# COMPARATIVE ANALYSIS OF VARIOUS GROWTH, IONS UPTAKE AND OTHER PHYSIOLOGICAL PERFORMANCE OF TWO RICE (*Oryza sativa* L.) GENOTYPES UNDER ALTERING SALINE CONDITIONS

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**A R T I C L E   I N F O**

**A B S T R A C T**

Soil salinity is a severe threat among various abiotic stresses that lowers down the growth and productivity of agricultural crops throughout the world due to the excessive use of poor quality irrigation water. Tolerance of plants involves various physiological, biochemical, and metabolic pathways, and a network of genes to express in saline soils. The present study was conducted to assess the genetic potential of two rice cultivars (Super Basmati and Basmati 515) in altering saline conditions i.e., 0, 40, 80 and 120 mM of NaCl. The data was recoded for height, fresh and dry weight of the plant, sodium, potassium, calcium, magnesium, phosphorus contents, total free amino acids and total proteins. The results showed that increase in salinity reduced the growth parameters in both cultivars of rice. The reduction was more prominent at higher concentrations of soluble salt. The application of salt stress also negatively affected the uptake of sodium, potassium, calcium, magnesium and phosphorus contents, and total proteins in contrast with total free amino acids that were increased with the application of salt stress. Basmati 515 showed better growth than Super Basmati under saline and non-saline conditions. Therefore, Basmati 515 could be suggested for planting to get better performance under salt-affected soils.

## Introduction

Soil salinity is one of the severe threats among abiotic stresses that disturbs the plant productivity worldwide due to the excessive use of poor quality or saline irrigation water [1, 2]. Total saline soils cover about 953 million hector area which is about 8 % of the surface of land [3, 4]. It has been evaluated that nearly 900 million ha of land has been damaged in all over the world, in which the cultivated area is about 20 percent, and the remaining area has been irrigated [5].

 It has been a well-established fact that salt stress reduced the growth and productivity of plants [6-8]. Salinity causes the accumulation of salts as a consequence of ionic and osmotic imbalance [9] resulting in lowering of water potential and osmotic potential in plants [10]. The development of plants relies on the rate of photosynthesis and uptake of nutrients from the soil. The increased concentrations of sodium and chloride ions ultimately decrease the uptake of essential nutrients [11]. All abiotic stresses like drought and salinity that adversely affect plant growth and development have a negative impact on the photosynthesis and uptake of these nutrients, which become suppressed under saline conditions [12, 13]. The lowering in photosynthesis rate directly results in the discount in the plant biomass under salinity [14, 15]. Different techniques have been used to increase the production of crops in the saline conditions; however, the screening of salt tolerant genotypes and the use of these genotypes in salt affected area has been one among the best options for getting higher crop productivity.

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Rice has a significant place in the universal food system because above than half of the population of the world consume rice in their daily food [16] as staple food. The production of rice has been estimated to be 8.9 billion in 2030 [17]. The rice seeds are commonly used for industrial purposes such as starch and beverages production. The hay of rice is used for feeding cattle. The straw of rice is also used for making mats. The Pakistani varieties like basmati and super basmati are famous throughout the world especially for the length of seeds and their fragrance [18]. The dry soil condition in Pakistan and other countries coupled with micronutrient deficiency has been the main problem throughout the world. Water scarcity and shortage of labor have been causing decline in the rice production [19, 20]. The rice transplantation requires mandatory water logging environments for its maturation. Due to the problem of water limitations, a technique has been developed to call adaptation to the aerobic rice [21]. The rice crop grown in aerobic environments showed a significant reduction in the production of grain and tillers. Keeping the significance of rice and salt stress in view, the current study was planned to observe the effect of salinity stress on the behavior of two rice cultivars for salt stress, and determine the level of uptake of essential nutrients and accumulation of metabolites in saline environments for having more production in salt-hit areas of the world.

