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SOIL SCIENCE

ALLEVIATION OF SALT STRESS BY USING POTASSIUM NITRATE IN DIFFERENT GENOTYPES OF SOYBEAN (*GLYCINE MAX* L.)

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ABSTRACT

The world is facing a biggest threat of salinity that are causing a severe reduction in crops growth and yield. In order to appraise this salinization an experiment in hydroponic was executed in the year 2022 with eleven (11) genotypes of soybean sown under the stress induced by salts. Raised nursery plants of soybean were shifted to metal tub having 200 Liters solution of Hoagland and plants were kept in solution for a period of two weeks. Treatments structure was consisted of different concentrations of NaCl alone and with mixing of both NaCl and KNO_3. NaCl levels were kept 0.0 mM, 60 mM and 120 mM, respectively, while mixing of NaCl with KNO_3 were kept as 60 mM sodium chloride (NaCl) with 6 mM potassium nitrate, 60 mM sodium chloride (NaCl) with 09 mM potassium nitrate (KNO_3), 120 mM sodium chloride (NaCl) with 6 mM potassium nitrate (KNO_3) and 120 mM sodium chloride (NaCl) with 09 mM potassium nitrate (KNO_3), and these levels were distributed in each tub. A significant decrease in parameters of growth along with in ions up-taking, H_2O balance and gas exchange were recorded in treatment structure. Least reduction was recorded in control treatment compared with highest reduction in 120 mM NaCl+09 mM KNO_3 treatment application trailed by where only 120 mM NaCl was applied. It was found that genotypes No. 62 and No. 13 exhibited most tolerance to salts and Ajmeri and William-82 as most sensitive to salts stress. It was also noted that by using KNO_3 in salt media enabled plants to alleviate the salt stress, however, best salt stress alleviation was observed at higher dose of potassium nitrate use in the growing media.

KEYWORDS: *Glycine max*; salinity; KNO_3; soybean; NaCl; salts; genotypes; Pakistan

INTRODUCTION

Due to salinization, arable land is reducing which may pose food security as population number is increased day by day around the globe. The research related to salt affected soils and their impact on plants growth has been boasted in the current era (Zora, 2006). Among abiotic stress higher salt concentrations has become a great danger to the agriculture globaly (Flowers and Colmer, 2008) particularly in the regions of dried and semi dried where temp is high with low rainfall, inferior water and soil management practices (Neto *et al.,* 2006). Due to salinization around the globe, almost 45mha of irrigated land has been affected and about 1.5 mha has gone out of cultivation each year (Munns and Tester, 2008).Salt stress is increased due to the availability of soluble salts. Salinization is responsible for nutrients and ionic imbalance, toxicity by ions, generation of ROS (reactive oxygen species) and cutting down in assimilation of nitrate and ammonium ions. Sodium (Na) brings changes in calcium bridging in the membranes of plants and hindering in nucleic acids and normal functions of the enzymes (Thitisaksakul and Maysaya, 2008). Although plant genotypes response is different towards salinity stress, however, some general reactions are occurring simultaneously in similarly in all genotypes. The elevated salinity not only cause harmful effects on plants but also be seen as the death of plants resultantly a reduction in yield as well as in productivity. Salinity stresses cause severe inhibition in growth and yield decline because of imbalance in cellular osmotic potential resulting in imbalance in water uptake, a decrease in water uptake in the cells of root causes homeostasis of ion disturbance in cells with membranes injury and slowdown of metabolism resulting a difference in strength to protect salts (Tester and Davenport, 2003, Tripler *et al.,* 2011). Salts in excess disturb the vital physiological mechanisms like photosynthesis, energy, metabolism of protein and lipid formation within a plant. Usually, two major approaches are existed to increase the production of crops under salt affected soils: 1st is the salt affected soils reclamation and 2nd approach is the isolation and bringing out the tolerant genotypes towards salinization (Blumwald *et al* and Yilmaz *et al.,* 2004). The earlier method is out of approach because of larger areas affected by salts with less good quality water availability, poor permeability of soil and elevated rates of amendments (Akhtar *et al.,* 2010). Hence, crop genotypes which are more tolerant to salts are found the best approach for using the verse saline soils (Iqbal *et al.,* 2007).While in comparison of salt sensitive plants with salt tolerant plants, tolerant plants are able to maintain a high potassium (K+) contents as compare to sodium (Na+) concentration (Azooz *et al*., 2004 and Rejile *et al*.,2007). This happens owing to the potassium pivotal role in balancing a number of potentials in plants like turgor, membrane etc. potentials as well as stoma working with tropisms and enzymes functioning (Cherel, 2004). The availability in the soil solution of the both Na+ and K+ and soil exchange sites exists simultaneously & both can synergies or antagonize each other in their absorption and translocation within plant under saline and sodic soil conditions (Hussain *et al.,* 2013). Thus, a well balanced sodium to potassium (Na+/K+) ratio is more decisive for the proper functioning of number of organs like normal functions of stomatas, enzymes activation, synthesis of protein, osmoregulation, metabolism of oxidants and most important of photosynthesis as well as turgor maintenance (Shabala *et al.,* 2003). Under sodic soil conditions, exogenous application of potassium nitrate application under sodic soil not only cope the production of reactive oxygen species productions but also enhance the activities of the antioxidant enzymes namely superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Cakmak, 2005; Liang *et al.,* 2007). The functioning of newly produced rubisco enzymes is increased by potassium resulting an increases in net photosynthetic rate. Potassium also increases the glutamine synthetase (GS) activities. Starch synthatase activity enhanced by potassium and the activity of the biosynthesis of starch (Bingsong, 2002; Kahrizi *et al.,* 2010). A better K+/Na+ ratio is maintained with the accumulation of potassium in plants stressed with salts. With the supplementary supply of potassium, the adverse effects of increasing salinity can be reduced very effectively (Ashraf, 2004; Kavitha *et al.,* 2012). A favorable response of soybean to KNO3 (potassium nitrate) application brings an increase in oil as well as contents of protein in seeds of treated plants (Tiwari *et al.,* 2002). The current research work was conducted to appraise the role of potassium nitrate on the physiological, morphological, and biochemical conditions of soybean plants grown under sodium chloride treatment conditions.

