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Full Length Article

Nitrogen Nutrition Effects on Growth, Protein and Oil Quality in Soybean (*Glycine max*) Genotypes under Saline Conditions

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Abstract

Nitrogen (N) application affects crop growth and development even under saline condition. This study compared the effects of nitrogenous fertilizer on growth, biochemical activities, proteins and oil quality of soybean (*Glycine max* L.) genotypes under saline conditions. The experiment was comprised of salinity levels (7.5 and 15 dS m^{-1} including control) and two nitrogen rates viz. 25 and 50 kg ha^{-1} using calcium nitrate and ammonium sulphate as a source for NO_3^- and NH_4^+. Soybean growth significantly reduced under adverse saline conditions while N application increased the plant biomass. The results indicated that the application of nitrogen fertilizer in both forms significantly increased the shoot and root fresh as well as dry weights, reducing the leaf Na^+ and enhancing K^+ concentration under normal and saline conditions. Protein and oil contents were also improved by nitrogen nutrition which were significantly reduced by salinity. It was concluded that soybean accession 2429-3130 and 3702 had better biomass production, protein content, oil percent and K^+ contents through N application then Lochlon and Ajmari under both saline and non-saline conditions. Comparing the N sources impact the application of N in the nitrate form was more effective under salt stressed conditions.

Keywords: Nitrogen; Soybean; Salinity; Calcium nitrate and ammonium sulphate; Protein and oil

Introduction

A fertilizer is a nourishing material of natural or synthetic origin which mainly provided to soils or tissues of plants. Thus, it enhances growth of plants (Haq and Mallarino 2005; Mannan 2014). For the purpose to achieve optimal growth balanced nutrients are required in soil solution to regulate adequate concentration for growth during plants developmental stages (Chen 2006). Thus, crops mineral nutrition efficiency can be improved by applying fertilizer through foliage or in growing medium (Mallarino *et al.* 2001). By applying fertilizer as nitrogen, phosphorus, potassium as well as additional nutrients that could affect many processes of physiological nature could be an opportunity to influence economical yield (Haq and Mallarino 2005). Though, beneath high and moderate salinity levels, fertilization affects growth of plants (Haq and Mallarino 2005; Murtaza *et al.* 2014). Hence in interactive studies of nitrogen and salinity, nitrogen supplied form is important (Murtaza *et al.* 2000). According to some studies, increase in nitrate concentration in plants reduce the uptake of chloride and also retard its accumulation (Murtaza *et al.* 2000; Bybordi 2010). During salinity stress in plants nitrate has valuable effects that are related to antagonism between of Na^+ and Cl^- ions (Munns 2002). The existence of higher concentration of NO_3^- enhanced cations translocation such as Ca, K, and Mg, whereas NH_4^+ has been shown to decrease cations concentration (Nadian *et al.* 2012; Murtaza *et al.* 2014). Hence with significant increase of nitrogen contents, sustain C/N ratio, designated to increase the photosynthesis as well as metabolism activities and ultimately increase biomass of plant (Dubey and Pessarakli 1995; Guan *et al.* 2011). Therefore, nitrogen additions to the plants showed symptoms of stress under salinity may improve their tolerance for salt, growth and yield (Jahangir *et al.* 2009; Nadian *et al.* 2012).

As soil salinity is a major abiotic stress with limiting effects on plant growth worldwide and whole to agriculture produced on salt affected areas. Approximately, 7% of total world's soil is affected by salinity and on approximate basis 20% of world to irrigated land (45 m ha) (Yamaguchi and Blumwald 2005; FAO 2007). As an abiotic stress, salinity impacts negatively approximately 20% of 310 million ha lands under irrigation used for crop production, causing an assessed annual loss of US$ 27 billion. It is cost-effective to invest in salt - induced land degradation for sustainable management, investments for active salt affected lands remediation, should be broader strategy in arrangement part, or for security of food and be defined as plans of national action (Qadir *et al.* 2014). Hence, soil salinity presence is

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even before of human existence and farming (Foolad 2004). Thus, more commonly in areas of low rainfall, high temperature, in arid and due to higher degrees of evapotranspiration in poor quality irrigated water areas, salty parent material and poor management practices resulted in net upward salts movement (Neto *et al.* 2006). In Pakistan, most serious environmental problem is salinity that is categorized at eighth in terms of extended area (FAO 2006).

Soybean is a leguminous oilseed crop all around the world by way of with its unique chemical composition and a high-quality digestible protein source (He and Chen 2013; Vagadia *et al.* 2017). It contains about 6 % ash, 17–24% oil, 29% carbohydrates and 37–42% good quality protein (Gibbs *et al.* 2004). It is a good source of essential fatty acids including saturated, and polyunsaturated fatty acids, fibers (USDA 2018), and contains secondary metabolites that are beneficial such as isoflavones, saponins and phenolic components including minerals, vitamins, and comprises of energy (Krishnan 2001; Sakthivelu *et al.* 2008; Khojely *et al.* 2018). By management of agronomic production practices for optimized growth, fertilizer nutrient (Haq and Mallarino 2005; Mannan 2014) and careful genotypes selection influences soybean yield (Matsuo *et al.* 2016; Gulluoglu *et al.* 2017).

