Effect of Potassium Nitrate and Salinity on Growth and Endogenous Gibberellins of *Glycine max* Var. Daewonkong.

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ABSTRACT:

Plant growth and development was affected greatly through salinity. Appropriate nutrients application on salinity condition can reduce its harmful effects and increased productivity of salinized soils. A pot experiment was carried out in 2007 in cs Lab., Department of Agronomy, Kyungpook National University, Korea to assess the effects of potassium nitrate on *Gycine max* (Var. Daewonkong) under different salinity levels. Experiment was complete randomized block design (CRBD), consisted of 9 treatments with 3 replications per treatment and each replication comprising 9plants. KNO_3 and NaCl were applied to soybean plants 14 days after sowing (14 DAS). KNO_3 was applied at the rates of 5.0 mM and 10.0 mM, each pot receiving150 ml of KNO_3 solution. For salt stress induction, single dose of 300 ml of NaCl solution of 70 mM and 140 mM strength was given to the plants. Plant length, shoot and root fresh and dry weights were measured for harvested soybean plants while chlorophyll content was estimated in fully expanded leaves. Plant samples were harvested after 24 h of KNO_3 and NaCl application for the analysis of endogenous bioactive Gibberellin. Results showed that all growth parameters (shoot length, fresh and dry shoot weight, fresh and dry root weight), chlorophyll content and endogenous bioactive GA_1 and GA_4 content of soybean cultivar Daewonkong were reduced under NaCl stress. KNO_3 application enhanced shoot length, fresh and dry shoot weight, fresh and dry root weight, chlorophyll contents and endogenous bioactive GA_1 and GA_4 content as compared to control treatment.

KEYWORDS: Salinity, Potassium nitrate, Endogenous Gibberellin, Chlorophyll.

INTRODUCTION:

Salinity is a serious problem that limiting the productivity of crop plants. Crop production rate decreases due to salinity toxicity every year [1]. It results from high level of soluble salts in the soil. High amounts of dissolved salts in soils can limit crop yield [2].High concentration of salts causes hyper osmotic stress and ion imbalance that produce secondary effects on plants growth [3] [4].Salt-affected soils currently account for 8% of the world's total land area [5], and the salt-affected agricultural land is predicted to double by 2050 for irrigated agriculture and some semi-arid areas [6] [7]. Most of the salt stresses in nature are due to Na^+ salts, particularly NaCl [8]. High salinity lowers water potential and induces ionic stress, and results in secondary oxidative stress. It severely limits growth and development of plants by affecting different metabolic processes such as CO_2 assimilation, oil and protein synthesis [9].

One approach to minimize effects of salinity is use of nutrient application or nutrient enrichment into soil or soilless culture to increase tolerance to plant salinity by alleviating Na^+ and Cl^- injury to plants [10] [11] [12]. Potassium (K) acts as a very essential and important nutrient for the plant growth and development. It is necessary in plants to improve the efficiency of photosynthesis and use of water [13]. In wheat, deficiency of K^+ causes so many problems and shows many deficiency symptoms in plants growth such as weaker straw, increased lodging and decrease in growth [13]. Application of K to wheat plants under saline conditions enhances the growth of the plants and decreases the effect of salinity [14].

Soybean (*Glycine max*) is a major agricultural crop that is used widely for providing human and animal food because of its high oil and protein content (18 and 38%, respectively) [15]. It is classified as a moderately salt-sensitive crop [2].It’s seed is a major source of high-quality protein and oil for human consumption [16]. It’s chemical composition has made it one of the most valuable agronomic crops worldwide [17]. Its protein has great potential as a major source of dietary protein. The oil produced from soybean is highly digestible and contains no cholesterol [18]. Growth, development and yield of soybean are the result of genetic potential interacting with environment.

Objectives:

This experiment was carried out in order to assess the effectiveness of combined supplementary K and N (as KNO_3) for overcoming salinity stress. The specific aim was to determine if potassium nitrate (KNO_3) would correct K and N deficiencies in the presence of high NaCl, and also to assess effects of combined K and N on soybean growth, chlorophyll content and endogenous gibberellins.

MATERIALS AND METHODS:

This experiment was complete randomized block design (CRBD), consisted of 9 treatments with 3 replications per treatment and each replication comprising 9plants.Seeds of high yielding Korean soybean cultivar Daewonkong were procured from Plant Genetics Lab., Department of Agronomy, Kyungpook National University, Korea.

General Procedure:

Seeds of soybean cultivar Daewonkong were surface sterilized with 5% NaClO for 15 min and then thoroughly rinsed with double de-ionized distilled water. Seeds were sown in plastic pots (5.5 liter) filled with horticulture soil on 25th June, 2007. The composition of horticulture soil was as follows; peat moss (13-18%), perlite (7-11%), coco-peat (63-68%) and zeolite (6-8%), while the macro-nutrients were present as follows: NH_4^+~90 mg/L; NO_3^- ~205 mg/L; P_2O_5 ~350 mg/L and K_2O~100 mg/L. Initially 5 seeds were sown, that were later thinned to three seedlings per pot. The experiment was conducted under controlled greenhouse environment. Soybean plants were harvested on 25th July, 2007 (30 DAS).

