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Nitrogen (N) and Potassium (K) Supplementation to Improve Salt Tolerance in Brassica (*Brassica juncea*, cv: Agati sarheen)

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Abstract To observe the mitigating effects of nitrogen and potassium under salinity on *Brassica juncea* (cv. Agati sarheen), N and K was supplemented at different stages of growth. The studies were conducted under control condition (gravel culture). Salinity (12 dS/m) was imposed by NaCl salt after three weeks of germination through irrigation (1/4th Hoagland solution). Nitrogen and Potassium was applied exogenously in the form of KNO_3 @ (T1. 30mM and T2 60mM) at vegetative and flowering stage. There was decrease in growth and yield under salinity. However; the relative reduction in plant height, slique length, grain wt/ 5 slique and grain yield/ 15 plants was comparatively low due foliar application of N and K as compared to non applicated plants. The overall accumulation of organic solutes (proline and glycine betaine) was significantly high under salinity. The response of N and K application was almost nil in case of proline accumulation. On the other hand application of KNO_3 @ 30mM showed increased accumulation of glycine betaine. The uptake of Na under salinity was less in plant having KNO_3 application @ 30mM (T1). Potassium content under saline condition was high in KNO_3 @ 60mM (i.e. T2 treatment), resulting in high K/Na ratio. The application of KNO_3 also found to increase in nitrogen content in brassica. It is therefore concluded that better performance of brassica genotype under salinity might be due to low Na uptake and high K/ Na ratio especially under T1 treatment (i.e KNO_3 @ 30mM).

Keywords Salinity, Brassica, Nitrogen, Potassium Supplementation

1. Introduction

Salinity is a major constrain in arid and semiarid areas of the world. About 6.8 million hectares, salt affected soils occupy in Pakistan (Khan,[1], Khan et al., [2], Shirazi, et al [3]), which is about 30% of the total cultivated area of Pakistan (Govt of Pakistans, [4]).

The ability of plant to grow and complete its life cycle on saline soils is regarded as salt tolerance, which vary among the plant type and within genotypes of same species. Rapeseed (B. napus L.) and mustard (B. juncea (L.) Czern. & Coss.) are the common brassica oil-seed crops grown for commercial purposes. Brassica species ranks as third among oilseed crops and are an important source of edible oil. It is categorized as medium salt tolerant crop (Maas and Hoffman, [5]. The salt tolerance among the brassica species varies due to their physiological response e.g. electrolyte leakage, proline accumulation and the K/Na ratio (Puppala et al., [6]; Mer et al., [7]; Bybordi, [8]; Tunuturk et al.,[9]; Zamani et al., [10]. It is reported that though the brassica species have high threshold values but the yield decrease is very high above the threshold values than most other crops in the tolerant category (Maas, [11] Ashraf and McNilly [12]).

It is therefore, necessary to adopt suitable strategies for the improvement of growth and yield performance of brassica species under saline conditions. To mitigate the adverse effects of salinity, different strategies are required, among which application of osmolytes, especially inorganic osmolytes such as nitrogen and potassium are encouraging as they are efficient and economical. Siddique et al., [13], suggested that addition of nitrogen (N) can play a significant role in reducing the adverse effects of salt stress by the accumulation of osmoprotectants, availability of essential nutrients and photosynthetic capacity of plant. Jabeen and Ahmed [14], reported that the application of KNO_3 improves growth and enzymatic activity (NRA), reduced increasing tendency of Na^+ and Cl^- and increased leaf area, fresh and dry weight per plant, NO_3^- and soluble protein concentration in safflower (Carthamus tinctorius L.)

and sunflower (Helianthus annuus L.). They also reported that the improvement of these parameters is irrespective of the plant grown under non saline or saline conditions. Alleviatory effects of foliar nutrient application of $Ca(NO_3)_2$ and KNO_3@10mM at 40mM salinity level in strawberry were also reported by Yildirim et al., [15] who observed approximately 50% increase in plant root and shoot dry weight. The present study is therefore designed to improve the yield performance of *Brassica juncea* (cv: Agati sarheen) grown under saline environment by the application of suitable doses of KNO_3.

2. Material and Methods

Gravel culture (net house) studies were conducted to improve the salt tolerance in Brassica species through foliar application of K and N. The KNO_3 was applied @ 30 and 60mM foliarly along with few drops of Triton x 100 to confirm its absorption properly. Crop was irrigated by nutrient solution. Two treatments (control and 9.0dSm^{-1} NaCl), were imposed after two weeks of germination. The salinity was imposed by NaCl salt applied with irrigation. The experiment was laid out according to randomized complete block design (RCBD with two replicates. Physiological observations were recorded after 2nd spray (at the time of flowering). Proline , Glycine betaine , Na, K and Cl were determine in green leaves after extracting in (0.5%) toulene water, according to Weimberg et al., [16]. Proline was estimated according to the method of Bates et al., [17]. Glycine-betaine was estimated according to Grieve and Gratan, [18]. Sodium (Na^+) and Potassium (K^+) contents were measured in toluene extract by flame photometer after making suitable dilution with D.W. and nitrogen was determined by Kjeldhal method according to the standard methods as reported by Jackson [19].