Production of soybean at local level is minor and as importation of it as meal and oil of soya has become prerequisite to meet the demand. The cultivation is on restricted areas with diminishing trend, need motivation and techniques to raise the cultivation of soybean to overcome import bill on the edible oil. Therefore, the present study was conducted to check that either nitrogen has ameliorative role in nutrition under saline and normal conditions. While the different sources of nitrogenous fertilizers might be performing better under saline stress.

Materials and Methods

Plant material and conditions for growth

Experiment was conducted in wire house, at University of Agriculture, Faisalabad, Pakistan. Two sets of soybeans (*Glycine max* L.) genotypes, No.2429-3130 and No. 3702 (salt tolerant), Lochlon and Ajmari as salt sensitive were used in the experiment. 12 kg soil was used in pots. Collected soil was dried in air, sieved in 2 mm mesh sized sieve and analysed for physical and chemical characteristics (pH=8.01, EC_e=1.3 d Sm^{-1}, SAR=5.04 (mmol $L^{-1})^{1/2}$ and sandy clay loam texture). Before filling of pots, different salinity levels of 7.5 and 15 dS m^{-1} settled in soil by mixing calculated amount of NaCl using mixer mechanical based on saturation percentage and soil ECe. However, nitrogen levels (0, 25 and 50 kg ha^{-1}) established by using two sources of nitrogen, calcium nitrate and ammonium sulphate at 25 day's intervals as recommended rates. The experiment was comprised of control (no added N), control + 25 kg ha^{-1} N (NO_3^-), control + 25 kg ha^{-1} N (NH_4^+), control + 50 kg ha^{-1} N (NO_3^-), control + 50 kg ha^{-1} N (NH_4^+), 7.5 dS m^{-1} (no added N), 7.5 dS m^{-1}+ 25 kg ha^{-1} N (NO_3), 7.5 dS m^{-1} + 25 kg ha^{-1} N (NH_4^+), 7.5 dS m^{-1}+ 50 kg ha^{-1} N (NO_3), 7.5 dS m^{-1}+ 50 kg ha^{-1} N (NH_4^+), 15 dS m^{-1} (no added N), 15 dS m^{-1}+ 25 kg ha^{-1} N (NO_3^-), 15 dS m^{-1} + 25 kg ha^{-1} N (NH_4^+), 15 dS m^{-1} + 50 kg ha^{-1} N (NO_3^-), 15 dS m^{-1} + 50 kg ha^{-1} N (NO_3^-). The tap water was used for irrigation (EC= 0.76 dS m^{-1}, RSC= 0.78 me L^{-1}, SAR= 4.2). The irrigation was applied when needed and total experimental duration was approximately four months (110 days).

Determination of Na^+ and K^+

Dried leaves of soybean plants were grounded in grinder. The 0.5 g sample was taken in digestion flasks and added 7 mL HNO_3+3 mL $HClO_4$ and digested on hot plate of 4 h and raised gradually. After once approximately 3 mL was left in flask then digestion was stopped. For filtrate Whatman filter paper (No.42) was used and final volume was obtained by addition of 50 mL by distilled water. Then sodium and potassium were determined using flame photometer after standardization (Jones *et al*. 1990).

Oil and protein contents measurement

The oil content measurement followed the method of Matthäus and Brühl (2001). The following formula was used to calculate the oil contents from soya seed.

$$C = +\frac{100 \times Qw}{\mathrm{W} \times (1 - \mathrm{moisture})}$$

The Qw (g) is the oil extracted from seed, W (g) is the weight of ground sample, and moisture % is the moisture percentage of the ground sample.

The sieved soy flours (0.5 g) were homogenized in a test tube with 5 mL of phosphate buffer (0.2 *M*, pH 7.8) for protein contents measurement. The mixture was stirred and centrifuged at 3000 rpm for 10 min. The supernatant (1 mL) was added with 5 mL coomassie brilliant blue G-250 (CBB) and this mixture was analyzed by the method of Soluble Protein Content Assay according to Bradford (1976).

Statistical analysis

Analysis of variance (ANOVA) was used for data analysis up to with two-way interaction using software "Statistix 8.1" for the analysis and presented by as mean of three replicates of ± SE and $P < 0.05$ value used for checked significance (Steel *et al*. 1997).

Results

Effect of salinity on growth and quality of soybean

There was a significant ($P < 0.05$) difference in soybean growth under salt stress and normal conditions. The highest shoot fresh and dry weights, root fresh and dry weights were