MATERIALS AND METHODS

The research trial was executed in house made of metallic wires having protection from rain, ISCES (Institute of Soil and Environmental Sciences), University of Agriculture, Faisalabad Pakistan. The performance of eleven (11) genotypes of soybean named as (William-82, NARC-2, Rawal-I, Ajmeri, Kwowgyo, Lakota, c-n-S. No. s-39-40, 13, 54, & 62) were tested within varying levels of sodium chloride (control (without salts), 60 &120 milli molar sodium chloride) only or in mixture of potassium nitrated levels (60 milli molar sodium chloride with 6 milli molar potassium nitrate, 60 milli molar sodium chloride with 09 milli molar potassium nitrate, 120 milli molar sodium chloride with 6 milli molar potassium nitrate and 120 milli molar sodium chloride with 09 milli molar potassium nitrate) in the year 2022. The seeds of different soybean genotypes were taken from NARC; National Agriculture Research Centre, Islamabad, Pakistan. Nursery plants were raised in trays of iron lined with polythene filled with sand of 2-inch layer. At the age and stage of two leaves, plants were shifted to two hundred liter capacity tubs with nutrients solution of Hoagland's were filled (Hoagland and Arnon, 1950) the joint of root shoot of tiny plants were wrapped with foam, transplanted them into thermopore sheets having holes, which was floating on Hoagland solution in two hundred liter capacity tubs. For aeration supply to the plants roots throughout the growing season of experiment, aeration pumps were used and with the changing of Hoagland's solution in the tubs every week. One week later of transplantation, treatments were applied to plants, keeping in control tub the similar amount of distilled water instead of any treatment was added. In order to elude the impact of nitrogen as added with the application of KNO_3 for each treatment, a complete calculation was made which might have reduced the exact nitrogen (N) amount effected by solution of Hoagland's in each tub, while in control tub, nitrogen was added as per the recipe of Hoagland's solution. A pH of 6.5±0.5 was maintained throughout the 30 days' experiment project to confirm nutrients availability. The plants in the tubs harvested exactly after salt stress given for 60 days. Experiment was