## Material and Methods

### Rice genotypes collection and growth experiments
Two cultivars of rice Basmati 515 and Super Basmati were analyzed in this study. After 40 days of sowing, the rice, the nursery plants were transplanted into plastic pots, each containing 8 kg clay loamy soils. Two cultivars of rice Basmati 515 and Super Basmati were subjected to different levels of salt (NaCl) stress. The pots were designed in a completely randomized design (CRD) with three replications of each.

### Salinity treatments
The treatments of salt stress were subjected after fifteen days of transplantation of plants. The rice cultivars i.e., Super Basmati and Basmati 515 were grown in 0 mM, 40 mM, 80 mM and 120 mM NaCl salt stress which were provided in a split form.

### Soil analysis
For the purpose of soil analysis, the soil was first grinded well then it was dried on a sheet under the sun light. A 300 g sample of soil was taken in a plastic beaker, and distilled water was added with continuously stirring with the help of spatula until a homogenized paste of soil was formed. Then the paste was used to obtain the extract by a soil extractor. This soil extract was used for various further analyses (Table 1).

**Table 1:** Physiochemical characteristics of soil and water used for growth of different rice cultivars (*Oryza sativa* L.) for the analysis of their salinity tolerance potential

| Soil characteristics | Mean readings | Water characteristics | Mean readings |
|---|---|---|---|
| Soil texture | Clay loam | EC( $\mu$S/cm) | 684 |
| EC( dS m$^{-1}$) | 0.9 - 1.4 | pH | 7.77 |
| pH | 7.52 | Ca$^{2+}$ (meqL$^{-1}$) | 5 |
| Ca (meqL$^{-1}$) | 5 | Mg (meq L$^{-1}$) | 5 |
| Mg (meq L$^{-1}$) | 4 | CO$_3$ (meq L$^{-1}$) | - |
| HCO$_3$ (meq L$^{-1}$) | 4-6 | Cl$^-$ (meq L$^{-1}$) | 4-5 |
| CO$_3$ (meq L$^{-1}$) | - | HCO$_3$ (meq L$^{-1}$) | 6 |
| K$^+$mg kg$^{-1}$ | 20 – 48 | K$^+$(mg kg$^{-1}$) | 3.0-4.0 |
| Cl$^-$ (meq L$^{-1}$) | 5-7 | Na$^+$(mg kg$^{-1}$) | 62 |

### Biochemical parameters
### Preparation of phosphate Buffer
The phosphate buffer (0.02M) was prepared by using NaH$_2$Po$_4$ (1M) and Na2HPo4 (1M) reagents. The 0.5 g material of the fresh plant was extracted with the phosphate buffer (10ml) at pH 7, and left for a night to estimate the total protein and amino acid contents.

### Total free amino acids
To measure the total free amino acids, the method of Hamilton and Van Slyke was used [22]. Briefly, 1 ml of the leaf extract was taken in a test tube of 25ml, and 1 ml of pyridine (10%) and 1 ml of ninhydrin (2%) were added in the test tube that containing the leaf extract. The test tubes were heated at 97$^0$C for 30 minutes on water bath,

till the color of the tube extract changed. After 20 minutes, when test tubes became cool, 10 ml of distilled water was added to all the test tubes for maintaining the volume. The optical density of the colored solutions was measured at 570 nm on spectrophotometer (Jenway 6700). The calculation was measured by using the following formula

Total free aminocids=Graph reading x volume of sample x dilution of factor/weight of tissues (g)x1000.

### Total soluble proteins

The total soluble proteins were calculated by the method of Lowry et al. [23]. The copper reagent was prepared to measure the total soluble proteins.

### Preparation of copper reagent

Solution "A" was prepared by dissolving 2.0 g $Na_2CO_3$, 0.2 g NaOH and 1.0 g sodium potassium tartarate in distilled water, and the volume of mixture was made 100 ml. Solution "B" was prepared by dissolving 0.5 g $CuSO_4.5H_2O$ in 100ml distilled water. Solution "C" (Copper reagent) was prepared by mixing 50ml Solution "A" and 1ml Solution "B". The leaf extract (1ml) taken from the test tube was mixed with 1 ml solution "C" and left for 30 minutes at room temperature. Then 0.5ml Folin-Phenol reagent was added in test tubes, and incubated at room temperature for 30 minutes. The optical density was measured at 620nm on spectrophotometer (Jenway, 6700).