Table 1: Shoot fresh weight (g plant^{-1}) of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	26.3 ± 0.49	23.8 ± 0.88	18.8 ± 0.58	18.2 ± 0.78
	NO_3^- kg ha^{-1}	25	34.0 ± 0.44	32.0 ± 0.55	25.5 ± 0.68	23.4 ± 0.76
	NH_4^+ kg ha^{-1}	25	32.4 ± 0.47	31.4 ± 0.45	23.9 ± 0.87	22 ± 0.69
	NO_3^- kg ha^{-1}	50	35.5 ± 0.75	34.7 ± 0.75	26.1 ± 1.06	25.5 ± 0.73
	NH_4^+ kg ha$^-$	50	36.8 ± 0.61	35.1 ± 0.61	25.4 ± 0.53	23.8 ± 0.91
7.5 dS m^{-1}		0	23.3 ± 1.06	20.8 ± 0.66	14.8 ± 1.09	13.2 ± 0.81
	NO_3^- kg ha^{-1}	25	26. ± 0.46	25.4 ± 0.73	17.2 ± 0.87	14.9 ± 0.67
	NH_{4+} kg ha^{-1}	25	25.4 ± 0.8	24.5 ± 0.7	16.5 ± 0.65	14.5 ± 0.57
	NO_3^- kg ha^{-1}	50	29.0 ± 0.73	27.0 ± 0.36	20.3 ± 0.61	18.3 ± 0.25
	NH_4^+ kg ha^{-1}	50	28.8 ± 0.34	26.2 ± 0.66	19.6 ± 1.24	16.8 ± 0.6
15 dS m^{-1}		0	19.9 ± 0.8	14.5 ± 0.4	14.5 ± 1.2	13.19 ± 0.9
	NO_3^- kg ha^{-1}	25	23.1 ± 0.80	22.1 ± 1.0	17.2 ± 0.9	15.6 ± 0.8
	NH_4^+ kg ha^{-1}	25	23.1 ± 0.5	21.8 ± 1.1	18.8 ± 0.2	14.8 ± 1.1
	NO_3^- kg ha^{-1}	50	25.5 ± 0.8	23.5 ± 1.2	18.8 ± 0.2	18.2 ± 0.5
	NH_4^+ kg ha^{-1}	50	23.9 ± 0.7	22.9 ± 0.9	16.2 ± 0.4	15.5 ± 0.7

(Each value is an average of three replicates± S.E)

Table 2: Shoot dry weight (g plant^{-1}) of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	5.6 ± 0.8	4.6 ± 0.1	2.8 ± 0.5	2.6 ± 0.2
	NO_3^- kg ha^{-1}	25	5.7 ± 0.2	5.6 ± 0.6	2.8 ± 0.3	2.47 ± 0.10
	NH_4^+ kg ha^{-1}	25	5.7 ± 0.1	4.6 ± 0.2	2.8 ± 0.1	2.7 ± 0.1
	NO_3^- kg ha^{-1}	50	5.7 ± 0.3	4.7 ± 0.1	2.9 ± 0.3	2.7 ± 0.3
	$NH_4^{\pm}$ kg ha$^-$	50	5 .7 ± 0.2	4.6 ± 0.2	2.8 ± 0.10	2.7 ± 0.1
7.5 dS m^{-1}		0	4.5 ± 0.1	3.5 ± 0.1	1.9 ± 0.1	2.1 ± 0.1
	NO_3^- kg ha^{-1}	25	4.6± 0.4	3.5 ± 0.	2.0 ± 0.2	2.1 ± 0.2
	NH_4^+ kg ha$^-$	25	4.6 ± 0.2	3.5 ± 0.2	1.9 ± 0.1	2.1 ± 0.6
	NO_3^- kg ha^{-1}	50	4.8 ± 0.1	3.6 ± 0.2	2.2 ± 0.2	2.1 ± 0.1
	NH_4^+ kg ha^{-1}	50	4.6 ± 0.2	3.5 ± 0.2	2.1 ± 0.2	2.1 ± 0.6
15 dS m^{-1}		0	3.3 ± 0.1	2.7 ± 0.1	1.4 ± 0.10	1.2 ± 0.01
	NO_{3-} kg ha^{-1}	25	3.3 ± 0.5	2.8 ± 0.4	1.5 ± 0.10	2.1 ± 0.05
	NH_4^+ kg ha^{-1}	25	3.3 ± 0.2	2.8 ± 0.2	1.4 ± 0.1	1.2 ± 0.17
	NO_3^- kg ha^{-1}	50	3.4 ± 0.1	2.8 ± 0.7	1.5 ± 0.2	1.3 ± 0.1
	NH_4^+ kg ha^{-1}	50	3.4 ± 0.8	2.8 ± 0.4	1.5 ± 0.4	1.3 ± 0.3

(Each value is an average of three replicates± S.E.)

observed under normal conditions and significantly decreased at EC 7.5 and 15 dS m^{-1}. In case of salt stressed conditions, the highest shoot and root traits were found in accession No.2429-3130 and lowest values for these traits in Ajmari at EC 15 dS m^{-1}. A significant difference was also observed in leaf Na^+ and K^+ concentration among the all these tested genotypes (Table 5–6). The increase in Na^+ was observed under both the salinity levels as compared to control. The highest Na^+ concentration was recorded in tolerant genotypes No.2429-3130 and No. 3702 rather than Lochlon and Ajmari. While K^+ concentration showed a significant reduction at 15 dS m^{-1} compared to respective control and 7.5 dS m^{-1} in all four soybean genotypes. The tolerant genotypes sustained relatively high K^+ concentration than the salt sensitive genotypes. Protein and oil% contents (Table 7 and 8), decreased under salt stress condition compared to their respective control.