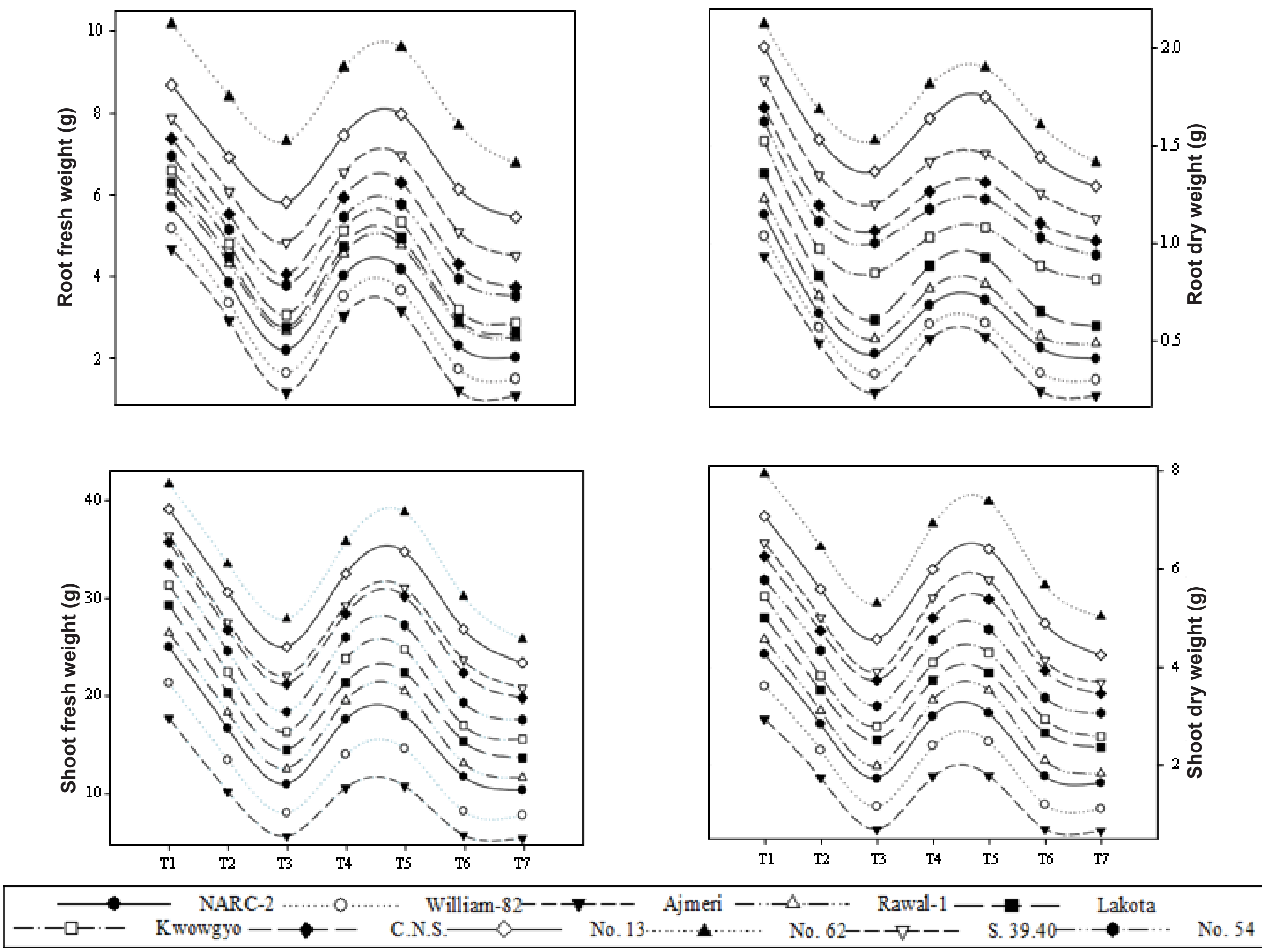

Fig. 1. Effect of salinity and potassium applications on shoot and root fresh and dry weights (g) of soybean varieties. T1: Control, T2: 60mM NaCl, T3: 120mM NaCl, T4: 60mM NaCl+6mM K, T5: 60mM NaCl+09mM K T6: 120mM NaCl+6mM K, T7: 120mM NaCl+09mM K

carried out under a statistical design of Complete Randomized Design CRD with three treatments in factorial arrangement.

MEASUREMENTS

Using chlorophyll meter SPAD, the data for chlorophyll contents were recorded (Minolta, Japan), similarly, LCA.4, ADC, IRGA-portable (infrared gas analyzer) used to measure the net photosynthetic rate (A), transpiration rate (E) as well as stomatal conductance and intercellular carbon dioxide concentration (ci). Standard procedures and methods were used to measure the membrane stability index (MSI), leaf area as well as relative water contents (RWC), dry and fresh root & shoot weights along with root & shoot lengths. Sodium along with potassium ions in the fully expanded younger leaves and mature roots were analyzed using the flame photometer method.Using the modern tools of Statistics software: Statistix 8.1®., all the collected data was analyzed. In order to indicate the significant differences among the treatments, least significant difference (LSD) multiple range test ($P \leq 0.05$) was used (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

Salinity & applied potassium effect on fresh & dry shoot, root weights (g) (morphological parameters) of soybean genotypes of soybean in number were grown under solution culture conditions exhibited different responses for NaCl (sodium chloride) and KNO_3 (potassium nitrate) treatments. weights of both fresh as well as dry root & shoots were significantly reduced in NaCl (sodium chloride) developed salinity & these maximum effects of decreases were recorded both of 120 milli molar sodium chloride and 09 milli molar potassium application was made in 11 genotypes (Fig. 1). The distinctive behavior of genotypes among different treatments was because of their varying

