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Modulation of Growth and Physicochemical Assays in Salt Stressed Rice by Application of Potassium and Salicylic Acid

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Abstract

Salinity stress adversely affected the rice growth, productivity and yield from last few decades. Here, in this study the ameliorative role of foliar application of potassium (K) and salicylic acid (SA) have been investigated against salinity stress in rice. Four rice varieties (Kashmir Basmati, Basmati-370, Super-Basmati and Basmati-515) were exposed to NaCl stress (0, 100mM) and sprayed with SA (0.02%), K (0.1%) and their combination (SA+K) at vegetative and flowering stages. Salinity stress caused reduction in plant fresh and dry weight, plant height, chlorophyll and carotenoids contents, and photosynthetic rate. Moreover, the induced oxidative stress was confirmed by remarkable changes in nitrogen metabolism (nitrate, nitrite reductase, proline, protein and total free amino acid), plant water relations (osmotic, turgor and water potential) and yield (1000 grain weight, seed yield per plant). However, among four varieties, Basmati-370 exhibited best performance as compared to others and showed tolerance against salinity. The foliar spray of SA and K was effective in enhancing the plant biomass, nitrogen metabolism and water potential in all varieties under saline conditions. It is concluded that salinity caused reduction in growth and yield of four varieties of rice. However, Basmati-370 showed better stress tolerance regarding growth, physiology and yield. The application of potassium and salicylic acid may be a good strategy in improving the performance of rice varieties under salt stress.

Keywords: Foliar spray; Potassium; Rice; Salinity; Physiological traits; biochemical traits

Introduction

Soil salinity is a worldwide problem limiting plant growth and productivity [1]. The loss of land due to salinity is rising and will reach up to 50% by 2050 [2,3]. Worldwide, more than 800Mha of arable lands are affected by salt [4]. In Pakistan, 4.8Mha of irrigated land is threatened by salinity stress [5]. Salinity is caused by high salt concentrations at the surface layers due to evaporation and perceived by the root system as osmotic stress from diminished availability of water. As a consequence, the water potential of the plant is lowered [6,7]. Hence, a high amount of sodium and chloride in the root zone affects many vital processes of plants such as water uptake, photosynthesis, enzyme activities, ion accumulation, hormone balance or cell division and expansion. In addition, ion toxicity results in a nutrient imbalance in the cytosol [8] and toxic amount of Na and Cl can accumulate in the leaves. The ions disorganize membrane structures by producing reactive oxygen species [9] thereby causing oxidative stress in plants. Persistent oxidative stress damages lipids, proteins, nucleic acids and ultimately the whole machinery of the cell [7].

Plants may adopt different strategies for stress tolerance. Exogenous application of growth substances and nutrients have been shown to reduce the toxic effect of salt stress [10] and in this respect, salicylic acid (SA) has been mentioned to induce stress tolerance in plants [11]. The exogenous spray of micro and macro nutrients is also practiced under saline conditions to enhance uptake of minerals [12] and in this regard potassium contributes greatly against salinity stress tolerance [13].

Salicylic acid is a water-soluble compound that regulates plant growth and plays an important role in the protection of plants against abiotic stress [14]. It regulates several physiological processes such as photosynthesis, stomatal closure, protein synthesis, nutrient uptake and also contributes in germination process [15,16]. The application of SA increases the soluble protein and total soluble sugar in *Vigna ungiculata* (cowpea), mineral content in *Oryza sativa* (rice) [17], *Phaseolus vulgaris* (bean) [18], *Solanum lycopersicum* (tomato) [19]) and *Zea mays* (maize) [20].

Potassium is a macronutrient that plays important role in maintaining cell membrane stability, turgidity, regulation of osmotic pressure and improvement of crop yield. While, potassium deficiency rendered the plant growth by reducing the plant size and number of leaves [21]. Under saline condition, potassium competes with sodium and increases plant water status therefore, enhances the tolerance against salt stress [13]. Foliar spray of potassium minimizes the damaging effect of salinity in *Solanum melongena* (eggplant) [22], *Beta vulgaris* (sugar beet) [23] and *Tritucum aestivum* (wheat) [24].

Rice is an important cereal crop and good source of carbohydrates. About 60% of the world population live in Asia and consume 90% of the rice produced worldwide as staple food [25,26]. The average yield of rice is comparatively low to meet the human need in many rice growing and consuming countries [27]. Rice is salt susceptible crop and salinity stress adversely affected the growth of rice especially at seedling stage [28], vegetative and later reproductive phase [29]. Salt stress significantly alters the physiological and biochemical characteristics of plants. As a result, rice crop yield is decreased in salt affected soils [30].

Keeping in view the constraints and facts, major objective of the current study was to assess the ameliorative effect of SA and K as foliar spray and rice genotypes for salinity stress tolerance based on physiological, biochemical and yield traits, so that saline land can be used for cultivation of rice crop and food production to ensure future food security.

Materials and Methods

This pot experiment was conducted to evaluate the influence of foliar application of K and SA on rice to counteract the effects of salinity. The seeds of four rice varieties (Super Basmati 370 (V1), Basmati (V2), Kashmir Basmati (V3) and Basmati 515 (V4) were obtained from the mutation breeding division, NIAB (Nuclear Institute of Agriculture and Biology), Faisalabad, Pakistan. Rice seeds were surface sterilized with 5% sodium hypochlorite for 10 minutes and then washed with water. Ten seeds were sown in plastic pots (28cm diameter), then after germination, thinning was done to select healthy and uniform seedlings. Healthy seedlings were transplanted after twenty days of seed germination in the earthen pots (capacity 10 kg) containing clay loam soil (pH 7.8; ECe 1.2dSm^{-1}). The pots were salinized before transplanting and six plants per pot were grown. Salinity treatments (control and 100mM) were created with NaCl on soil saturation percentage basis. The pots were kept in net house under natural conditions and average temperature was (33/25°C day night temperature) and relative humidity was (60±5%). The experiment contains three replicates per cultivars and treatment. The optimum concentration of potassium (1000mg L^{-1}) and salicylic acid (200mg L^{-1}) was selected as foliar spray after determining the effective treatment by conducting the dose optimization experiment for rice. The first foliar spray of SA (0, 200mgL^{-1}), K (0, 1000mgL^{-1}) and their combination (SA+K) was applied at the vegetative stage after forty-five days of transplantation. Second spray was given at the reproductive stage after two weeks of panicle appearance. Samples were collected after the 15 days of foliar spray at each stage. The yield data was also recorded at maturity by counting the thousand seed per plant and measuring the total seed weight (g) per plant by the help of electronic balance.