Concentration and Time of KNO_3 and NaCl Application:

KNO_3 and NaCl were applied to soybean plants 14 days after sowing (14DAS). KNO_3 was applied at the rates of 5.0 mM and 10.0 mM, each pot receiving150 ml of KNO_3 solution. For salt stress induction, single dose of 300 ml of NaCl solution of 70 mM and 140 mM strength was given to the plants.

Analysis of Endogenous Bioactive Gibberellin:

Plant samples were harvested after 24 h of KNO_3 and NaCl application and were immediately frozen in liquid nitrogen and stored at minus 80ºC. Plant samples were lyophilized in freeze drier (Virtis, SP Industries Inc.). Leaves of the lyophilized plant samples were crushed to powder form and used for analysis of endogenous bioactive gibberellin.

Extraction and quantification methods:

Measurement of Growth Component:

Plant length, shoot and root fresh and dry weights were measured for harvested soybean plants while chlorophyll content of fully expanded leaves was analyzed with the help of chlorophyll meter (Minolta Co., Ltd, Japan). Four replicates of 6 plants each per treatment were randomly selected for measuring growth parameters. Dry weights were measured after drying the samples at 70ºC for 48 h in an oven.

Statistical Analysis of Data:

The data was subjected to analysis of variance (ANOVA SAS release 9.1; SAS, NC, USA) and Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION:

Effect of KNO_3 on Soybean Growth under Salt Stress:

Many factors involved in lowering the crop growth and yield, such as drought, salinity, and high temperatures [19] [20]. Salinity adversely affect the plant growth and development [21].Our study showed that all growth parameters (shoot length, fresh and dry shoot weight, fresh and dry root weight) of soybean cultivar Daewonkong were reduced under NaCl stress (Table 1). Decrease in plant lengths was done by the effect of salinity and this phenomenon was observed by a number of investigators [22] [23] [24]. Similar investigations were also reported by kausar et al., [25]. They find out that lengths of plants were adversely affected by salinity stress. Growth of wheat plant was reduced by lower fixation of carbon dioxide, reduced stomatal conductance, photochemical and biochemical capacity or combinations of all other factors in stress environments [26].Several reports are available in agreement of the present studies that fresh biomass production reduced by the salinity stress as compared to non-saline conditions [25] [27] [28].Reduction in dry weight reflects the increased metabolic energy cost and reduced carbon gain. It also reflects salt impacts on tissues [29] reduction in photosynthetic rates [30] [31] and attainment of maximum salt concentration tolerated by the fully expanded leaves [32].

KNO_3 application enhanced shoot length, fresh and dry shoot weight, fresh and dry root weight as compared to control treatment so its application alleviated the detrimental effect of NaCl on soybean growth parameters (Table 1).Foliar supply of KNO_3 to the salt treated plants may reduce toxic ions uptake as well improve K and N status of salt treated plants. Potassium has role in ionic balance which is reflected in nitrate metabolism [33]. Nitrogen plays role of chlorophyll and protein so act as an essential element for plant growth. Potassium application result an increase in leaf potassium content which was accompanied by increased rates of photosynthesis, photorespiration and RuBP carboxylase activity. Ebert *et al.*,[34] found that supplying of $Ca(NO_3)_2$ at 10 mM had a beneficial effect on growth and metabolism of NaCl treated guava seedlings. Akram *et al.*,[35] observed an improvement in growth of sunflower due to the foliar spray of K2 SO4 and KNO3 at 1.25% under saline concentration of 150 mMNaCl.

Present investigation confirmed that salt stress markedly decreases leaf chlorophyll content during vegetative growth stage (Table 1). These results are in agreement with those of Azooz *et al.*,[36] for sorghum and Dager *et al.*,[37] for *Salvadora persica*. The reduction in leaf chlorophyll content under NaCl stress has been attributed to the destruction of chlorophyll pigments and the instability of the pigment protein complex [38]. Another cause for the reduction is the interference of salt ions with the de novo synthesis of proteins, the structural component of chlorophyll, rather than the breakdown of chlorophyll [39]. Potassium and nitrogen supplemented in the form of KNO_3 can significantly improve the variables affected by high salinity and can also correct both K and N deficiencies. Addition of KNO_3 successfully ameliorated leaf chlorophyll and carotenoid contents of plant grown in salt stress. Stress conditions result in limited photosynthesis due to a decline in Rubisco activity and reduced gas exchange [40]. Application of SA or KNO_3 to salt and water stress treated barley plants enhanced the contents of photosynthetic pigments. The decrease Na^+ and increase K^+ in stressed barley plants in response to application of SA or KNO_3 may be caused maintenance of photosynthesis.