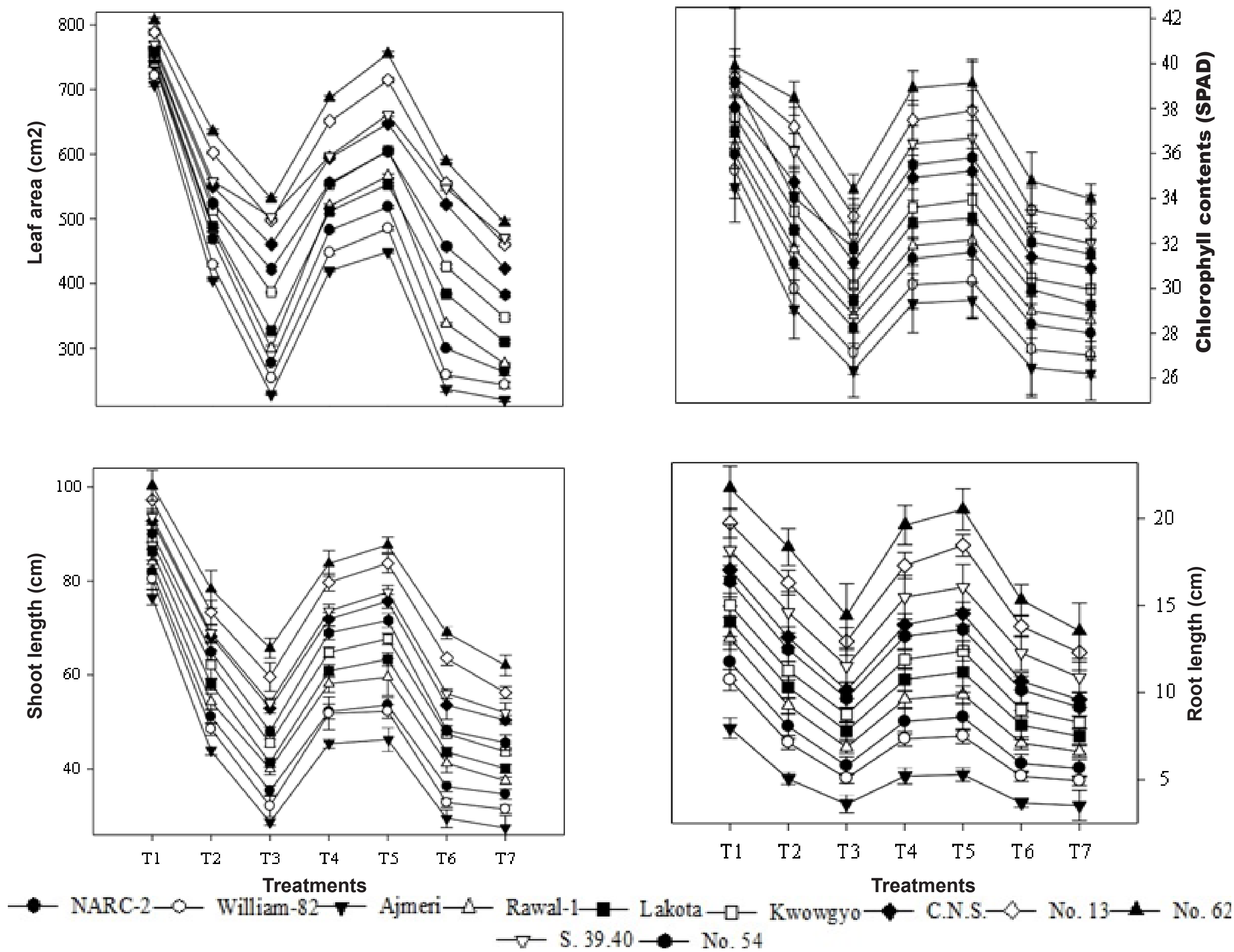

Fig. 2. Effect of salinity and potassium applications on shoot and root lengths (cm), leaf area (cm²) and chlorophyll contents (spad) of soybean varieties. T1: Control, T2: 60mM NaCl, T3: 120mM NaCl, T4: 60mM NaCl+6mM K, T5: 60mM NaCl+09mM K T6: 120mM NaCl+6mM K, T7: 120mM NaCl+09mM K

abilities toward salt tolerance. Least decrease in fresh weight of shoot (62%), dry weight of shoot (63%), fresh weigh of root (67%) and dry weight of root (67%) ware noted in No. 62 genotype taken after by No. 13 genotype, found as salt tolerant genotypes, whereas genotypes sensitive to salt Ajmeri appeared most extreme diminishment, which was 30, 22, 23 and 23% of control individually over parameters taken after by William-82 at 120 milli molar NaCl+09 milli molar KNO_3, while comparing with control. Potassium application made strides salt resistance capacity of all genotypes & critical advancement in parameters described above was watched, that were more at higher application of potassium level. Genotypes tolerant to salts like No. 13 & 62 both gave way best comes about in reaction of the application of potassium with contrast to salt sensitive genotypes (William.82 & Ajmeri) in all levels of potassium applications.ot, root length (cm) and leaf area (cm^2) As the salinization level increased there were a parallel reduction noticed in length of shoot, root as well as in area of leaves among all genotypes of soybean under test (Fig. 2). Maximum increase in lengths of shoot, root and leaf area were recorded in control (salt free) respectively, whereas in 120 milli molar NaCl+09 milli molar KNO_3, minimum increase in same parameters of was recorded. Thus showing best resistance to stress of salts with least reduction was by varieties No. 62 and No 13 respectively however, the genotypes William-82 and Ajmeri showed more reduction. This proved that K most effectively provide a good stand to plants growth against adverse bad effects of NaCl salinization. Genotypes No. 62 and Ajmeri

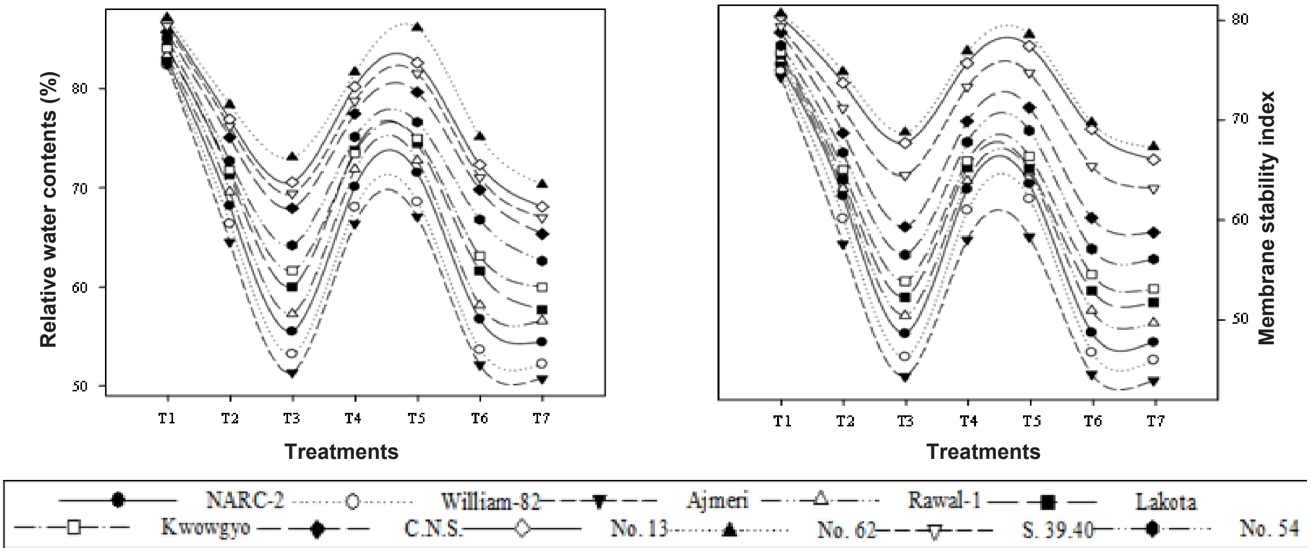

Fig. 3. Effect of salinity and potassium applications on relative water contents (%) and membrane stability index of soybean varieties. T1: Control, T2: 60mM NaCl, T3: 120mM NaCl, T4: 60mM NaCl+6mM K, T5: 60mM NaCl+09mM K T6: 120mM NaCl+6mM K, T7: 120mM NaCl+09mM K

made increase in its shoot, root lengths side by in its leaf areas at 60 milli molar NaCl+09 milli molar KNO_3 in the pattern of 14, 12, 19% and 5, 5, 11%, respectively in related to 60 milli molar NaCl comparison alone, lead to prove positive role of K for managing salinization. With the combined application of highest levels i.e., 09 milli molar of potassium nitrate and sodium chloride i.e.,120 milli molar , the response was noticed quite opposite. To overcome effects of salinity the utilization of potassium was better in genotypes tolerant to salts as compared to genotypes sensitive to salts.