### Ion analyses

The rice plants were grown for 120 days, and then uprooted. Their lengths and fresh weights were determined immediately, and then oven dried for seven days to carry out ions analysis.

### Digestion

The material for digestion was oven dried for 7 days. The rice plants' dried materials (0.5g) were taken in digestion tubes. Concentrated $H_2SO_4$ was added to the digestion tube, and left overnight at room temperature. The next day, 0.5 ml of 35% $H_2O_2$ was added, and the digestion tubes were transferred to the digestion block for heating. The tubes were heated at 350ºC until the fumes of $H^2O^2$ began to come out from the digestion tubes. The digestion tubes were then removed from the digestion block, and allowed to cool for 5 minutes. After cooling, 0.5 ml $H_2O_2$ was added. This procedure was repeated for five times, till the digested material became completely colorless. The digested material was filtered, and the volume was made 50 ml by the addition of distilled water. Aliquot was used for the determination of Na and K on flame photometer (Jenway, PFP7), and P was calculated from the digested leaves by Wolf method [24].

### Barton`s reagent preparation

Preparation of solution "A": Ammonium molybdate (25g) was dissolved in 400 ml water.

Preparation of solution B: Ammonium metavandate (1.25g) was dissolved in 300ml water. These two solutions were mixed thoroughly to prepare Barton`s reagent. Barton`s reagent of 2ml and aliquot 2ml were mixed, and the volume was made 50ml with water. After 30 minutes, the optical density of mixture was recorded at 470 nm on spectrophotometer (Jenway, 6700). Phosphorus estimation was determined from the standard curve.

### Statistical Analysis

All of the statistical analyses were performed with software package COSTAT (v. 6.3, Cohort software, California). The effect of salt stress on essential nutrient uptake and growth response of rice cultivars were calculated by multiway-analysis of variance (ANOVA) of data with the level of significance set at p≤0.05.

### Results

### Growth attributes

The plant height was reduced with increasing the concentrations of sodium chloride in both cultivars of rice (Fig. 1a). The varying salinity concentrations behaved differently. The maximum height of plants was estimated under normal conditions in both rice cultivars. The tallest cultivar was Super Basmati (59.02 cm) in non-saline conditions. However, the maximum reduction in height was measured at 120 mM, followed by 80 mM and 40 mM. The differences between height in normal and saline conditions were significant (Tab.2). The response towards salt stress was significant in both rice cultivars (Table 2). In salt stress conditions, the maximum height was recorded in Basmati 515 (52.67 cm) as compared to Super Basmati (51.48 cm). The cultivar interaction and treatments were non-significant which showed that the rice cultivars retorted similarly to the different levels of salinity. It was recorded that under non saline medium, the length of plants was more as compared to the stressed plants (Table 2, Figure 1a).

In both rice cultivars, the fresh and dry weights were negatively affected by saline conditions (Figure 1b, and 1c) at all the applied concentrations of sodium chloride. The varying salinity environments affected variably. The maximum fresh weight (16.82 g) of plants was determined at non-saline condition in both rice cultivars. However, the highest reduction was recorded at 120 mM (41 %) followed by 80 mM (29 %) and 40 mM (17%) salt stress in both rice cultivars. The effect of salinity on fresh and dry weights in non-saline and saline concentrations was found to be significantly different (Table 2). The utmost fresh and dry weight was estimated in rice cultivar Basmati 515, and the lowest was observed in Super Ba0smati. The interactions between the treatments and cultivars were non-significant, which presented the similar behavior of both cultivars of rice under the saline conditions. The data indicated that the rice cultivars exhibited the highest value of fresh and dry weight of rice plants under normal environments, however, the lowest value was recorded in salt stressed environments (Table 2).

