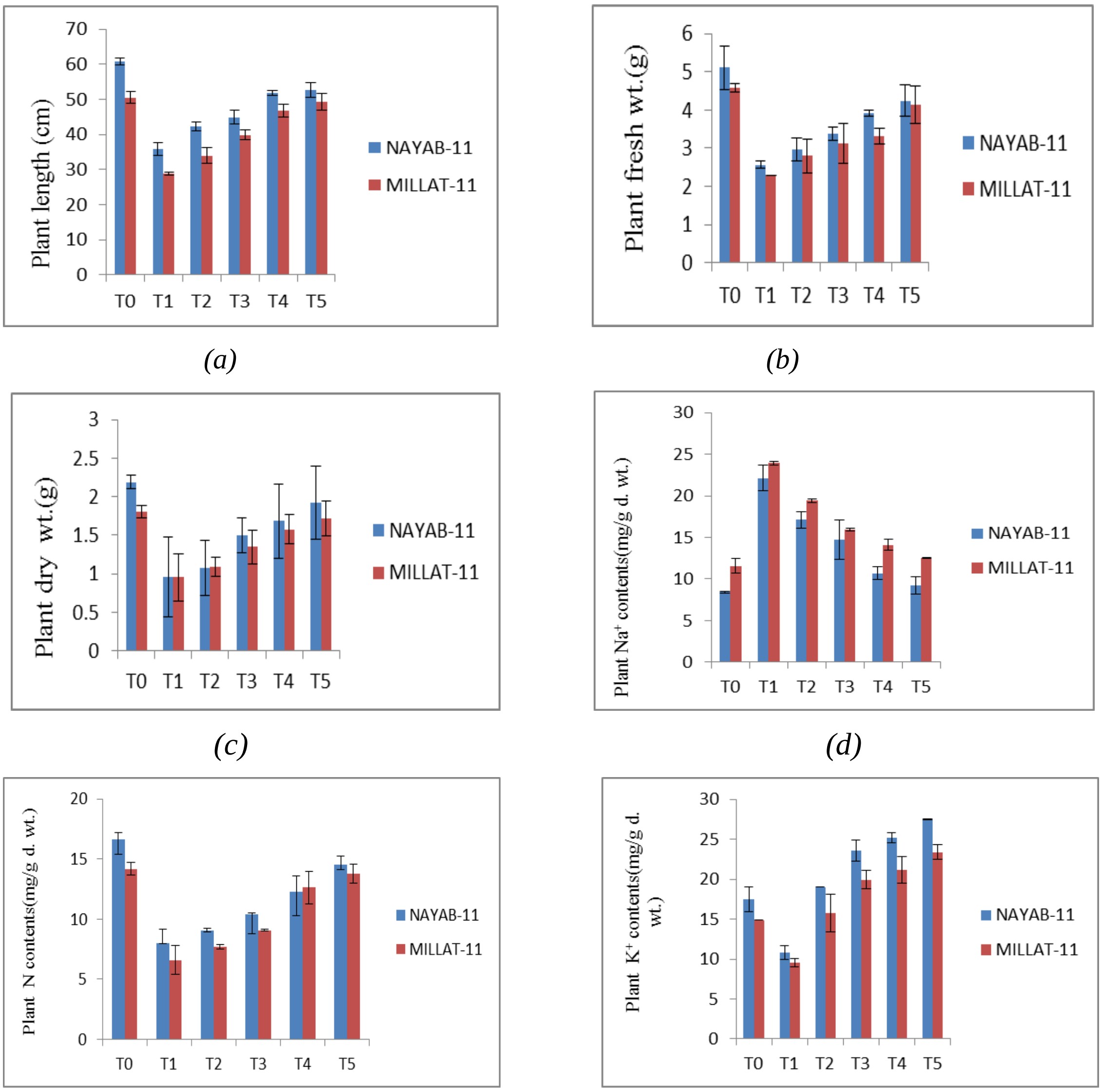
Cl^- concentrations than those growing under normal conditions (*Fig. 2 c*). Application of K_2SO_4 reduced sodium contens in NAYAB-11 more adversely than MILLAT-11 (*Fig. 1d*). Interactions between treatments and cultivars presented highly significant performances in wheat cultivars (*Table 3*). Accumulation of Na^+ (*Fig. 1d*) and Cl^- (*Fig. 2c*) decreased with increasing K_2SO_4 levels in salt stress environments.