Impact of salinity and potassium applications on (SPAD) chlorophyll contents, (RWP %) relative water contents & (MSI) membrane stability index A noteworthy decline in SPAD (chlorophyll contents) in all soybean genotypes along with RWP-relative water contents & similarly in MSI was recorded with increasing levels of salinization of 60 and 120 milli molar NaCl (Fig. 2 and 3). This Reduction was more in genotypes William-82 and Ajmeri sensitive to salt stress when compared with No. 62 and No. 13 genotypes the salt tolerant. when plants were subjected to 120 milli molar NaCl+09 milli molar KNO_3, highest decrease in growth with respect to control was observed. Chlorophyll contents was non-significantly affected by potassium application in all potassium treatments contrary to other parameters like H_2O contents and stability in membrane, a significant enhancement was detected. The 60 milli molar sodium chloride with and without 09 milli molar potassium, the values of RWC and MSI contents in genotype No. 62 were 86.21, 78.51,78.39 and 74.79, respectively.

Impact of salinity & potassium applications on parameters of gas exchange In 11 genotypes of soybean, photosynthetic rate and other physiological parameters like, conductance of stomata, rate of transpiration and CO_2 concentration within the cells showed almost a similar reducing trend towards salinization (Fig. 4). A maximum of reduction in these physiological parameters were observed at where both NaCl and KNO3 were combined in the ratio of 120 milli molar and + 09 milli molar, respectively. Simultaneously genotypes No. 13& 62 found tolerant to salts as exhibited minimum decrease in gas exchange parameters over the two genotypes of William-82 and Ajmeri which behaved as salt sensitive. The tubs where salinization was induced along with supplemented supply of potassium, here the plants showed an ability not only to overcome the salt stress but also helped in enhancing all types of gas exchange parameters. This enhancement in gas exchange parameters was more where the highest level 60 milli molar NaCl was applied as compared to the treatment where 09 milli molar KNO_3 was added to level of 120 milli molar sodium chloride, a decrease in parameter was observed. Better utilization of potassium was observed by salt tolerant genotypes in comparison to genotypes sensitive to salt.Impact of salinity and applied potassium on Na^+, K^+ (mg /g) contents & sodium potassium ratio in 11 genotypes of soybean leaves and rootsAlmost in all soybean genotype's root and shoots showed a significant variation in the concentration of Na^+ among all the treatments. Least Na^+ concentration was recorded in control treatment which was gradually higher in the treatments of NaCl of 60 milli molar and 120 milli