**Table 2:** Records of the mean squares analysis of variance (ANOVA) of data for height, fresh and dry weight, and sodium and potassium contents of two rice cultivars (*Oryza sativa* L.) under altering saline conditions indicating the comparative analysis of growth parameters.

| S.V. | Degree of freedom | Plant height (cm) | Plant Fresh Weight (g) | Plant dry weight (g) | Sodium (mg g-1 D. W.) | Potassium (mg g-1 D. W.) |
|---|---|---|---|---|---|---|
| Rice cultivars (V) | 1 | 8.833 ** | 3.26** | 0.11** | 1.43 *** | 1.24* |
| NaCl treatments (S) | 3 | 233.87*** | 38.32*** | 0.01** | 177.71*** | 109.97*** |
| V x S | 3 | 0.233 ns | 0.13ns | 0.001$^{NS}$ | 0.006 ns | 0.066 ns |
| Error | 16 | 0.755 | 0.65 | 0.000 | 0.073 | 0.162 |
| Total | 23 | | | | | |

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively., Ns =Non-significant, D. W= Dry Weight



(a)
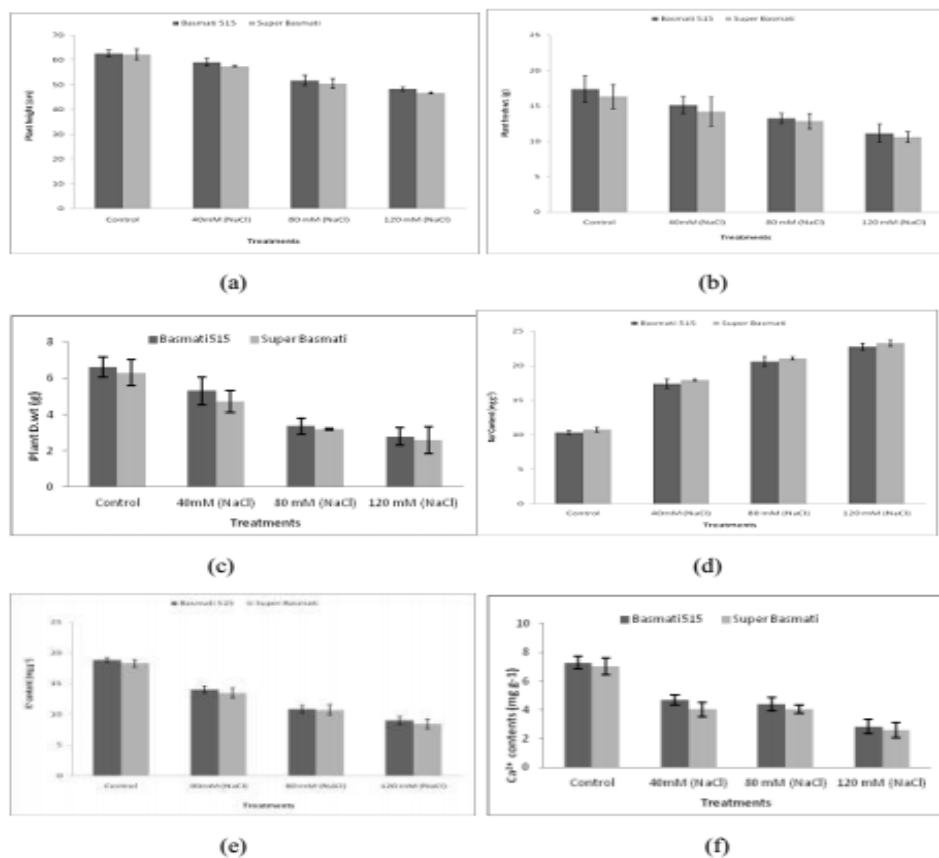
(b)

(c)

(d)

(e)

(f)

**Figure 1:** Plant growth parameters, plant height (a), fresh weights (b), dry weight (c) and essential nutrients uptake sodium (d), potassium (e) and calcium (f) contents according to the altering concentration and treatments with NaCl salinity in two rice cultivars. Data are means ±standard error of different independent experiments. Data with different letters are significantly different between the treatments at p≤0.05.