Effect of nitrogenous nutrition on soybean growth and quality

The application of N increased the shoot fresh and dry weights, root fresh and dry weights under both saline and normal condition however there was a significant ($P < 0.05$) difference between the soybean genotypes under salt stressed conditions (Table 1–4). Plant leaf ionic contents very significantly affected by treatments and with high significant difference between the genotypes. The interactive effect of nitrogen treatment was highly significant. The application of both nitrate and ammonium form significantly reduced concentration of leaf Na^+ and increased the leaf K^+ concentration, and consequently the growth. Increased nitrogen supply led towards increased ratio of leaf K: Na in salinity stress condition (Table 9). The salt-tolerant genotypes responded more efficiently to nitrogen application at significant values than salt-sensitive genotypes. The effect of nitrate application was more as compared to ammonium and in the same way more to salt tolerant than to sensitive. Application of nitrogen at both sources (NO_3^- and NH_4^+) and levels (25 and 50 kg ha^{-1}) had significant differences and increased the protein and oil content of soybean. However, response was pronounced in tolerant genotypes as compared to sensitive (Lochlon and Ajmari) ones.

Discussion

Salinity is worldwide problem causing threat to agricultural food production and sustainability. It also encourages other stresses that negatively impacts crop

Table 3: Root fresh weight (g $plant^{-1}$) of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	5.2 ± 0.09	4.1 ± 0.06	3.9 ± 0.19	2.8 ± 0.02
	NO_3^- kg ha^{-1}	25	6.5 ± 0.26	5.2 ± 0.13	4.3 ± 0.31	3.4 ± 0.20
	NH_4^+ kg ha^{-1}	25	5.6 ± 0.30	4.9 ± 0.16	4.1 ± 0.45	3.1 ± 0.05
	NO_3^- kg ha^{-1}	50	7.8 ± 0.28	6.6 ± 0.35	5.1 ± 0.19	5.1 ± 0.15
	NH_4^+ kg ha^-	50	7.7 ± 0.26	6.1 ± 0.32	4.8 ± 0.20	3.9 ± 0.22
7.5 dS m^{-1}		0	5.3 ± 0.55	4.5 ± 0.29	2.2 ± 0.16	2.2 ± 0.18
	NO_3^- kg ha^{-1}	25	6.7 ± 0.21	5.3 ± 0.15	2.6 ± 0.25	2.3 ± 0.26
	NH_4^+ kg ha^-	25	5.4 ± 0.17	5.0 ± 0.09	2.2 ± 0.20	2.0 ± 0.5
	NO_3^- kg ha^{-1}	50	6.2 ± 0.05	5.6 ± 0.24	3.5 ± 0.13	3.6 ± 0.22
	NH_4^+ kg ha^{-1}	50	5.9 ± 0.16	5.2 ± 0.03	3.4 ± 0.17	3.1 ± 0.36
15 dS m^{-1}		0	3.9 ± 0.36	3.5 ± 0.36	2.2 ± 0.20	1.6 ± 0.28
	NO_3^- kg ha^{-1}	25	4.5 ± 0.24	4.0 ± 0.09	2.2 ± 0.20	2.1 ± 0.07
	NH_4^+ kg ha^{-1}	25	4.2 ± 0.10	3.9 ± 0.03	2.4 ± 0.07	1.9 ± 0.07
	NO_3^- kg ha^{-1}	50	5.0 ± 0.34	5.0 ± 0.34	2.5 ± 0.19	2.2 ± 0.29
	NH_4^+ kg ha^{-1}	50	4.4 ± 0.06	4.3 ± 0.29	2.5 ± 0.19	2.0 ± 0.06

(Each value is an average of three replicates± S.E)

Table 4: Root dry weight (g $plant^{-1}$) of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	0.88 ± 0.04	0.70 ± 0.06	0.35 ± 0.07	0.26 ± 0.03
	NO_3^- kg ha^{-1}	25	1.05 ± 0.06	0.90 ± 0.01	0.56 ± 0.03	0.40 ± 0.04
	NH_4^+ kg ha^{-1}	25	0.91 ± 0.09	0.84 ± 0.06	0.49 ± 0.03	0.37 ± 0.04
	NO_3^- kg ha^{-1}	50	1.31 ± 0.15	1.55 ± 0.12	0.81 ± 0.06	0.63 ± 0.03
	NH_4^+ kg ha^-	50	1.18 ± 0.09	1.40 ± 0.04	0.75 ± 0.04	0.49 ± 0.04
7.5 dS m^{-1}		0	0.75 ± 0.07	0.79 ± 0.04	0.45 ± 0.03	0.36 ± 0.03
	NO_3^- kg ha^{-1}	25	0.92 ± 0.10	0.84 ± 0.07	0.49 ± 0.03	0.42 ± 0.01
	NH_4^+ kg ha^-	25	0.87 ± 0.07	0..82 ± 0.01	0.43 ± 0.05	0.39 ± 0.04
	NO_3^- kg ha^{-1}	50	1.05 ± 0.05	0.96 ± 0.02	0.63 ± 0.06	0.49 ± 0.04
	NH_4^+ kg ha^{-1}	50	0.98 ± 0.10	0.80 ± 0.05	0.42 ± 0.05	0.45 ± 0.03
15 dS m^{-1}		0	0.34 ± 0.01	0.36 ± 0.03	0.42 ± 0.05	0.16 ± 0.01
	NO_3^- kg ha^{-1}	25	0.38 ± 0.01	0.37. ± 0.01	0.21 ± 0.03	0.18 ± 0.03
	NH_4^+ kg ha^{-1}	25	0.33 ± 0.02	0.28 ± 0.00	0.24 ± 0.04	0.14 ± 0.01
	NO_3^- kg ha^{-1}	50	0.41 ± 0.04	0.37 ± 0.01	0.20 ± 0.01	0.23 ± 0.03
	NH_4^+ kg ha^{-1}	50	0.35 ± 0.01	0.32 ± 0.01	0.19 ± 0.01	0.17 ± 0.01