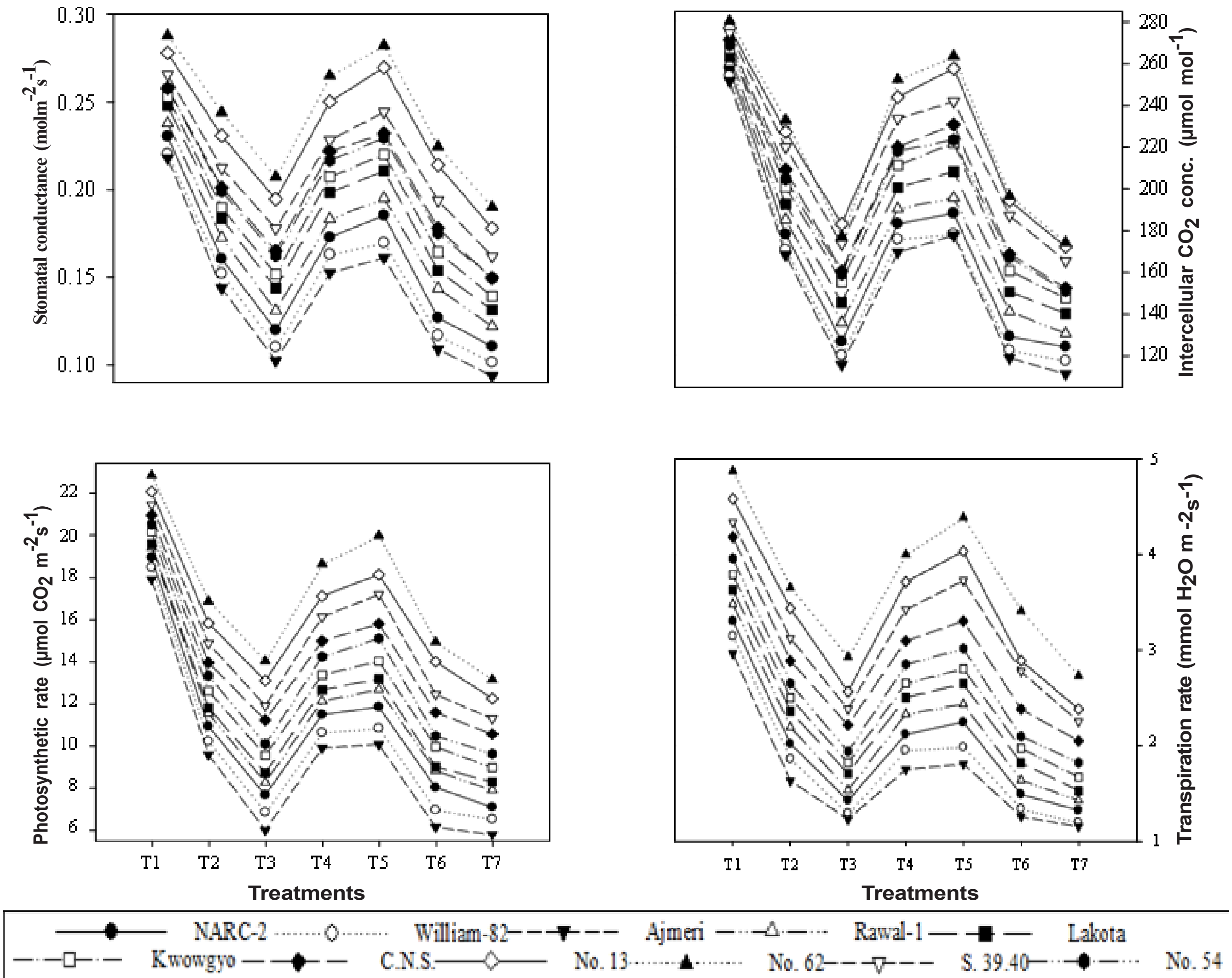

Fig. 4. Effect of salinity and potassium applications on photosynthetic rate (µmol CO2 $m^{-2}s^{-1}$), transpiration rate (mmol H2O $m^{-2}s^{-1}$), stomatal conductance ($molm^{-2}s^{-1}$) and intercellular CO2 conc. (µmol mol^{-1}) of soybean varieties. T1: control, T2: 60mM NaCl, T3: 120mM NaCl, T4: 60mM NaCl + 6mM K, T5: 60mM NaCl + 09mM K T6: 120mM NaCl + 6mM K, T7: 120mM

molar respectively. The genotypes like No. 62 and No. 13 which showed resistibility to salt stress, increment was less whereas in Ajmeri it was maximum after by William.82; demonstrated as salts sensitive. As K^+ adding was made Na^+ concentration was simultaneously decreased in both leaves & roots. However, where the highest level 09 milli molar of K was added in 60 milli molar NaCl, decrease was observed maximum over where 09 milli molar KNO_3 alongwith 120 milli molar sodium chloride was applied contributed contradictory results in the shape of high contents of Na^+ in both leaves & roots.Owing to having less contents of Na in leaves and roots with the addition of K, the No. 62 and No. 13 genotypes were found the best genotypes (Fig. 5). As the NaCl concentration increased within the growth medium, both in leaves and roots the critical K^+ concentration is affected. (Fig. 5). It was observed that either salinity level may be of moderate or higher it significantly will reduce the concentration of K+ in both roots and leaves. That is why K^+ contents were found maximum in control as compared to where NaCl and KNO_3 highest levels applied together. In this way the No. 62 genotype maintained K^+ highest concentration over Ajmeri genotype with lower K^+ contents. Thus the genotypes which were already treated with salts, with the application of K^+ enables themselves in enhancing K concentration in both roots and leaves. This enhancement was observed more at higher KNO_3 level alongwith 60 milli molar sodium chloride.As the level of NaCl increased there was observed a gradual increase in Na^+:K^+ equally in leaf & root, however at the combined level of 120 milli