For growth parameters, the fresh weight was determined by harvesting the two plants from each pot and weight was measured (g). These plants were further used to estimate the dry weight per plant. The plants were kept in oven at 65°C to get constant weight. A meter rod was used to record the plant height (cm) by measuring from base to top of the leaf.

The chlorophyll and carotenoids extraction were carried out as described by [31,32]. Fresh green leaves were chopped into fine segments (5cm) and extracted with 80 % acetone and then kept overnight at 10°Ċ. The extract was centrifuged at 1400g for 5min. Absorption was measured at 480, 645nm and 663nm using spectrophotometer (Hitachi, U-2001, Japan). Concentration of chlorophyll (a, b, total) and carotenoids was calculated by formulas and expressed as mg g^{-1} FW.

Water potential (ψ_w) was determined by using the third leaf from top (i.e., the youngest fully expanded leaf) of three plants per treatment. The measurements were made between 8.00 am and 10.00 am with Scholander type pressure chamber (1505D-EXP, PMS Instruments Company). The same leaf was frozen at -20°C for seven days that was used for the measurement of water potential. The cell sap was extracted from the frozen leaf with the help of disposable syringe. Extract was used to estimate

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the osmotic potential (ψ_s) with an osmometer (Wescor, 5500). Turgor potential (ψ_t) was calculated as the difference between water potential values (ψ_w) and osmotic value (ψ_s) determined by pressure chamber and osmometer, respectively.

The measurement of the net CO_2 assimilation rate (A), transpiration rate (E) and stomatal conductance (*gs*) were made on second fully expanded leaf of each plant between 10:00 am and 2:00 pm with a portable infrared gas analyzer (Model Cl-340, Analytical Development Company, Hoddesdon, England).

Total free amino acids were determined according to [33] method. The nitrate reductase activity (NRA) and nitrite reductase (NiRA) were determined according to [34] method. Total soluble proteins were determined by the method of [35]. Proline from rice leaf was determined according to the method of [36] (Table 1).

Table 1: Analysis of variance (ANOVA) to determine the effect of foliar spray of SA and K on fresh /dry weight, height, Chl. a, b, total, carotenoids, Ψw, Ψs, ΨT, E, gs, A, TFAA, protein, NRA, NiRA, proline, grain yield and 1000 grain wt. of four rice varieties under salinity.

SOV	DF	Fresh Weight	Dry Weight	Plant Height
Treatment (T)	4	386.21***	132.74***	330.01***
Varieties (V)	3	12.47**	14.9	77.08**
T X V	12	1.01**	1.09**	3.06
Error	40	3.43	6.7	4.76
SOV	DF	Ψw	Ψs	ΨT
Treatment (T)	4	2.70***	3.20***	0.05***
Varieties (V)	3	0.04**	0.01*	0.04**
Stages (S)	1	1.76**	0.33**	0.03*
T X V	12	0.17**	0.11*	0.04**
V X S	3	0.21*	0.002	0.01
T X S	4	0.13	0.12**	0.13**
T X S X V	12	0.02*	0.01*	0.09*
Error	80	0.04	0.02	0.04
SOV	DF	Chl.a	Chl.b	Total Chl
Treatment (T)	4	1.318***	0.29***	4.57***
Varieties (V)	3	1.212*	0.32**	0.77**
Stages (S)	1	0.431**	0.75*	4.80**
T X V	12	0.09**	0.01	0.08*
V X S	3	0.08	0.03**	0.02
T X S	4	0.04*	0.02*	0.41**
T X S X V	12	0.025**	0.01	0.05**
Error	80	0.177	0.03	0.24
SOV	DF	Carotenoids	E	A
Treatment (T)	4	0.578***	76.429***	4.24***
Varieties (V)	3	0.113**	1.529**	264.20**
Stages (S)	1	0.134*	21.164*	10.41*
T X V	12	0.011**	0.48**	8.24*
V X S	3	0.044	0.54**	7.93**
T X S	4	0.02*	0.71	1.33**
T X S X V	12	0.08**	0.842*	11.14*
Error	80	0.02	0.04	0.11
SOV	DF	gs	TFAA	TSP
Treatment (T)	4	615.80***	41.23***	2.22***
Varieties (V)	3	7.65***	0.51***	1.03**
Stages (S)	1	9756.10*	653.28*	43.44*
T X V	12	24.87**	0.23**	0.08**
V X S	3	2.57	0.11	0.01

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T X S	4	0.60**	8.85**	7.66**
T X S X V	12	0.74**	0.24*	0.04**
Error	80	0.22	0.067	0.01
SOV	DF	NRA	NiRA	Proline
Treatment (T)	4	3.86***	4.79***	16.65***
Varieties (V)	3	1.47**	0.34**	1.08**
Stages (S)	1	8.54**	33.20**	18.53***
T X V	12	0.41*	0.013*	0.03*
V X S	3	0.71**	0.15*	0.11
T X S	4	0.07	0.17*	0.86**
T X S X V	12	0.04*	0.04	0.12*
Error	80	0.17	0.11	0.05
SOV	DF	1000 Grain Wt.	Yield Plant-1	
Treatment (T)	4	73.10***	63.59***	
Varieties (V)	3	7.66**	3.27***	
T X V	12	0.89**	0.39*	
Error	40			

* Significant at P<0.05; ** Significant at P<0.01; *** Significant at P<0.001; NS = non-significant.

Statistical Analysis

The data was statistically analysed by using software Statistix 8.1 at (P<0.05). The mean significance was tested by using Least Significant Difference test at 5% probability level [37]. The Microsoft Excel was used to calculate standard error and to make graphs for presenting data.