(Each value is an average of three replicates± S.E)

Table 5: Leaf Na^+ (mg/g DW) content of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	4.9 ± 0.4	4.7 ± 0.4	2.3 ± 0.1	2.8 ± 0.4
	NO_3^- kg ha^{-1}	25	4.1 ± 0.2	3.4 ± 0.1	1.8 ± 0.4	2.10 ± 0.2
	NH_4^+ kg ha^{-1}	25	5.4 ± 0.3	4.2 ± 0.6	2.1 ± 0.1	7.4 ± 0.6
	NO_3^- kg ha^{-1}	50	3.5 ± 0.2	2.7 ± 0.1	1.5. ± 0.7	2.4 ± 0.3
	NH_4^+ kg ha^-	50	3.9 ± 0.1	3.2 ± 0.5	1.6 ± 0.1	1.7 ± 0.1
7.5 dS m^{-1}		0	7.4 ± 0.2	6.3 ± 0.2	4.5 ± 0.5	4.2 ± 0.1
	NO_3^- kg ha^{-1}	25	6.1 ± 0.2	5.8 ± 0.2	3.9 ± 0.1	3.8 ± 0.3
	NH_4^+ kg ha^-	25	6.6 ± 0.2	6.0 ± 0.2	4.2 ± 0.4	4.0 ± 0.5
	NO_3- kg ha^{-1}	50	4.7 ± 0.3	4.7 ± 0.8	3.2 ± 0.1	3.0 ± 0.2
	NH_4^+ kg ha^{-1}	50	5.9 ± 0.2	5.6 ± 0.2	3.8 ± 0.3	3.6 ± 0.7
15 dS m^{-1}		0	8.3 ± 0.3	7.6 ± 0.3	6.1 ± 0.20	5.6 ± 0.6
	NO_3^- kg ha^{-1}	25	8.0 ± 0.3	7.3 ± 0.2	5.5 ± 0.20	5.2 ± 0.1
	NH_4^+ kg ha^{-1}	25	8.2± 0.3	7.4 ± 0.7	5.9 ± 0.2	5.4 ± 0.5
	NO_3^- kg ha^{-1}	50	6.1 ± 0.2	4.5 ± 0.2	4.1 ± 0.2	3.6 ± 0.5
	NH_4^+ kg ha^{-1}	50	7.8 ± 0.3	7.1 ± 0.3	5.4 ± 0.9	5.0 ± 0.3

(Each value is an average of three replicates± S.E)

growth like osmotic, nutrient deficiency and specific ion toxicity, through upsetting growth of plant and development by varying physiological and biochemical mechanisms associated (Sairam *et al.* 2002; Chen 2006; Hanin *et al.* 2016; Shu *et al.* 2017). The increase in concentrations of NaCl decreased the development of plants of soybean in both types of genotypes tolerant and sensitive; it could be due to osmotic stress and specific ion toxicity of Na^+ as well as Cl^- in the root which also hinders the uptake of other ions and nutrients (Parveen *et al.* 2016).

Additionally, soybean plants have a large harvest index for nitrogen as compared to other legumes. The soybean cultivars exposed to nitrogen application had an optimistic effect on yield of soya seed (Jahangir *et al.* 2009; Maw *et al.* 2011). This study results are similar to Maw *et al.* (2011) that application of nitrate to soybean cultivars increased the yield and this increase were mainly attributed to accumulation of dry matter in leaves at fifth stage of vegetative growth. Tshivhandekano and Lewis (1993) revealed that maize and wheat fed within NH_4^+ more