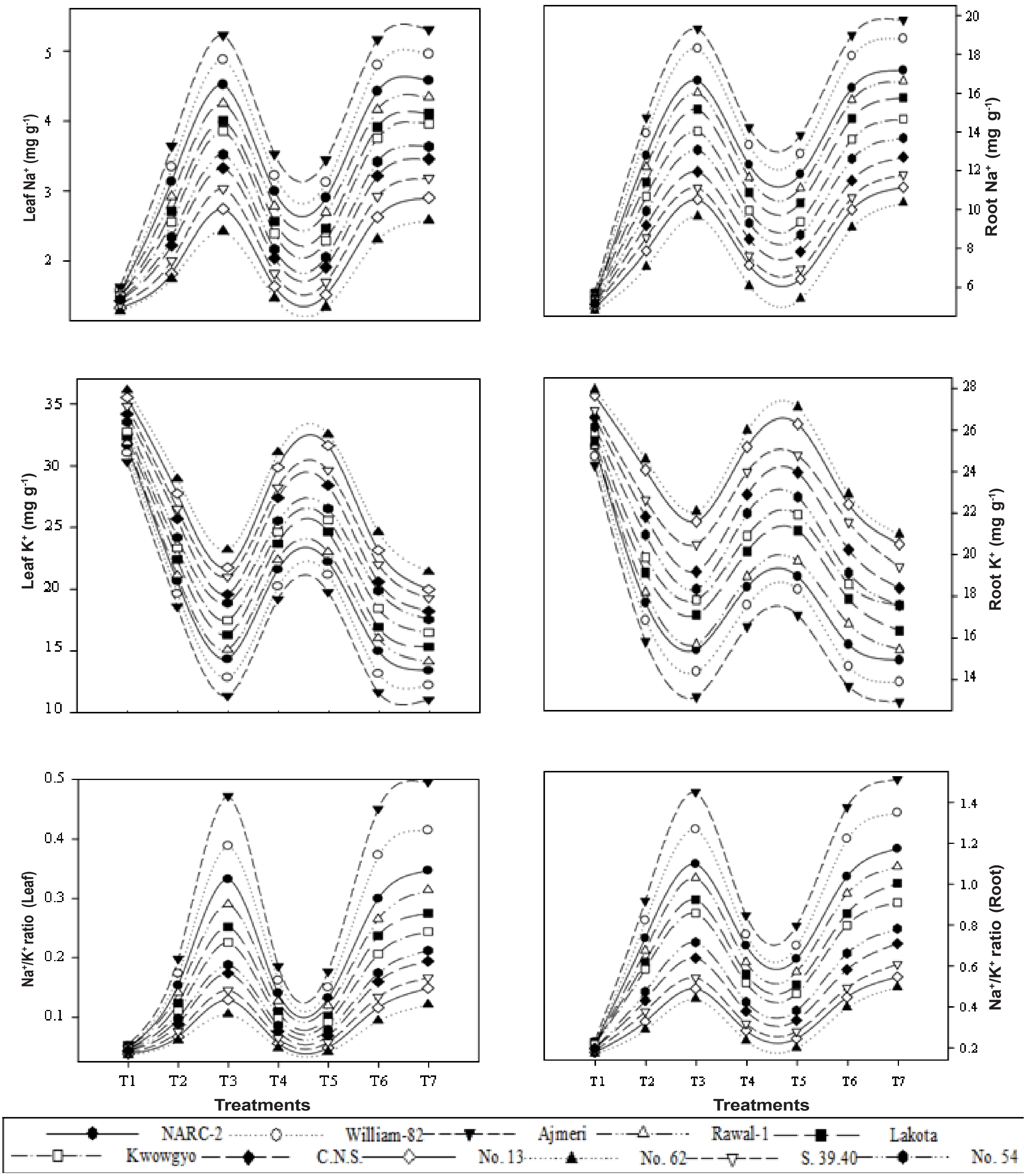

Fig. 5. Effect of salinity and potassium applications on leaf and root contents of Na^+ (mg g^{-1}), K^+ (mg g^{-1}) and Na^+/K^+ ratio of soybean varieties.

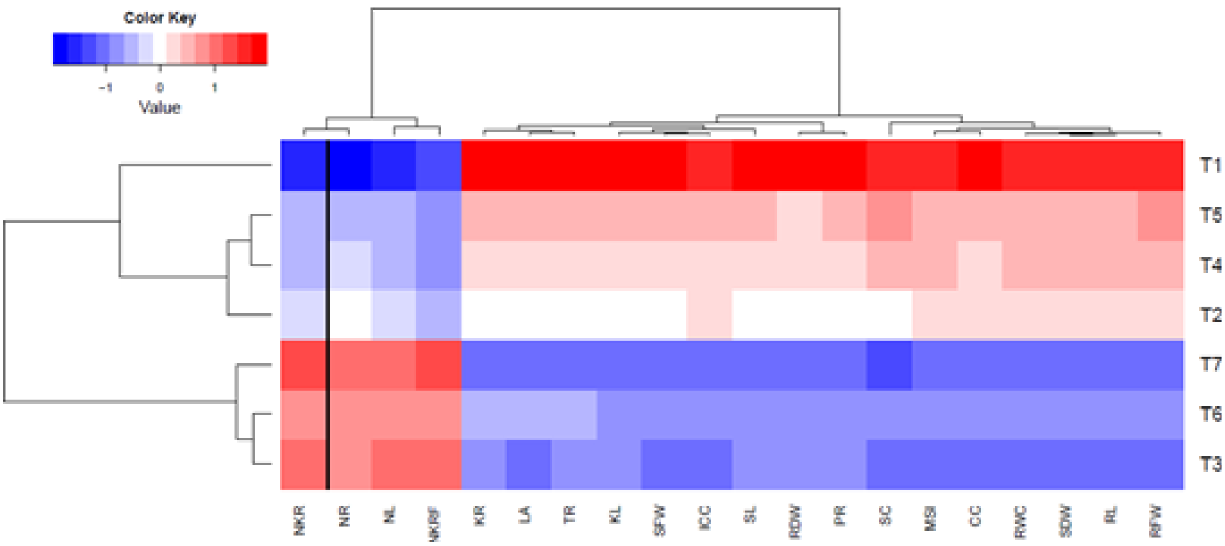

Fig. 6: Performance of treatments on the basis of growth, gas exchange and ionic parameters by heat map ggplot statistical package