Salinity adversely affected the N contents in both wheat cultivars. Maximum N content was noted in plants grown in controlled environment, however, cultivar NAYAB-11 had higher N than culivar MILLAT-11 (Fig 1e). Application of K_2SO_4 significantly enhanced the uptake of N. Maximum N uptake was recoded at 200mM and the minimum at 50 mM K_2SO_4 in salt stressed plants of both cultivars of wheat (*Fig. 1e*). Maximum reduction in N (52%) was recorded in the plants growing at 150 mM sodium chloride level, while minimum reduction about 24% was in the plants treated with 200 mM K_2SO_4 in both the cultivars of wheat under NaCl stress (*Fig 1e*).



(a) *(b)*

(c) *(d)*

(e) *(f)*

***Figure 1**. Effect of different levels of K_2SO_4 on plant length (a), fresh weight(b), dry weight (c) Na^+ (d), N (e) and K^+ (f) contents in plants of two wheat cultivars at 0 and 150 mM of NaCl stress*

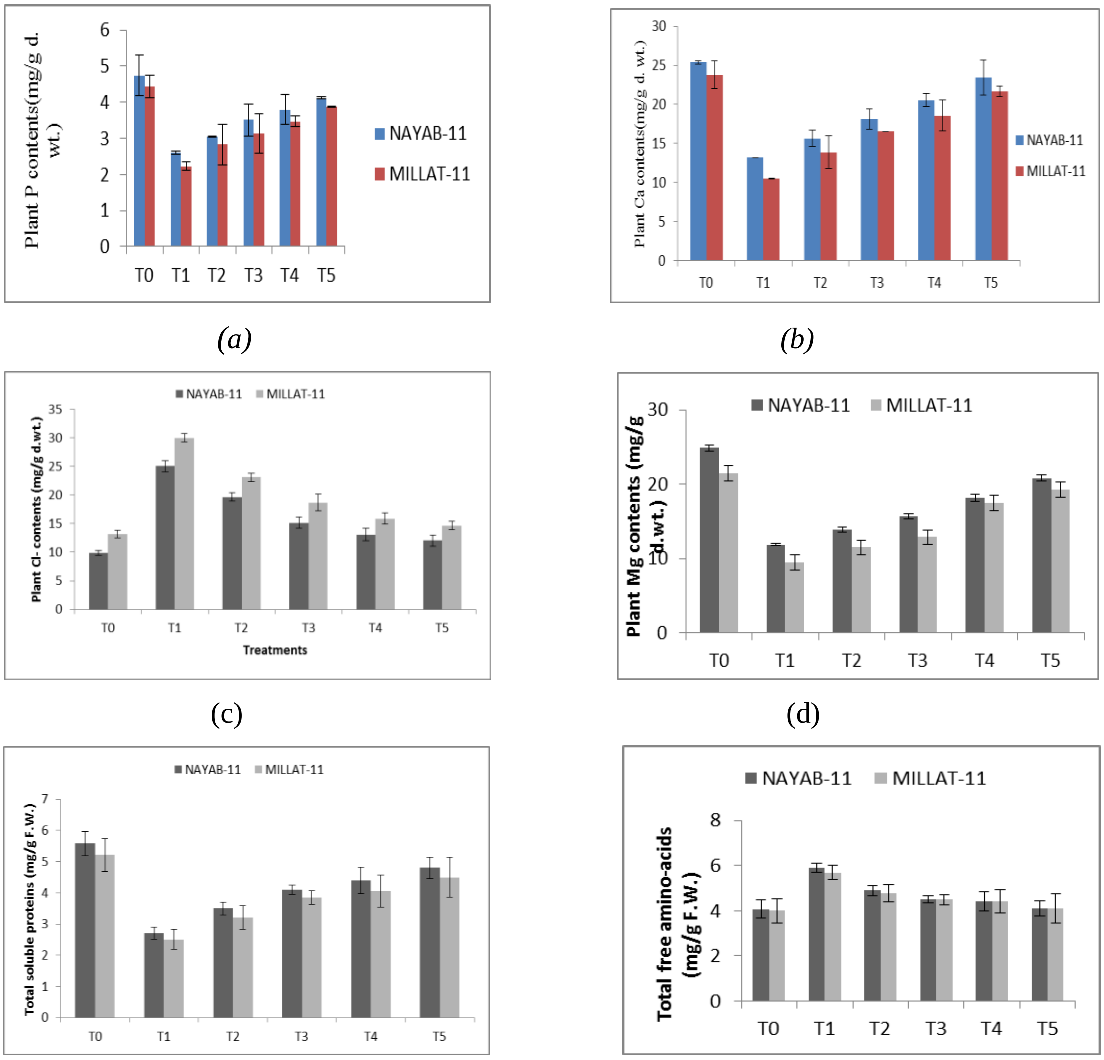
Analysis of variance of the data indicated that K^+ contents significantly decreased in both wheat cultivars growing under saline environments. Maximum decrease in K^+ was noted in plants of cultivar MILLAT 11 and the least in NAYAB 11 growing under normal conditions. Potassium uptake increased with the application of K_2SO_4 fertilizer on wheat plants under salt stress, but the minimum increase was exhibited by wheat cultivar MILLAT 11 (*Fig. 1f*). Similarly, maximum reduction in K (37.5%) was observed in plants growing at 150 mM NaCl, however, an increase in K uptake was noted at 200 mM K_2SO_4 under NaCl salinity stress (*Fig. 1f*). Under salinity stress, plants of both wheat cultivars maintained lower shoot K^+ contents. Wheat cultivars responded positively to K_2SO_4 fertilizer. Adverse effects of saline medium were also detected on P uptake (*Fig.2a*). On addition of K_2SO_4 plants exhibited an increase in the uptake of P under NaCl salinity stress in both NAYAB 11 and MILLAT 11. Wheat Cultivar MILLAT-11 showed the least uptake of P as compared to NAYAB-11. Maximum reduction (54.5%) in P uptake was observed at 150 mM of salt stress, however, the least reduction (18.2%) was observed at 200 mM K_2SO_4 under saline conditions (*Fig. 2a*).