Table 6: Leaf K^+ (mg/g DW) content of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	40.6 ± 1.7	37.5 ± 1.1	21.0 ± 1.6	20.20 ± 1.0
	NO_3^- kg ha^{-1}	25	45.8 ± 1.3	42.8 ± 1.1	24.4 ± 0.9	23.50 ± 0.5
	NH_4^+ kg ha^{-1}	25	44.2 ± 1.2	41.2 ± 2.1	23.1 ± 1..2	21.90 ± 1.1
	NO_3^- kg ha^{-1}	50	51.3 ± 2..7	47 ± 1.7	28.3 ± 0.8	27.20 ± 0.9
	NH_4^+ kg ha^-	50	48.0 ± 1.8	45.4 ± 2.0	26.4 ± 0.9	25.50 ± 0.7
7.5 dS m^{-1}		0	34.3 ± 0.9	30.1 ± 1.0	14.5 ± 0.5	13.20 ± 0.9
	NO_3^- kg ha^{-1}	25	38.9 ± 1.4	34.8 ± 1.4	16.2 ± 0.5	16.40 ± 0.3
	NH_4^+ kg ha^-	25	37.0 ± 1.4	33.3 ± 1.4	15.2 ± 0.6	14.8 ± 0.5
	NO_3^- kg ha^{-1}	50	45.1 ± 1.0	41.4 ± 0.9	17.9 ± 0.8	18.8 ± 0.7
	NH_4^+ kg ha^{-1}	50	41.8 ± 1.7	38.3 ± 1.1	16.1 ± 1.0	16.4 ± 0.8
15 dS m^{-1}		0	25.1 ± 0.70	23,3 ± 1.2	9.2 ± 0.00	8.6 ± 0.2
	NO_3^- kg ha^{-1}	25	29.3 ± 1.03	27.1 ± 0.9	12.0 ± 0.70	11.8 ± 0.3
	NH_4^+ kg ha^{-1}	25	27.2 ± 1.0	25.0 ± 0.6	11.2 ± 0.6	10.6 ± 0.3
	NO_3^- kg ha^{-1}	50	34.3 ± 1.1	32.2 ± 1.1	13.9 ± 0.2	14.1 ± 0.4
	NH_4^+ kg ha^{-1}	50	31.8 ± 1.7	29.2 ± 0.7	12.6 ± 0.3	12.4 ± 0.9

(Each value is an average of three replicates± S.E)

Table 7: Protein content (%) of soybean seed to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	43.43 ± 0.57	42.11 ± 0.77	41.76 ± 0.35	40.24 ± 0.43
	NO_3^- kg ha^{-1}	25	45.76 ± 1.46.	44.93 ± 1.24	43.52 ± 2.63	43.90 ± 1.10
	NH_4^+ kg ha^{-1}	25	45.10 ± 0.86	44.60 ± 0.73	45.10 ± 0.57	43.60 ± 1.04
	NO_3^- kg ha^{-1}	50	49.60 ± 0.89	47.26 ± 0.72	46.10 ± 1.55	45.60 ± 1.03
	NH_4^+ kg ha^{-1}	50	49.21 ± 0.89	47.06 ± 0.80	45.96 ± 1.09	44.13 ± 1.93
7.5 dS m^{-1}		0	41.86 ± 0.34	41.60 ± 0.41	42.10 ± 0.68	39.36 ± 0.95
	NO_3^- kg ha^{-1}	25	46.76 ± .74	44.26 ± 0.43	43.80 ± 2.99	41.89 ± 2.30
	NH_4^+ kg ha^{-1}	25	45.76 ± 0.87	46.93 ± 0.73	44.10 ± 1.84	43.26 ± 0.73
	NO_3^- kg ha^{-1}	50	47.76 ± 0.50	48 06 ± 0.66	45.76 ± 2.00	44.33 ± 1.02
	NH_4^+ kg ha^{-1}	50	46.10 ± 1.84	45.26 ± 1.00	45.10 ± 1.62	41.93 ± 0.90
15 dS m^{-1}		0	42.10 ± 0.68	41.70 ± 0.85	39.43 ± 0.87	36.26 ± 1.05
	NO_3^- kg ha^{-1}	25	45.20 ± 0.50	43.66 ± 0.60	42.76 ± 0.50	39.26 ± 0.56
	NH_4^+ kg ha^{-1}	25	44.80 ± 1.17	43.26 ± 1.41	43.76 ± 0.46	37.60 ± 1.20
	NO_3^- kg ha^{-1}	50	46.43 ± 1.54	44.60 ± 0.62	45.76 ± 0.65	41.60 ± 1.61
	NH_4^+ kg ha^{-1}	50	45.10 ± 0.88	42.60 ± 0.75	44.43 ± 0.65	39.96 ± 1.31

(Each value is an average of three replicates± S.E)