Results

Salinity stress significantly (P≤ 0.05) reduced the plant fresh and dry weight and height in four varieties of rice (Figure 1). Under saline conditions, all these growth characteristics (fresh /dry weight and plant height) were remarkably decreased in cultivars Super-Basmati (V_2) and Basmati-515 (V_4) while application of SA+K improved growth parameters in Basmati-370 (V_1) and Kashmir-Basmati (V_3). The interaction between varieties and treatment was significant. The cultivars sprayed with combine application of SA+K showed better results than individual spray of SA and K. The results clearly indicated that all these growth attributes were considerably increased in Basmati-370 (V_1) and Kashmir-Basmati (V_3) as compared to Super-Basmati (V_2) and Basmati-515 (V_4).

The osmotic potential, water potential and turgor pressure were significantly (P≤ 0.05) affected by salt stress (Figure 2). The osmotic potential and water potential were decreased (more negative) under salinity stress. All cultivars exhibited significant reduction in osmotic and water potential in saline media. However, Basmati-370 (V_1) showed maximum reduction in osmotic potential and water potential than Kashmir-Basmati (V_3), Super-Basmati (V_2) and Basmati-515 (V_4). The turgor potential was increased under stress and variety Basmati-370 (V_1) maintained maximum turgor potential as compared to others. The influence of exogenous spray of K and SA on all varieties was significant regarding osmotic and water potential. However, turgor potential remains unchanged by the application of SA and K under saline conditions (Figure 2c).

Salt stress induced a significant (P≤ 0.05) reduction in chlorophyll (Chl a) of all rice cultivars (Figure 3). Under saline conditions, Basmati-370 showed maximum decrease in chlorophyll a content while those cultivars (Kashmir-Basmati (V_3), Super-Basmati (V_2) and Basmati-515) which were sprayed with SA+K exhibited a clear increase in chlorophyll 'a'. The interaction among varieties, treatment and stages was significant. The combine application of K and SA enhanced chlorophyll 'a' content in cultivar Basmati-370 at the vegetative stage and in cultivar Kashmir-Basmati at the flowering stage (Figure 3a). The chlorophyll 'b' contents were also decreased under salinity stress (P≤ 0.05) in all tested varieties (Figure 3b). Maximum decrease was observed in Basmati-515 under saline media while highest concentration was observed in Basmati-370 when sprayed with SA+K (Figure 3b). The concentration of total chlorophyll was also affected by salinity stress (Figure 3d). Salt stress significantly decreased the total chlorophyll contents in all varieties, but maximum diminution was observed in Super Basmati. The interaction was significant among stages, varieties and treatment. The combine application of K and SA increased total chlorophyll contents in Basmati-370 and Kashmir Basmati at the vegetative and the flowering stage under saline conditions (Figure 3c). The carotenoids were also decreased significantly (P≤

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0.05) under salinity (Figure 3d). Maximum reduction was noted in Basmati-515 under salinity while Basmati-370 exhibited higher concentration of carotenoids when treated with foliar spray of SA and K. The interaction between varieties and treatment was significant (Figure 3d).