Table 1. Effect of KNO_3 and salinity on growth parameters and chlorophyll content of *Glycine max* Var. Daewonkong

Treatment	Solution strength (mM)	Plant length (cm)		Shoot weight (g plant^{-1})		Root weight (g plant^{-1})		Chl. content
		Shoot	Root	F.W	D.W	F.W	D.W	(plant^{-1})
Control	0	38.2±0.7^{a}	44.9±1.2^{a}	7.19±0.4^{ab}	1.16±0.04^{abc}	2.22±0.2^{ab}	0.28±0.03^{a}	28.3±0.5^{ab}
NaCl	70	37.3±1.0^{ab}	44.8±7.5^{a}	6.38±0.6^{bc}	0.75±0.1^{cd}	2.19±0.02^{ab}	0.24±0.02^{a}	25.4±1.1^{abcd}
	140	34.6±1.5^{b}	40.2±2.3^{a}	4.05±0.9^{c}	0.64±0.14^{d}	1.73±0.3^{b}	0.21±0.01^{a}	22.0±2^{cd}
KNO_3	5.0	39.5±1.4^{ab}	37.3±6.5^{a}	7.84±0.6^{ab}	1.19±0.09^{abc}	2.76±0.6^{ab}	0.39±0.09^{a}	28.6±0.6^{ab}
	10.0	42.3±4.0^{ab}	45.1±5.9^{a}	9.3±1.7^{a}	1.36±0.2^{ab}	3.33±0.9^{a}	0.46±0.09^{a}	29.3±0.3^{a}
NaCl + KNO_3	70+5.0	38.9±1.7^{ab}	44.7±3.8^{a}	9.07±0.2^{a}	1.4±0.01^{a}	3.12±0.2^{ab}	0.38±0.03^{a}	26.5±1^{abcd}
	70+10.0	40.3±0.9^{ab}	38.7±8.7^{a}	7.67±0.4^{ab}	1.0±0.08^{abcd}	2.29±0.02^{ab}	0.29±0.02^{a}	26.9±0.2^{abc}
	140+5.0	37.8±1.3^{ab}	43.1±7.0^{a}	6.25±0.3^{bc}	0.83±0.1^{cd}	2.23±0.2^{ab}	0.23±0.04^{a}	25.7±1.7^{abc}
	140+10.0	38.6±1.1^{ab}	43.7±5.9^{a}	6.79±0.3^{bc}	0.96±0.04^{cd}	1.87±0.1^{ab}	0.26±0.02^{a}	26.1±1.1^{bcd}

*In a column, treatment means having a common letter(s) are not significantly different at the 5% level by DMRT.

Endogenous Bioactive Gibberellin Content of Daewonkong:

The endogenous bioactive GA_1 and GA_4 content of soybean leaves showed a steady decline in response to elevated NaCl nutrition. Single application of KNO_3 greatly enhanced bioactive GA_1 and GA_4. Reduction of GA levels and signalling has been shown to contribute to plant growth restriction on exposure to several stresses, including cold, salt and osmotic stress [41]. The endogenous bioactive GA_1 and GA_4 content of soybean leaves showed a steady decline in response to elevated NaCl nutrition. Single application of KNO_3greatly enhanced bioactive GA_1 and GA_4. Application of KNO_3 to salt stressed plants yielded high GA_4 content while GA_1 content was not significantly affected; soybean thus showing a differential response in bioactive gibberellin metabolism to KNO_3 nutrition. Our results also demonstrated that application of KNO_3 was more effective at lower salt stress level as GA_1 and GA_4 contents in soybean leaves were higher with basic NaCl (70 mM) as compared to double NaCl (140 mM) treated plants. The concentration of GA_4 was much higher than GA_1 in all treatments (Fig. 1).

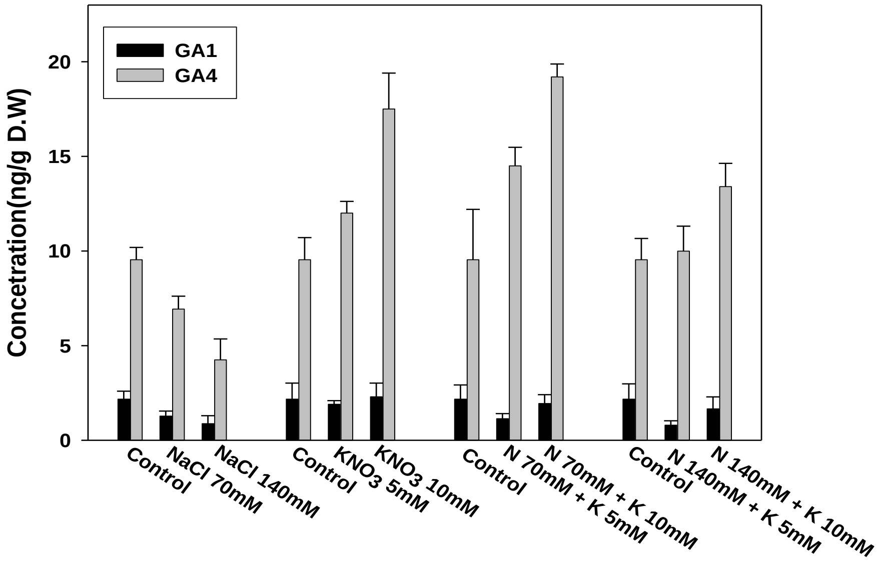

Figure1 Level of endogenous bioactive GA_1 and GA_4 content in response to elevated KNO_3 and NaCl.

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