Table 8: Oil (% DM) of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen		No. 2429-3130	No. 3702	Lochlon	Ajmari
Control		0	18.89 ± 0.29	18.19 ± 0.39	17.67 ± 0.52	16.68 ± 0.15
	NO_3^- kg ha^{-1}	25	19.56 ± 0.59	18.86 ± 0.34	18.34 ± 0.75	17.00 ± 0.53
	NH_4^+ kg ha^{-1}	25	19.22 ± 0.23	18.52 ± 0.61	18.01 ± 1.23	17.00 ± 0.67
	NO_3^- kg ha^{-1}	50	20.22 ± 0.91	19.52 ± 0.34	19.01 ± 1.76	18.00 ± 1.73
	NH_4^+ kg ha^{-1}	50	19.89 ± 0.30	19.19 ± 0.51	18.68 ± 0.89	17.33 ± 1.00
7.5 dS m^{-1}		0	18.22 ± 0.78	17.52 ± 0.51	17.01 ± 0.89	16.02 ± 1.00
	NO_3^- kg ha^{-1}	25	19.47 ± 0.40	18.52 ± 0.66	18.01 ± 0.73	17.22 ± 0.67
	NH_4^+ kg ha^{-1}	25	18.89 ± 0.59	18.19 ± 0.65	17.68 ± 1.45	16.67 ± 0.44
	NO_3^- kg ha^{-1}	50	20.22 ± 0.67	19 66 ± 0.44	19.38 ± 0.74	17.23 ± 1.00
	NH_4^+ kg ha^{-1}	50	19.89 ± 0.38	19.19 ± 0.63	18.86 ± 0.66	17.14 ± 1.12
15 dS m^{-1}		0	16.89 ± 0.62	16.52 ± 1.06	15.01 ± 0.65	13.33 ± 0.36
	NO_3^- kg ha^{-1}	25	17.22 ± 0.93	16.86 ± 0.59	16.34 ± 0.23	14.67 ± 0.78
	NH_4^+ kg ha^{-1}	25	17.16 ± 0.36	16.69 ± 0.18	16.21 ± 0.37	14.40 ± 0.45
	NO_3^- kg ha^{-1}	50	17.56 ± 0.58	17.19 ± 0.88	16.74 ± 0.95	15.17 ± 0.27
	NH_4^+ kg ha^{-1}	50	17.39 ± 0.35	17.02 ± 0.84	16.54 ± 0.42	14.83 ± 0.60

(Each value is an average of three replicates± S.E)

sensitive to salinity than plants fed with NO_3^- when grown in solution culture.

The dry matter of cotton and corn decreases by increase in salinity but by application of nitrogen increases (Homaee *et al.* 2002) the growth. The salt tolerant genotypes-maintained K^+ higher levels and enhanced growth than salt sensitive genotypes. The previously this has been reported in various crops including wheat, rice (Murtaza *et al.* 2014), tomato (Amjad *et al.* 2014), spinach, strawberry (Kaya *et al.* 2001, 2003) and soybean (Jahangir *et al.* 2009; Parveen *et al.* 2016). Parveen *et al.* (2016) reported that salinity severely reduced the growth of soybean plants and yield by upsetting morphological, physiological processes in all soybean genotypes yet more pronounced effect was on sensitive plant as compare to tolerant. Nadian *et al.* (2012) found that by increasing salinity noticeably decreased root and shoot growth. The high Na inhibitory effect on K uptake concentrations and also on growth of plant improved with increased nitrogen supply and this led to increase in ratio K: Na in leaf under

Table 9: K^+/Na^+ ratio of soybean genotypes to different levels of NaCl and Nitrogen after 110 days of stress

Salinity	Nitrogen form	Level	No.2429-3130	No. 3702	Lochlon	Ajmari
Control		0	8.28 ± 0.7	7.97 ± 0.1	9.13 ± 1.6	7.21 ± 1.0
	NO_3^- kg ha^{-1}	25	11.17 ± 0.3	12.60 ± 0.1	13.55 ± 0.9	11.19 ± 0.5
	NH_4^+ kg ha^{-1}	25	8.18 ± 0.2	9.81 ± 1.1	10.95 ± 1..2	7.25 ± 0.5
	NO_3 kg ha^{-1}	50	14.65 ± 0..7	17.40 ± 1.7	18.86 ± 0.8	11.33 ± 0.7
	NH_4^+ kg ha^{-1}	50	12.30 ± 0.8	14.20 ± 2.0	16.50 ± 0.9	15.00 ± 0.7
7.5 dS m^{-1}		0	4.64 ± 0.9	4.77 ± 0.0	3.22 ± 0.5	3.14 ± 0.9
	NO_3^- kg ha^{-1}	25	6.38 ± 0.14	5.52 ± 0.4	4.15 ± 0.5	4.32 ± 0.3
	NH_4^+ kg ha^{-1}	25	5.6 ± 0.4	5.55 ± 1.4	3.62 ± 0.6	3.70 ± 0.5
	NO_3 kg ha^{-1}	50	9.59 ± 1.0	8.80 ± 0.9	5.59 ± 0.8	6.27 ± 0.7
	NH_4^+ kg ha^{-1}	50	7.08 ± 0.7	6.83 ± 1.1	4.20 ± 1.0	4.55 ± 0.8
15 dS m^{-1}		0	3.02 ± 0.70	3.06 ± .08.	1.51 ± 0.00	1.54 ± 0.2
	NO_3^- kg ha^{-1}	25	3.66 ± 0.03	3.71 ± 0.9	2.19 ± 0.70	2.27 ± 0.3
	NH_4^+ kg ha^{-1}	25	3.32 ± 0.0	3.37 ± 0.6	1.90 ± 0.6	1.96 ± 0.3
	NO_3 kg ha^{-1}	50	5.62 ± 0.9	7.10 ± 1.1	3.39 ± 0.2	3.92 ± 0.4
	NH_4^+ kg ha^{-1}	50	4.0 ± 0.27	4.11 ± 0.7	2.33 ± 0.3	2.48 ± 0.9

(Each value is an average of three replicates± S.E)

conditions of stress. In fact, nitrogen applications more than recommended rate compensate the detrimental effects under salinity stress.