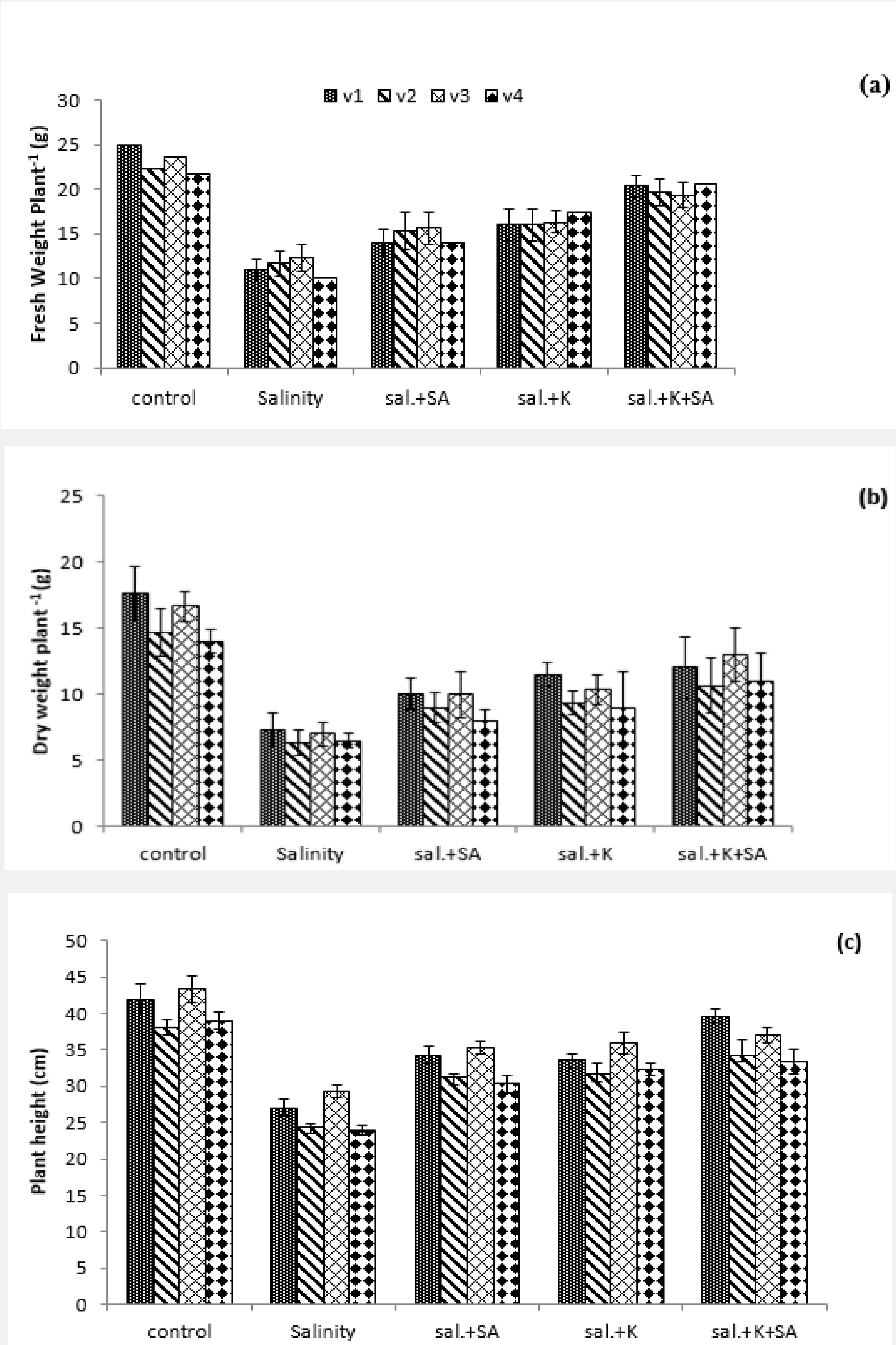

Figure 1: Effect of foliar applied SA and K on (a) fresh weight, (b) dry weight and (c) plant height in different rice varieties grown under normal and saline conditions.

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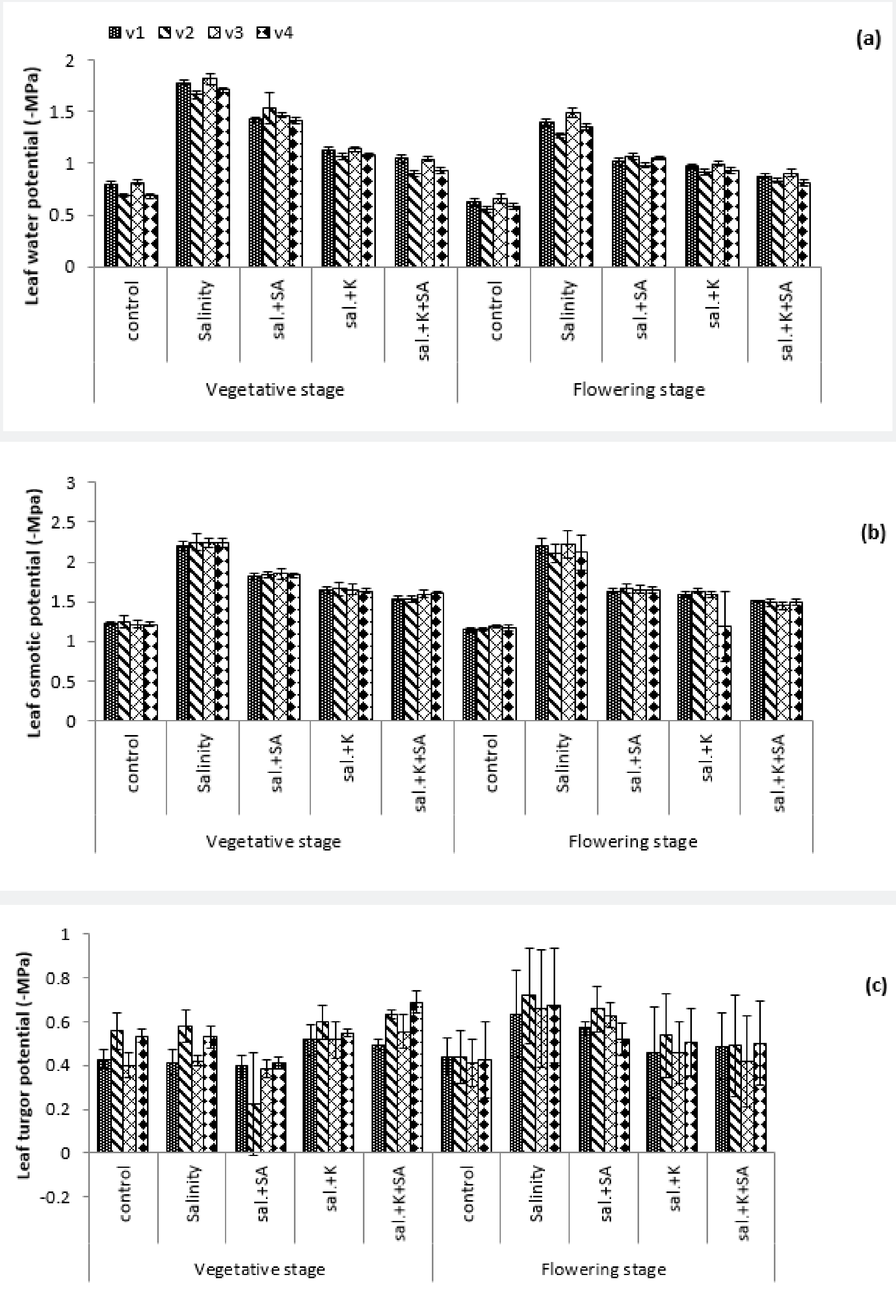

Figure 2: Effects of SA and K as foliar spray on water potential (a), osmotic potential (b) and leaf turgor pressure (c). Vegetative and flowering stages of four rice cultivars (V1-V4) grown under saline and control conditions.