***Table 4.** Mean square values from analysis of variance (ANOVA) of data for phosphorus (P), potassium (K^+), sodium (Na^+) and nitrogen (N) contents at different levels of K_2SO_4 in wheat cultivars under NaCl stress*

Source of variation	**df**	**Plant P contents**	**Plant K^+ contents**	**Plant Na^+ contents**	**Plant N contents**
Treament	5	0.7363912 ns	169.75993***	169.75993***	24.917318***
Cultivars	1	1.9904507*	0.0879219 ns	0.0879219 ns	25.662667**
Treament x Cultivar	5	2.602809***	36.436648***	36.436648***	37.879022***
Error	24	0.3628611	4.0945417	4.0945417	3.1693667
Total	35				

*** , **, * , ns = Significant at 0.05, 01, .001 %, non-significant respectively

Application of K_2SO_4 has improved the Ca^{2+} and Mg^{2+} contents under saline medium, however, maximum was in the plants growing in normal environment in both cultivars (*Fig 2b*). The contents increased with increasing level of K_2SO_4 fertilizer. The maximum Ca^{2+} content was observed at 200 mM K_2SO_4 in NIAB-11followed by MILLAT-11 under NaCl stress as compared to other treatments of K_2SO_4 (2b). Interactive effect of cultivars and treatment showed non significant behavior for Ca^{2+} content (*Table 5*). An increase in nutient uptake like K^+, Ca^{2+}, Mg^{2+}, N and P was observed by addition of K_2SO_4 fertilizer in plants of both the cultivars grown under saline conditions. The increase was further prominent with an increase in K_2SO_4 levels. The increase was further prominent with an increase in fertilizer application. The application of salinity decreased significantly the total proteins and increased the total free aminoacids in both wheat cultivars. However with the utilization of fertilizer there was less decrease in total proteins (*Fig. 2 e)* and total free aminoacids and with the increasing concentrations of potash fertilizer (*Fig. 2 f*).



(a) *(b)*

(c) (d)

(e) *(f)*

Figure 2*. Effect of different levels of K_2SO_4 on phosphorus (a) calcium (b), chloride (c)and magnesium (d), total soluble proteins and (total free aminoacids) of plants of two wheat cultivars at 150 mM of NaCl stress*

Table 5*. Mean square values from analysis of variance (ANOVA) of data for chloride (Cl^-) and magnesium(Mg^{2+}) contents at different levels of K_2SO_4 on two wheat cultivars under 150 mM of NaCl stress*

Source of variation	**df**	**Plant Cl^- contents**	**PlantMg $^{2+}$contents**	**Total soluble proteins (mg/g F.W)**	**Total free aminoacids (mg/g F.W)**
Treament	5	109.55973***	27.031512***	4.16732***	4.76432**
Cultivars	1	0.0679228 ns	41.212351**	5.43261***	5.32451**
Treament x Cultivar	5	30.336628***	86.539162***	2.9245**	1.45621*
Error	24	3.0744515	3.1274646	1.09821	0.12431
Total	35				