Thus, beneath salinity stress conditions nitrate valuable effects are related to antagonism between of Na^+ and Cl^- ions (Munns 2002). The existence of higher concentration of NO_3^- enhanced cations translocation such as Ca, K, and Mg, whereas NH_4^+ has been shown to decreased cations concentration (Nadian *et al.* 2012; Murtaza *et al.* 2014). Hence with significant increase of nitrogen content, C/N ratio decreased, designated by increased the photosynthesis as well as with metabolism activity and ultimately increase in biomass of plant (Dubey and Pessarakli 1995; Guan *et al.* 2011). Therefore, nitrogen additions to the plants show symptoms of stress under salinity improved their tolerance to salt, growth and finally yield (Jahangir *et al.* 2009; Nadian *et al.* 2012).

As form of nitrogen application effect, the growth, with mixed addition of NO_3^-/NH_4^+ produced highest yields under saline and normal conditions of soil (Cox and Reisenauer 1973; Botella *et al.* 1997; Drihem and Pilbeam 2002). Also, stromal contents and proteins of thylakoid increased by improved nitrogen supply in the chloroplast of leaf and finally enhanced leaves photosynthetic capacity (Homaee *et al.* 2002). Accumulation of solutes takes place under the sufficient nitrogen supply, important role of these in osmoticum adjustments as glycinebetaine, glutamate, proline, carnitine, sorbitol, fructans, polyols, trehalose, sucrose and oligosaccharides also increased by potassium and phosphorus added nutrition (Nadian *et al.* 2012). As osmolytes precisely produced by plants and counter the salinity osmotic deficit efficiently through solutes accumulation in cytoplasm and in vacuole by seizing the toxic ions (Knight *et al.* 2000; Munns and Tester 2008). Fertilizer application at optimum rates to soils under salinity moderately lighten the adversial salinity effects on photosynthesis and also on photosynthesis-related parameters and yield components by full filling the nutritional demands of salt effected plants (Albassam 2001; Sultana *et al.* 2001). The appropriate and suitable use of nitrogen fertilizer in all types of soil is vital, but mostly in saline soils, where nitrogen use may minimize the damaging effects of salinity on growth of plant and yield (Shen *et al.* 1994; Flores *et al.* 2001; Abdelgadir *et al.* 2005).

Thus addition of NH_4^+ in place of NO_3^- in structures can reduces the uptake of other cations, like Mg^{2+}, Ca^{2+} and K^+, that could be described by antagonism between cations and NH_4^+ The proportion of these effect differ according to factors between regulations made in the ionic balance of nutrients and growing conditions. Consequently, a vigilant use of NH_4^+ is suggested for crops which are sensitive to Ca deficiency including sweet pepper and tomato (Sonneveld and Voogt 2009).

As it is recognizable, that salt stress affected the soybean plants physiology significantly that resulted to decreased growth; nevertheless, better growth maintained in salt tolerant genotypes. Nitrogen application decreased the NaCl toxic effects which result in low levels of Na^+ to tissue and activities of antioxidant enzymes in favorable conditions, enhanced photosynthetic features and consequently enhanced growth of plants. Thus, highest levels of nitrogen addition and nitrate form can be used as a good amendment facilitator against salt stress and also as a remedy for sensitive species/varieties for production of crop in stressed environment. Reduced crop productivity at high salinity generally triggered by an ionic imbalance causing toxicity, due to osmotic stress and ROS production in soybean plants (Akhtar *et al*. 2010; Jahangir *et al.* 2009; You and Chan 2015; Parveen *et al.* 2016). Salinity stress delayed the flowering and pod maturity enhanced in soybean ultimately effect grain development, causing it to shrivel (Jahangir *et al.* 2009; Parveen *et al.* 2016). Thus, this response was steady for salt tolerant and genotypes in flowering, reproductive and grain-filling stages, with significantly fewer pods per plant and leading towards lower grain yield (grain plant^{-1}) (Mannan *et al.* 2013). The salinity stress negatively affect yield and quality mainly due to short duration for protein and accumulation of oil by reducing seed yield per plant (Krasensky and Jonak 2012; Sabagh *et al.* 2015a, b).

Conclusion

Salinity stress adversely reduced the growth of all genotypes while the application of N increased the plant growth under both saline and non-saline conditions. The application of N was more beneficial for accession tolerant soybean genotype which produced drier biomass production, protein content and oil percent and K content through N application rather than sensitive genotypes under both saline and non-saline conditions. Application of nitrate form increased the plant growth and improved the protein and oil percent and K^+ content as compared to ammonium form. Hence, it was concluded that the application of N fertilizers in the nitrate form is more beneficial for soybean crop under saline conditions rather than NH_4.

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Author Contributions

Azhar S and MAU Haq designed the experiment; Azhar S conducted experiment, collected data and analyze the samples with the coordination of MAU Haq; MAU Haq wrote the manuscript; Azhar S and J.Akhtar drafted the manuscript and EAWariach review it before submission.

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