**Ion analysis**

Salt stress increased the concentrations of sodium in cultivars of rice (Figure 1d). The varying saline conditions affected differently. The maximum sodium contents (22.98 mg g-1 D.W.) were recorded in 120 mM of salt stress, followed by 40 mM (17.63 mg g-1 D.W) and 80 mM (20.81 mg g-1 D.W). While, the lowest value was noted under non- saline medium (10.52 mg g-1 D.W) in both rice cultivars. The difference between sodium contents in saline and normal medium was significant. The cultivar response for NaCl stress was also non-significant (Table 2). The variations among the sodium contents were non- significantly different in both cultivars of rice. However, the interactions for salt treatments and cultivars were also non-significant. It was noted that that both rice cultivars responded similarly to the salt stress. Ion analysis data showed that the highest sodium contents in both rice cultivars were found in saline medium as compared to the control (Table 2).

The salinity adversely affected the potassium accumulation in both rice cultivars (Figure 1e). Maximum potassium was accumulated (18.56 mg g$^{-1}$ D.W.) under non- saline conditions in both rice cultivars. In saline environments, the minimum accumulation was recorded at the 120 mM (57 %) NaCl salt stress followed by the treatment of 80 mM (47 %), and then 40 mM (26 %) in both cultivars of rice. The difference between potassium accumulation in non-saline and saline medium was also significant. The response against salt stress of both rice cultivars was also non-significant. The highest accumulation of potassium was recorded in rice cultivar Basmati 515 (13.16 mg g-1 D.W.), while the lowest was found in Super Basmati (12.72 mg g$^{-1}$). The interactions between salinity applications and rice cultivars were non-significant which showed that rice cultivars similarly responded to the salinity. It was observed that the maximum value of potassium contents was found in both rice cultivars under the control conditions as compared to that recorded in the NaCl salinity stress treatments (Table 2).

Salinity stress also negatively affected the calcium and magnesium ions uptake in both the cultivars of rice (Figure 1f and 2a, Table 3). The accumulations of essential ions were decreased with increasing salt concentrations, and the maximum was found in non-saline conditions in both rice cultivars. All levels of NaCl salinity stress adversely affected the phosphorous accumulation in both rice cultivars (Figure 2b). Maximum phosphorous was accumulated (6.03 mg g$^{-1}$ D.W.) under the normal environments in both rice cultivars. Under salinity stress, the maximum reduction was observed at 120 mM, which was about 75 % followed by 80mM (58 %), and 40 mM about 41 % in both rice cultivars. The difference between the accumulation of phosphorous content under non-saline and saline medium was also significant (Table 3). The maximum phosphorous accumulated in rice cultivar Basmati 515 (3.57 mg g$^{-1}$ D.W.), and the lowest was observed in Super Basmati (3.33 mg g$^{-1}$ D.W.). The variation in the phosphorous contents between rice cultivars was found non- significant. The interaction between salt treatments and the cultivars was also recorded non-significant which showed that both rice cultivars nearly performed similar behavior under salinity stress for phosphorous contents.

**Biochemical attributes**

Salinity decreased the total soluble proteins in both cultivars of rice (Figure 2c). The different levels of salinity affected differently the total soluble protein contents. The maximum total soluble proteins (13.52 mg g$^{-1}$ D.W.) were recorded under normal conditions. In distinction, the highest decrease was calculated at 120 mM (64 %) followed by 80 mM (42 %) and 40 mM (28 %) in both rice cultivars. The response of both rice cultivars toward NaCl stress was non-significant. The utmost total soluble protein was observed in Basmati 515 (9.58 mg g$^{-1}$ D.W.), and the lowest in Super Basmati (8.74 mg g$^{-1}$ D.W.). The difference between the rice cultivars for total soluble proteins was significant. The interactions between salt treatments and cultivars were also non-significant which exposed that both rice cultivars behaved similarly under the salt stress conditions. It was established that the rice cultivars presented the highest value of total soluble proteins in non -saline conditions in contrast to the salt stressed conditions (Table 3). The varying salt concentrations decreased the accumulation of total free amino acids in rice variously (Figure 2d). The maximum total free amino acids (10.55 mg g$^{-1}$ D.W.) were found under the salt stress level of 120 mM. In contrast, the lowest value of total free amino acids was observed in non- saline environments which was about 4.77 mg g$^{-1}$ D.W., followed by the 40 mM (7.11 mg g$^{-1}$D.W.) and 80 mM (9.19 mg g$^{-1}$ D.W.) in both rice cultivars.