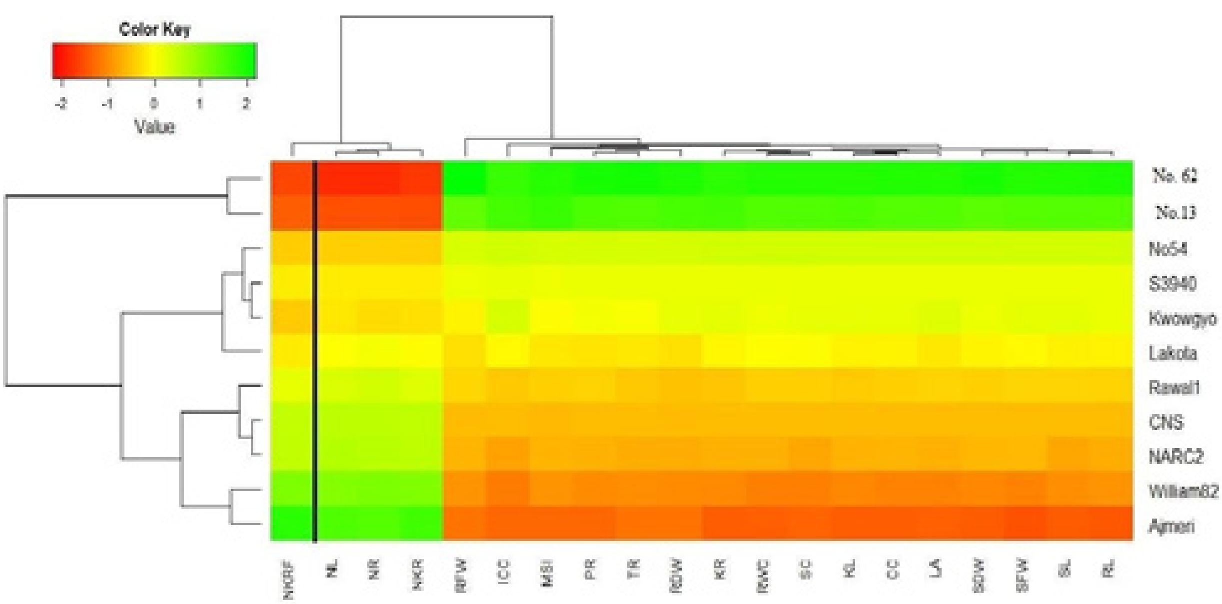

Fig. 7: Performance of soybean varieties/genotypes trend on the basis of growth, gas exchange and ionic parameters by heat map ggplot statistical package

T1: control, T2: EC 60 mM, T3: EC 120 mM, T4: EC 60 mM+ 6 mM KNO_3, T5: EC 60 mM+ 09 mM KNO_3, T6: EC 120 mM+ 6 mM KNO_3, T7: EC 120 mM+ 09 mM KNO_3

molar NaCl+09 milli molar KNO_3, the increment was found highest. A noteworthy variation with respect to different treatments in 11 genotypes under Na^+:K^+ was also observed in both leaf and root. At salinity both levels, the genotype Ajmeri kept highest, while variety No. 62 maintained least ratio of Na^+/K^+ as compared to all other genotypes. In contrast to this, the application of potassium decreases the ratio of Na^+/K^+ in all genotypes in the way that in genotypes tolerant to salts like genotype 62 and 3, reduction was maximum, while in salt sensitive varieties like Ajmeri and William-82, a minimum reduction was observed. Both Greenway and Munns in 1980 also reported that a total of eleven soybean genotypes grown under solution culture of salinity either in alone or in combination with potassium significantly affected the physiological as well as morphological and biochemical parameters. Due to salinity a salient decrease in soybean 11 genotypes growth and yield attributes was detected which was certainly due to excessive salts which causes reduction of H_2O potential in the root zone areas, also cause an imbalance in nutrition with specific ion, which resultantly become a ultimate cause of photosynthetic process reduction in plants and due to this lowering of photosynthetic activity, the overall decline in growth, transpiration and in the contents of chlorophyll as well as water potential and stomatal conductance occurred. In 1993, Munns also reported that either moderate or low salinity only reduce the photosynthetic area and not reduce the photosynthesis per unit leaf area while, Brugnoli and Lauteri in 1991 observed that At high salinity, photosynthetic process in leaf are reduced which may be due to water imbalance and reduced stomatal conductance or it may be also due to some non-stomatal factors and toxicity of ions. It has been observed that when plants under salt stress were given potassium, significant increase in growth is responded which may be due to the potassium ability to overcome salt stress.

Similarly, in 1991, Kefu *et al.* also revealed that adventitious roots are significantly promoted with the application of potassium. However, Freitas *et al* in 2001 and Ashraf in 2004 reported that for the synthesis of protein and for photosynthesis process, potassium is as essential to maintain cellular turgor and osmoregulation. For cell membranes integrity, its proper functioning along with cell membranes maintenance, Wei *et al.* in 2003 described that it is the key role of potassium only. Similarly, Munns in 2002 and Carden *et al.* in 2003, reported that adequate potassium is maintained by plants in their body to manage Na^+, which is maintained through selective uptake of K^+, Na^+ and K^+ selective compartmentation and its distribution within the plant body.Response of all genotypes to salinity and potassium application was extremely variable in the pattern that genotype No. 62 and No. 13 found conversely the best salt tolerant, William-82 and Ajmeri found genotypes that are most salt sensitive. This difference might be due to their different genetic makeup for salts tolerance. Similar results, in stress environment by salts, were likewise stated by Rashid *et al* (1999), Munns, James and Wei *et al* (2003) which they showed that difference in their growth might be due to keep high K^+ amount and less Na^+ amount in their cellular environment and by keeping low ratio of Na^+/K^+ in leaves. In the same way the Cl^- like Na^+ when present in excess hinder uptake of K^+ and may cause deficiency of potassium under extreme conditions.In the present study, the genotype No. 62 and genotype No. 13 were recognized as salt tolerant as both the genotypes maintained lower Na^+, low ratio of Na^+/K^+ with higher K^+ in their cellular environment, which in turn helped plant for better stomatal conductance and photosynthetic rate which are important for better growth. Related findings were also reported by Khadri, Asch *et al* in 2007 and Zhu in 2001, in rice and beans plants and argued it was mere due to reduced ratio of Na^+/K^+. In future screening of more genotypes of soybean may also brought under experimentation against higher levels of salts stress.

CONCLUSION

Application of potassium nitrate improve the mechanism of salt tolerance in soybean varieties/ genotypes. Potassium nitrate dose optimization under saline soils is basic criteria essential to grow soybean under Pakistani soils.

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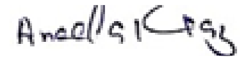
Sr. No.	Author's name	Contribution	Signature
1.	Parveen	Conducted the experiment and wrote the manuscript	
2.	Muhammad Aftab	Reviewed the manuscript	
3.	Ghulam Sarwar	Assisted in result and discussion writeup	
4.	Muhammad Arif	Statistically analysed the data	
5.	Aneela Riaz	Helped in data collection	
6.	Safdar Ali	Supervised the experiments	
7.	Muhammad Anwar ul Haq	Helped in material collection from different source	
8.	Iqra Nawaz	Assisted in conducting the research experiment