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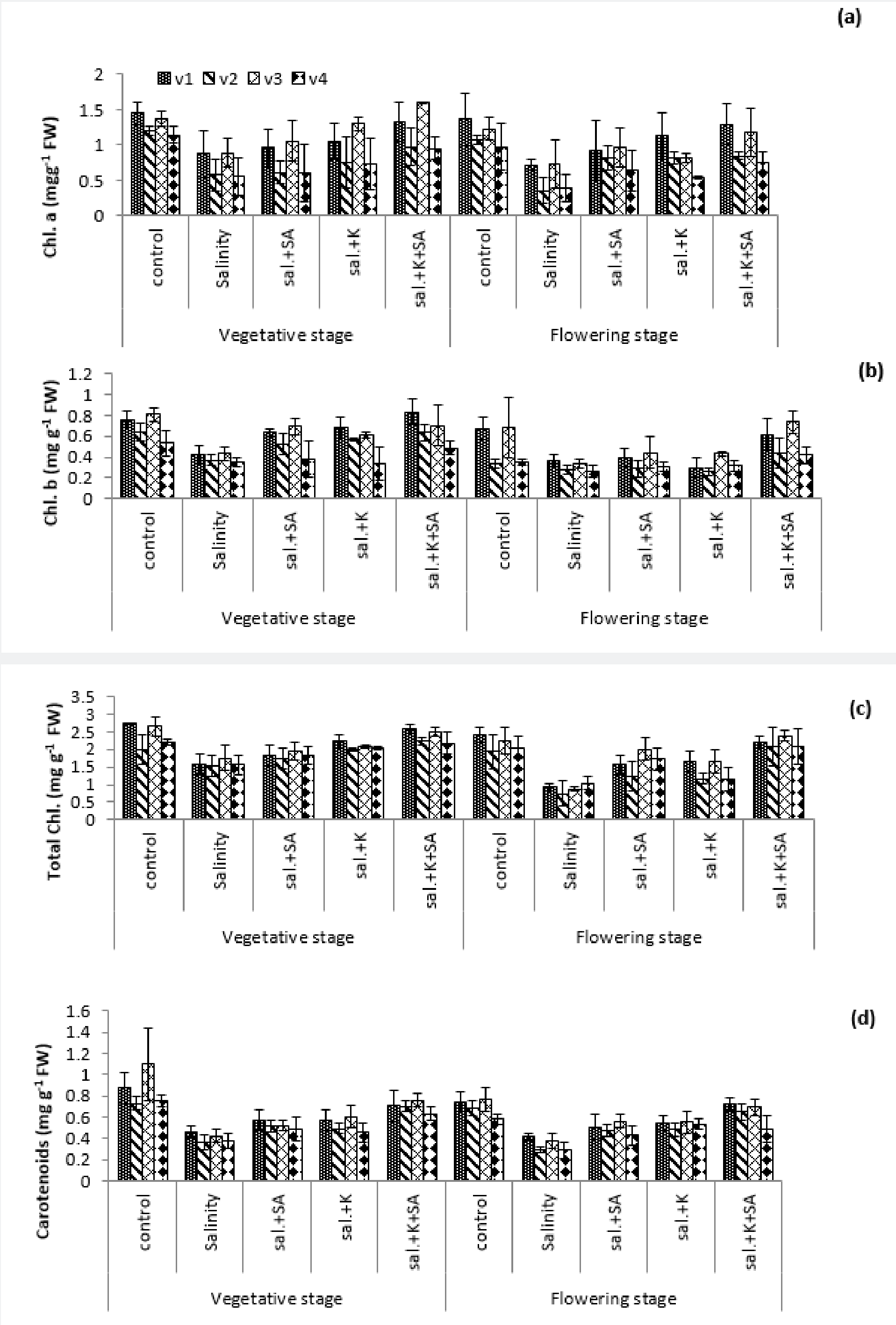

Figure 3: Foliar application of SA and K to different rice cultivars (V1 to V4) grown under saline and non-saline conditions. Effects on (a) Chl.a, (b) Chl.b, (c) total Chl and (d) Carotenoids contents.

All plants of rice cultivars under salinity stress showed a significant ($P \leq 0.05$) reduction in photosynthetic rate, transpiration rate and stomatal conductance) (Figure 4). The combine application of K and SA as a foliar spray enhanced the transpiration rate at the vegetative and the flowering stage (Figure 4a). The application of potassium improved the photosynthetic rate, transpiration rate and stomatal conductance in Kashmir-Basmati at the vegetative stage while in Basmati-515 at flowering stage. Application of SA increased the transpiration rate in Kashmir-Basmati at flowering stage (Figure 4a). However, Basmati-370 and Kashmir-Basmati performed better after the combine application of K and SA. From the results, it is clear that SA and K, separately and combine, showed an improvement in stomatal conductance and photosynthetic rate of all four rice cultivars under saline conditions.

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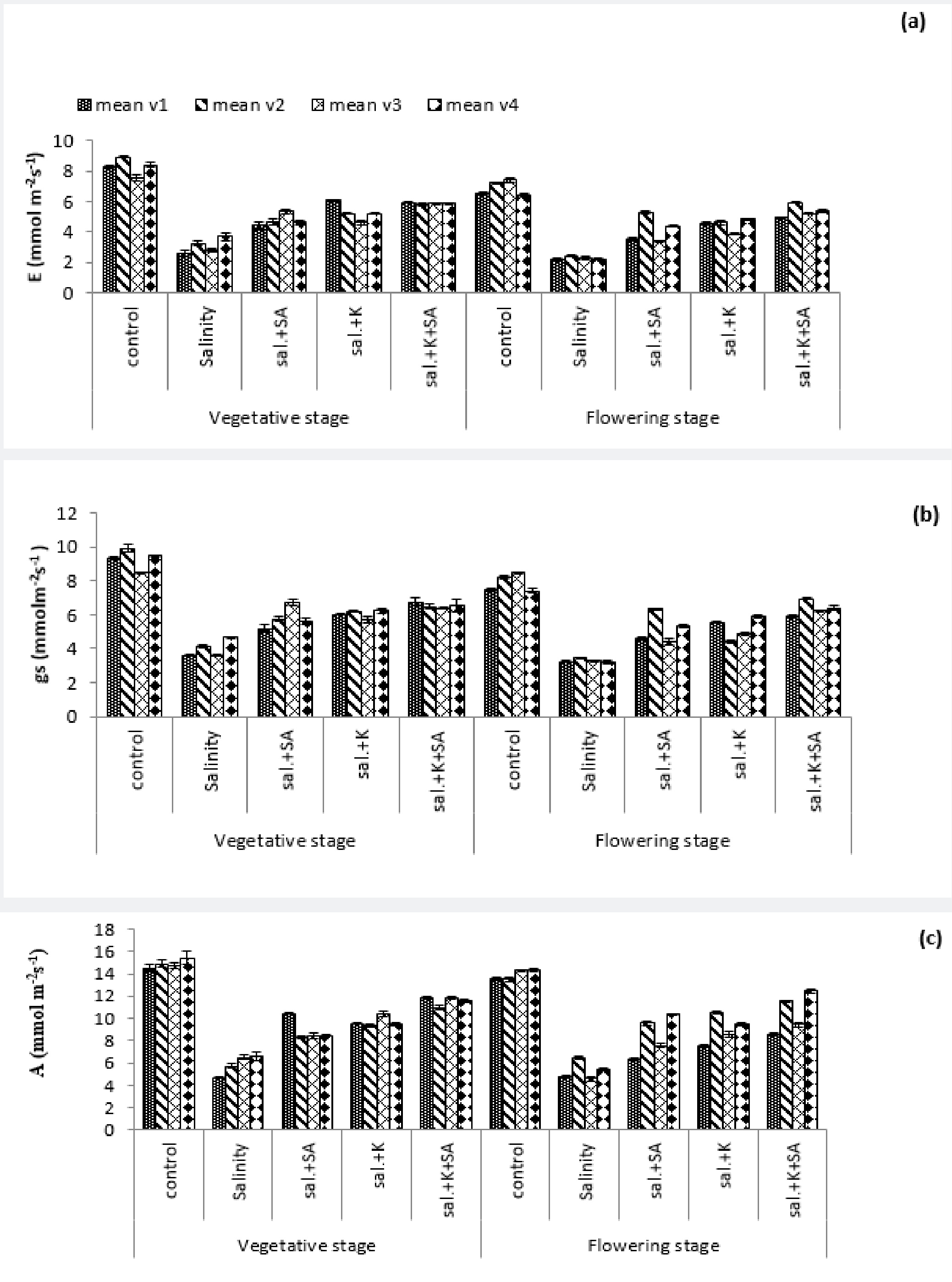