The difference between total free amino acids in non-saline and the saline conditions was also significant. The response of rice cultivars for salinity stress was non-significant (Table 3). The lowest value was measured in Basmati 515 (7.66 mg g$^{-1}$ D.W.), and the maximum was observed in Super Basmati (8.15 mg g$^{-1}$ D.W.). The interactions between salt treatments and cultivars were also non-significant. It was shown that both rice cultivars

performed similarly under the salinity stress. It was noted that the maximum value of the total free amino acids was observed in salt stress conditions as compared to the non-stress conditions (Table 3).

**Table 3:** Records of the mean squares analysis of variance (ANOVA) of data for calcium, magnesium, phosphorus content, total soluble proteins and total free amino acids of two rice cultivars (*Oryza sativa* L.) under altering saline conditions.

| S.V | Degree of freedom | Calcium ($Ca^{2+}$) (mg g$^{-1}$ D. W.) | Magnessium ($Mg^{2+}$) (mg g$^{-1}$ D. W.) | Phosphorus (P) (mg g$^{-1}$ D. W.) | Total soluble proteins (mg g$^{-1}$ F. W.) | Total free amino acids (mg g$^{-1}$ F. W.) |
|---|---|---|---|---|---|---|
| Rice cultivars (V) | 1 | 8.28** | 39.01** | 0.34 ns | 4.50*** | 2.66*** |
| NaCl treatments (S) | 3 | 0.58$^{NS}$ | 0.23$^{NS}$ | 22.23 *** | 76.93*** | 38.53*** |
| V x S | 3 | 0.38$^{NS}$ | 0.95$^{NS}$ | 0.015 ns | 0.21 ns | 0.30ns |
| Error | 16 | 1.08 | 0.62 | 0.106 | 0.24 | 0.112 |
| Total | 23 | | | | | |

*, **, *** = significant at 0.05, 0.01 and 0.001 levels, respectively., Ns =Non-significant, D. W= Dry Weight
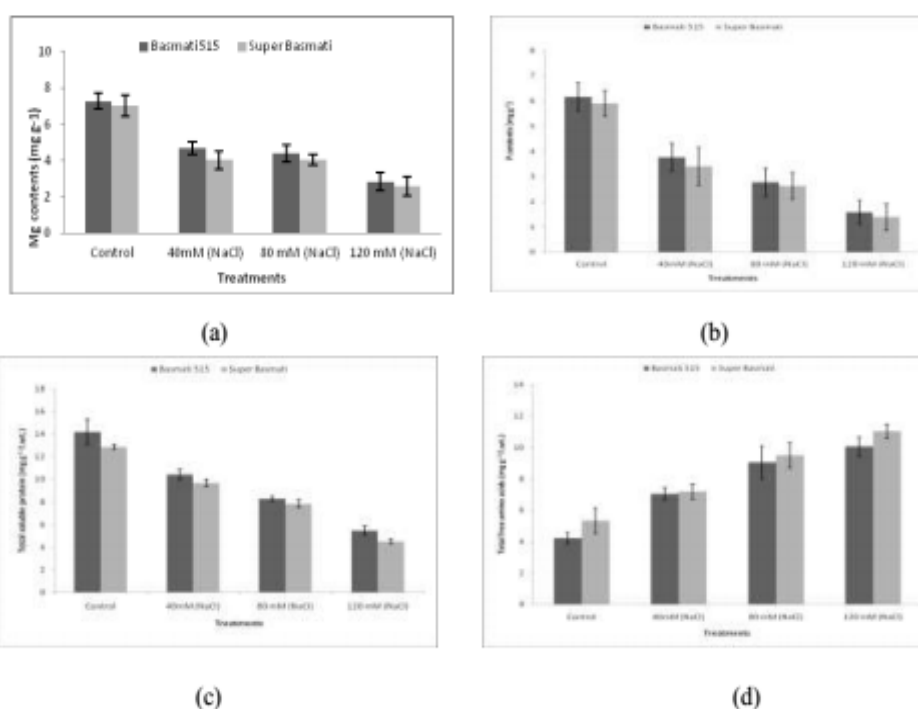