*** , **, * , ns = Significant at 0.05, 01, .001 %, non significant respectively

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Discussion

Environmental stresses are the main problem to plant growth and development and salinity is one of the major stresses causing severe losses in crop productivity all over the world (Hakeem et al., 2013; Zafar et al., 2015). However, there are other environmental factors like; drought, water logging, heavy metal toxicity and low or high temperatures, which are also involved in decreasing the growth and productivity of crops (Bray et al., 2000; Hakeem et al., 2013). In the present study fresh and dry biomass decreased by salinity stress (*Fig. 1b and c*) as indicated by many investigators (Ashraf et al., 2011; Ali et al., 2012; Kausar et al., 2012; Kausar and Gull, 2014b). The reason for this could be due to the lowering of stomatal conductance, fixation of carbon dioxide, and disturbance in biochemical reactions/activities (Ashraf et al., 2011; Ashraf et al., 2013, 2015). The application of K_2SO_4 alleviated the adverse effect of salinity stress on the plant height and was effective in enhancing the growth of wheat in stress medium (*Fig. 1a*). Similar results have been reported by Tzortzakis (2010) and Ashraf et al. (2013), who showed that application of potash fertilizer decreases the toxic effects of salinity on growth, as well as plant biochemical and physiological processes.

It is a well known fact that use of K_2SO_4 in saline soils is effective in having high fresh biomass production in many crops (Hussain et al., 2013; Saffa et al., 2013; Ashraf et al., 2015), because of the activation of some enzymes necessary for plant growth. Addition of K_2SO_4 in growth medium is an excellent way for working of enzymes by mainting pH required for proper growth of plant cell (Hussain et al., 2013, Saffa et al., 2013; Ashraf et al., 2015). A reduction in the biomass and uptake of essential nutrients under salt stress may be due to the presence of excessive Na^+ and Cl^- concentrations in the growth medium (Hussain et al., 2013; Saffa et al., 2013; Ashraf et al., 2015). Similarly a reduction in dry weight of the plant due to salt stress can be alleviated by an application of potash fertilizer in order to mitigate the toxic effects of salts (Kausar et al., 2012; Kausar et al., 2014; Ashraf et al., 2013). These findings are in agreement with those of other workers (Tzortzakis, 2010; Kausar et al., 2012, Ashraf et al., 2013). In saline conditions the uptake, absorption and accumulation of toxic ions (Na^+ or Cl^-) are enhanced which reduce the uptake and translocation of essential nutrients in plants resulting in a decrease in leaf water potential, rate of photosynthesis, growth and overall plant productivity (Ali et al., 2012; Hussain et al., 2013, Saffa et al., 2013).

Soils affected by salinity are in need of more nutrient uptake predominantly P, Mg^{2+} and K^+, which play key role in physiological and biochemical processes (Kausar and Gull, 2014a). Development of plant depends particularly on the accessibility of appropriate amounts of potassium fertilizer (Kausar and Gull, 2014b), which improves plant growth and productivity (Hussain et al., 2013, Saffa et al., 2013) especially in the plants facing salt stress. These findings too are in accordance with those of Ashraf et al. (2003, 2015), and Kausar et al. (2014). They showed that N and K^+ contents decrease in stressed environment and with the application of nutrients like potassium, the uptake of essential nutrients i.e., Ca^{2+} , K^+ and Mg^{2+} can increase followed by proper regulation of all metabolic activities, and resulting in an improvement of growth and productivity. Tzortzakis (2010) and Ashraf et al. (2015) also state that K^+ is a vital nutrient and growth of plants depends upon its availability. Both wheat cultivars showed positive response to K_2SO_4 and absorption of potassium nutrients (*Fig. 2 a, b and d*), as these enhance growth leading towards an improvement in fresh and dry biomass production. These results are in agreement with those of Ashraf and Sarwar (2002); Ashraf et al.

(2003, 2013); Golldack et al. (2003); Hussain et al. (2013); Saffa et al. (2013) and Kausar and Gull (2014b).

In the present study, potassium sulphate was used to alleviate the negative effects of salt stress and an improvement in plant height, fresh and dry weights was recorded in both wheat cultivars. Positive influence of potassium on plant growth and productivity under saline environments has been reported by Rashid et al. (2001) and Hussain et al. (2013) as well. Indirect evidences are also available indicating that potassium and sulphate have central role in the construction of some important proteins (Khan et al., 2008; Khan et al., 2010), which are necessary to impart stress tolerance in plants (Lee et al., 2009). Our findings too have confirmed that K_2SO_4 increases both growth and productivity of wheat plants by increasing the absorption of essential nutrients in saline and non saline medium. On the bases of earlier and present findings K_2SO_4 application in saline soils can be recommended to alleviate the adverse effects of salinity and to have economical crop yield.

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