Figure 4: Effects of foliar application of SA and potassium on (a) transpiration rate, (b) stomatal conductance and (c) photosynthetic rate. Vegetative and flowering stages of four different rice cultivars (V1-V4) grown under saline and control media.

The effect of salinity stress was significant ($P \leq 0.05$) on nitrate, nitrite reductase activity, proline, total free amino acid and protein concentration in all plants (Figure 5). The spray of K and SA improved the nitrate activity in Basmati-370 at both the vegetative and the flowering stage (Figure 5a & 5b)while in the case of nitrite reductase activity, the application of potassium and SA increased the activity preferentially at the vegetative stage than flowering stage (Figure 5a & 5b). Salinity caused a significant reduction in total soluble protein at both growth stages of rice. However, application of K and SA induced protein accumulation at the vegetative stage in Kashmir-Basmati (Figure 5c). Proline contents significantly increased in rice cultivars under saline growth conditions. The proline accumulation was recorded preferentially higher in Kashmir-Basmati at the vegetative stage

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when compared to the flowering stage. However, in Kashmir-Basmati, the application of K only as a foliar spray induced proline accumulation at the flowering stage (Figure 5d). Salinity stress significantly (P≤ 0.05) increased amino acids accumulation in all rice cultivars (Figure 6). The accumulation was higher in Basmati-370 when sprayed with SA and K. Minimum accumulation was recorded in Basmati-515 at the vegetative stage as compared to the flowering stage (Figure 6). The interaction between stages and varieties was significant. The results clearly showed that Basmati-370 performed better than other varieties under stress conditions.