(a)



(b)



(c)



(d)

**Figure 2:** Magnesium (a), phosphorus (b), total proteins(c) and total free amino acids (d) of two rice cultivars under altering saline conditions according to the concentration and treatments with NaCl. Data are means ± standard error of different independent experiments. Data with different letters are significantly different between treatments at p≤0.05.

**Discussion**

The decrease in plant growth and development has been directly associated with many physiological and biochemical processes which are disrupted due to the abiotic stresses in plants [25]. The results of the present investigation exhibited that salt stress seriously reduced the plant biomass, essential ions uptake and certain metabolites in rice cultivars [26, 27]. Similar results were also depicted in other crops [28, 29]. A reduction in the plant heights, fresh and dry weights was also detected under 75 mM concentration of sodium chloride [30, 31]. The salt stress induced a decline in the rate of photosynthesis and essential nutrients uptake, and as a result, the growth of plants was inhibited by the application of salt stress [32]. Salt stress was reported to reduce in biomass and productivity in rice [33]. The accumulation of micro and macro-nutrients changed under saline stress. The sodium accumulation increased in salinity, while the other nutrients like phosphorus and potassium decreased as previously recorded by the other researchers [34]. The decrease in ions uptake might be due to the presence of

toxic ions in the soil which were absorbed by the plants that disrupted the absorption and accumulation of the other essential micro and macro nutrients, and as a consequence, it hindered the various metabolic activities of plants, and ultimately inhibited the rice plant growth and development [35], or in severe conditions, there might be plant death. Previous studies also showed the relevant evidences [36].

The reduction of phosphorus, potassium, magnesium and calcium in plants directly affected the rate of synthesis of certain other metabolites [37]. When the accumulation of toxic ions increased in root system, it adversely affected the water potential of the leaves [38]. The regulation of stomatal membrane integrity and water adjustment was disturbed by the deficiency of potassium. As a result, there was a decrease in the respiration and photosynthesis which ultimately lowered the photosynthesis in plant tissues [39, 40]. Research by the other scientists also noted that the growth of rice crops was adversely effected under the salt stress. The emergence of panicle was found less affected by the salt stress; however, the panicle lengths and the number of tillers were seriously affected in rice. It has been observed that under salinity stress, the protein contents were reduced [41, 42], while the total free amino acids' concentration increased under the salinity stress as reported in previous investigations [43]. The decrease in the concentration of protein, and the increase in the amino acids' contents was also observed in chick pea plant against the salinity stress [44].

## Conclusions

The study was conducted to determine the comparative effect of salinity stress on two rice cultivars. It was well evident that saline soils reduced the uptake of the essential nutrients by the antagonistic effects of ions with sodium and chloride which were more accumulated, and as a result, disrupted many physiological and biochemical pathways in rice plants. The saline soils also reduced the proteins, and increased the total free amino acids for osmotic adjustment in plant tissues. The current studies have proved that salt tolerant varieties might be used to get better plant growth in the salt affected areas, and might be used for further breeding purpose by the breeders. The data analysis indicated that rice genotype Basmati 515 showed better growth than genotypes Super basmati under saline and non saline conditions. So, rice cultivar Basmati 515 could be used in future plans to obtain the best yield in salt stress agricultural lands.

## Conflict of interest

Authors would hereby like to declare that there was no conflict of interests that could possibly arise.

## Acknowledgements

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