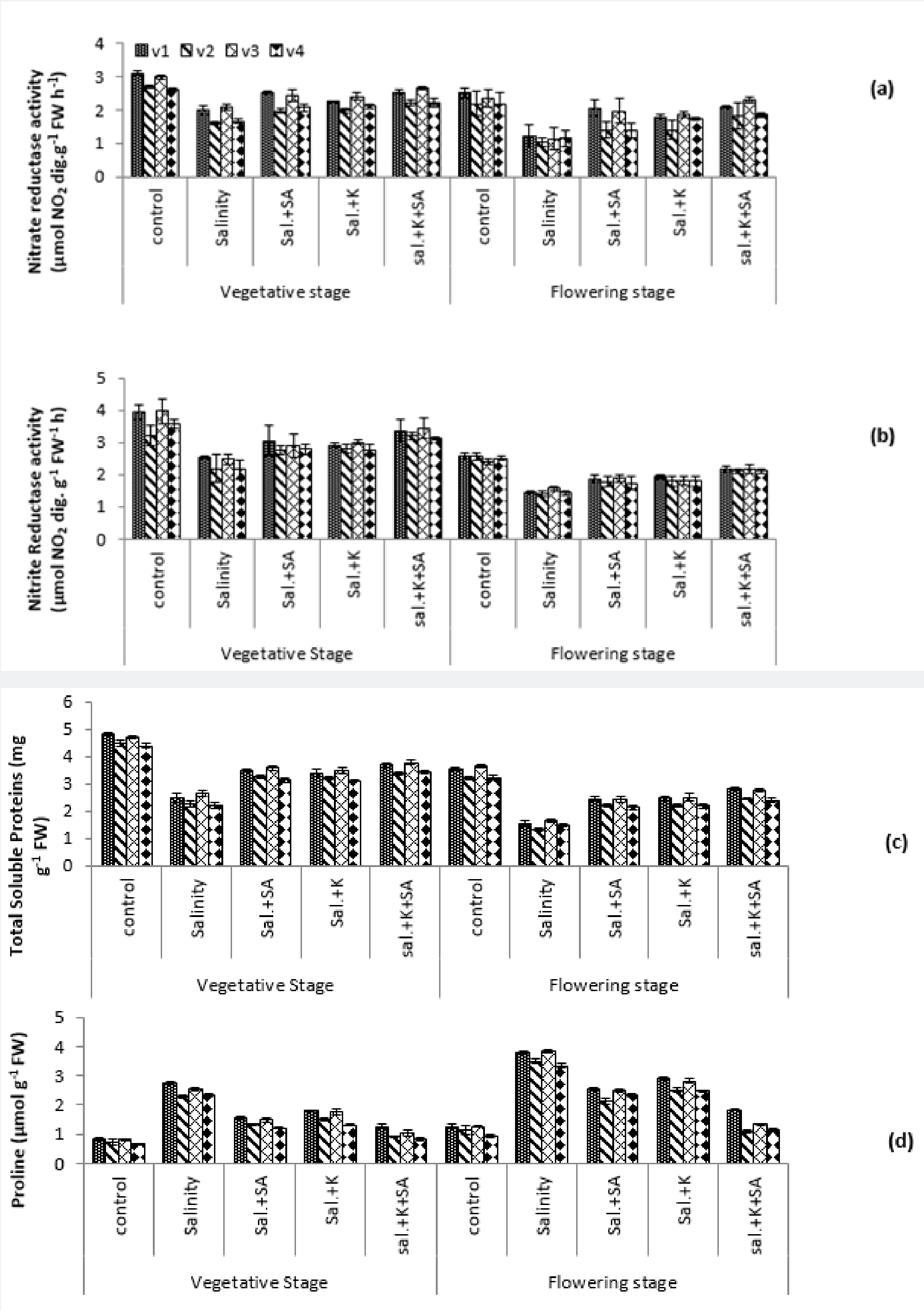

Figure 5: Effects of SA and K on Nitrate reductase (a), Nitrite reductase (b), total soluble protein (c) and proline (d). Four different rice cultivars (V1-V4) grown under saline and non-saline conditions.

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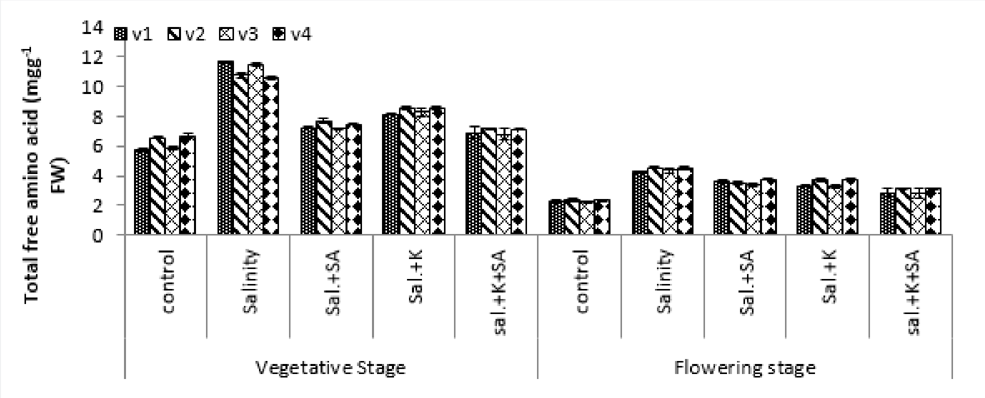

Figure 6: Effects of SA and K on total free amino acid accumulation of four different rice cultivars (V1-V4) grown under saline and non-saline conditions.

The yield and yield attributes (1000 seed weight per plant, seed yield per plant) were significantly ($P \leq 0.05$) decreased under salinity stress in all cultivars of rice (Figure 7). The varieties Basmati-515 and Super-Basmati showed maximum reduction under saline conditions. The foliar spray of SA and K improved the yield in Basmati-370 and Kashmir-Basmati. The interaction between varieties and treatment was significant. The effect of combine spray of SA+K was more effective in Basmati-370.

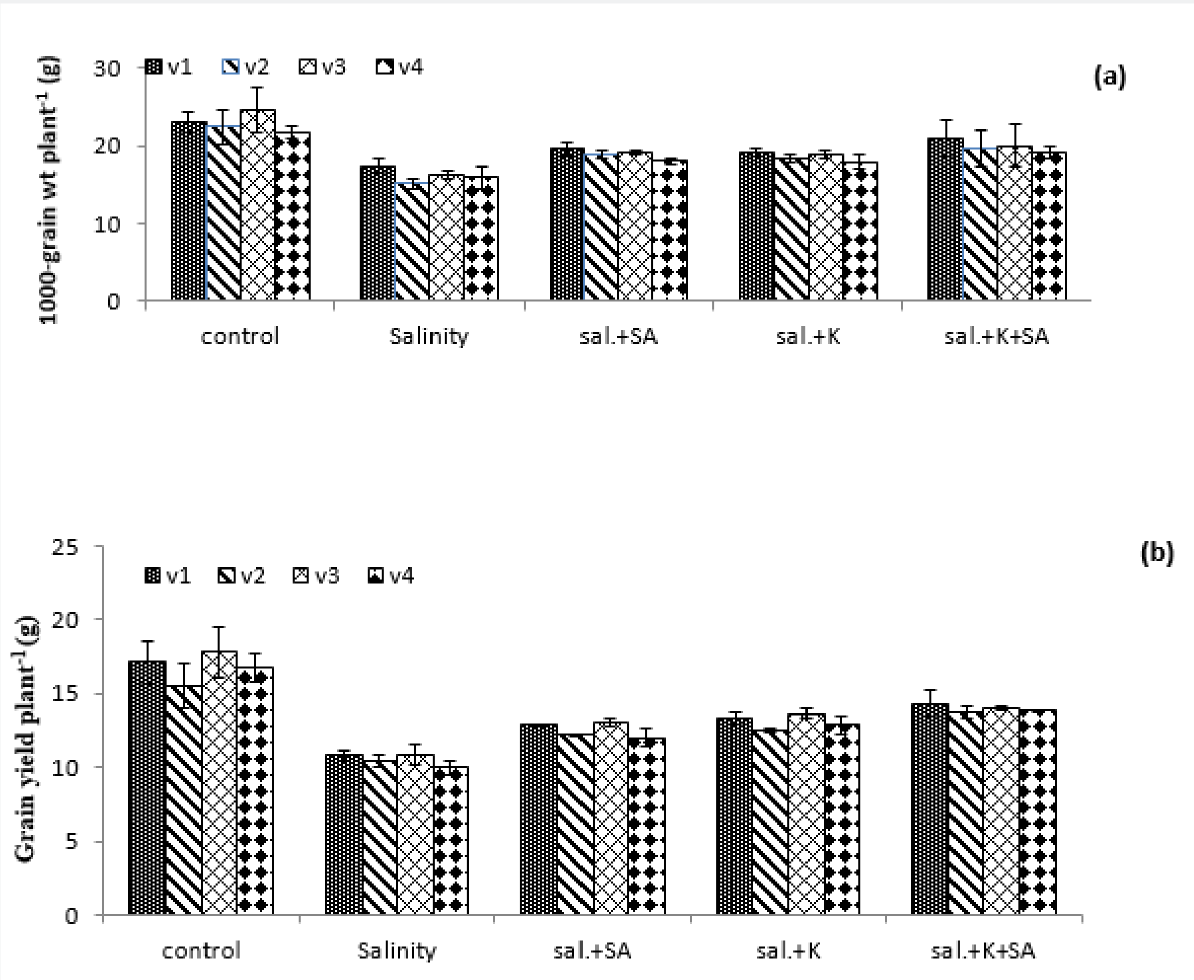

Figure 7: Effects of SA and K on (a) grain yield per plant (b) 1000 grain weight of four different rice cultivars (V1-V4) grown under saline and non-saline conditions.

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Discussion

Salinity stress decreased the biomass, physiological attributes and yield of four varieties of rice (Figure 1-7). The performance of variety (Basmati-370) was better than other varieties under saline conditions. The inhibitory effect of salinity on growth and physiological characteristics of mungbean and wheat have been also reported by [38,39]. Salt induced growth inhibition was due to water deficient conditions, specific ion effect [40] and low rate of cell division and elongation [41]. The growth and biomass were improved under salinity by foliar application of K and SA in all tested rice varieties (Figure 1). Similar reports were obtained from the previous studies on *Oryza sativa* (rice) and *Triticum aestivum* (wheat) [42,43]. The increase in growth was due to activation of some salt resistant genes [44]. The greater biomass helps to maintain physiological characteristics (chlorophyll concentration, photosynthetic rate, stomatal conductance) which ultimately improve the yield (Figure 2-7).

Salt stress reduced the chlorophyll (a, b, total Chl.) and carotenoid contents in rice. Similar results were also obtained for *Carthamus tinctorius* (Safflower), *Zea mays* (maize) and *Triticum aestivum* (wheat) [45-47]. The salt-induced reduction in chlorophyll contents is probably due to increased activity of chlorophyllase [48], low stomatal conductance, minimum uptake of water and maximum absorption of sodium ions which causes osmotic stress [9] and decreased crop yield [43]. Under salt stress, chlorophyll contents were increased with foliar application of SA and K in rice. Our findings are in accordance with earlier reports on wheat, *Helianthus annuus* (sunflower) and safflower [43,49]. The application of SA and K increases the chlorophyll contents by stimulating various physiological and biochemical activities and regulating the metabolic processes thus enhancing the salt stress tolerance in plants [50]. The higher concentration of salt may develop osmotic stress which reduces the uptake of water as a result the osmotic potential and water potential decreased [51]. The tolerant varieties may accumulate osmolytes to stimulate normal growth and maintain cell turgor as maintenance of turgor is necessary for adequate productivity [52]. The application of SA and K improved the plant water relation under stress condition and crop yield. The application of SA and K also improves the osmolytes accumulation under salinity stress. Photosynthesis, stomatal conductance and transpiration rate were declined under salinity in all varieties of rice (Figure 4). Our results are in line with findings of [53,54] in rice. At increasing levels of stress, the internal CO_2 and reduced stomatal activities influence the functioning of RuBisCo thereby decline the carboxylation and photosynthetic rate [55]. Low photosynthetic rate decreases the carbon fixation and plant biomass [56]. However, exogenous spray of SA and K increase these characteristics under salinity stress. Similar findings were obtained from previous reports on *Triticum aestivum* (wheat) [57]. SA play defensive role under stress condition as it maintain the functionality and stability of chloroplast membrane [58]. The application of K increases the photosynthetic rate by stimulating the ATPase which ultimately produces ATP. The adequate supply of K improves the performance of ATPase enzymes. K also regulates removal of water and entry of CO_2 in intercellular spaces; thereby maintain the stomatal regulation because Co_2 concentration is related with amount of photosynthates production [59]. The application of SA and K is helpful to scavenge ROS therefore improve the photosynthetic machinery and RuBP production [60]. Nitrogen metabolism of rice plants decreased under salinity. Similar reports were investigated by [61], in *Pisum sativum* (pea). The decrease was due to osmotic imbalance which resulted from low uptake of water. The nitrogen metabolism increased under salinity by foliar application of SA and K. These findings are similar with previous studies on maize and wheat under salinity stress [62,63]. The application of SA promote physiological and biochemical activities thereby influences the NRA inhibitors which increase the nitrate reductase activities [64]. The application of K also regulates metabolic activities, as it bound with inactive site of enzymes and stimulate the activation of enzymes thereby produce biomolecules like protein, phenols and increase the abiotic stress resistance in cells [65]. The yield and yield components were decreased under salinity stress in all rice cultivars. These results are in line with findings of [66], in sunflower under stress. The decrease in yield components was caused by low rate of photosynthesis [67], stomatal conductance, low osmotic potential and reduced nitrogen metabolism. The foliar application of SA and K improved the yield of rice under salinity stress. Similar findings were shared by [68], in maize. The application of K and SA increases the uptake of K ions and reduced the supply of sodium as well as influence the biochemical and physiological activities therefore resulted in improved crop yield.

These findings support the growth promoting effect of salicylic acid and potassium in rice under saline condition due to improved growth, physiochemical assays and yield attributes.

Conclusion

Salt tolerance potential of four varieties of rice was tested by foliar application of salicylic acid and potassium. Among four varieties, Basmati-370 performed better against salinity based on physiological, biochemical and yield traits and considered as salt tolerant variety. Furthermore, foliar application of salicylic acid + potassium improved growth and development of rice by improving the physiological and biochemical attributes. Therefore, it is recommended that Basmati-370 can be cultivated in saline soils and combine application of salicylic acid + potassium may a good approach to mitigate the effects of salinity in rice.

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