Growth observations were recorded at the time of crop harvest in terms of plant height (cm), slique length (cm), grain weight /5 slique (g) and grain yield / 15 plants (g). The data regarding all the growth parameters and biochemical aspects were analyzed statistically for analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) using MSTAT-C computer package.

3. Results

Growth Performance

The decrease in plant height was approximately 30% under salinity. There was a gradual increase in plant height under normal condition with the increasing dose of K and N application i.e. less at 30mM and more at 60mM KNO_3. Under saline condition the response of KNO_3 @ 30mM (T1) was better than KNO_3@ 60mM (T2), where maximum plant height was observed. However, the relative decrease was bit high under T1 (29.44%) than at T2 ((28.0%) treatment.

The response of KNO_3 was also better in case of slique length. There was a gradual increase in slique length with increasing dose of KNO_3 i.e. more under (T2= 60mM KNO_3) than (T1= 30mM KNO_3) treatment. The relative decrease was also less under T2 treatment (i.e. only 12.65% decrease).

The application of KNO_3 did not show any significant effect in improving the grain weight / 5 slique of brassica genotype i.e. grain weight/ 5 slique was bit higher under T0 (DW) treatments (both under normal and saline soil condition as well). However the relative decrease under salinity was less both under T1 and T2 treatments. The values for relative decrease under T1 and T2 treatments were recorded as 28.57 and 28.87%, respectively.

The application of KNO_3 was also found to improve the grain yield of brassica, both under normal and saline conditions as well. Under saline condition the values for the grain yield were comparatively high under T1 treatment i.e. KNO_3@ 30mM then T2 treatment (KNO_3@ 60mM). The mean reduction was also less under lower dose of KNO_3. i.e. 76% as compared to T0 (79.57) and T2 (80.75) treatments.

Solute Accumulation

Proline accumulation under normal soil condition was comparatively less, but its accumulation increased significantly in all the treatments under salinity (Figure 1a). Maximum increase was observed in T0 treatment followed by T1 and T2 treatments. Application of KNO_3 @ 30mM (T1) improved the accumulation of proline plant under salinity. The relative increase in T1 treatment was maximum i.e. 86.54%.

The trend in the accumulation of glycine betaine was also similar, where significant increase was observed under salinity (Figure 1b). However it varies among the treatments. Under normal soil condition maximum accumulation of proline was observed under T0 followed by T1 (KNO_3 @30mM.) and T2 (KNO_3 @60mM.), treatments. Under saline condition application of N and K at @ 30mM (T1) was found more effective to improve the accumulation of glycine betaine with maximum values of 53.15µ mole g^{-1} F.wt. The relative increase in T1 treatment was also maximum i.e. 31.58% followed by 15.18% in T2 treatment.

For better growth of plant under salinity, restricted uptake of Na ions is necessary. There was increase in Na uptake by brassica genotype under salinity ((Figure 1c). Under normal soil conditions the values for sodium accumulation were almost same under all the three treatments. Under saline conditions quite encouraging response of N and K application was observed in T1 treatment. Sodium uptake under T1 treatment was minimum i.e. 0.44%. The relative increase in T1 treatment was also less i.e. 54%. On the other hand no response was observed under T2 treatment, where the values for Na uptake were similar to T0 treatment i.e. 0.52% having 58%

increased as compared to non-saline condition.

Proper regulation of potassium is also necessary for normal growth of plant under salinity. There was a significant decrease in K uptake due to increase in Na accumulation under salinity (Figure 1d). Application of K and N proved to increase the uptake of K in plants. Comparatively higher K accumulation was observed in T1 and T2 treatments under both growing environments (i.e. non-saline and saline).

Increased Na accumulation and decreased K uptake under salinity resulted in the decrease in K/Na ratio in plant leaves (Figure 1e). K/Na ratio under normal conditions was higher ranging as 1.95-2.75. Significant decrease was observed in all the treatments under saline environments. The K/Na ratio was comparatively more under T1 followed by T2. The K/Na ration under salinity in T1 and T2 was recorded as 0.61 and 0.56, respectively. It was also observed that though the values of K/Na ratio in T1 were the maximum but the relative decrease in T2 was comparatively less. i.e. 74%.

Foliar application of N containing salt (i.e. KNO_3) did not showed any significant increase in N under normal soil conditions (Fig.1f). However, under saline conditions the response was quite encouraging. Here again the response of low KNO_3 dose (T1) was comparatively better than the higher one (i.e. T2). Total nitrogen under T1 was maximum (4.96%) followed by T2 (4.22%). The relative decrease under T1 and T2 was also minimum i.e. 10.66 and 20.76%, respectively.

4. Discussions

Nitrogen and potassium play a vital role in plant growth. The availability of these major nutrients depressed under salinity environment. According to Pooja and Kumar [20], ion toxicity and imbalanced nutrition in saline conditions are the main constraints for plant growth. It is reported that in saline conditions, nutrient imbalances can result through various ways: From the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant or may be caused by physiological inactivation of a given nutrient (such as K) resulting in an increase in the plant's internal requirement for that essential element (Ashraf, [21]. Under saline/ drought conditions, foliar application of nutrients to stressed wheat might offer a way to alleviate these stresses (Schmidhadhalter *et al,* [22]). In the present studies significant effects of salinity were observed on growth (plant height, slique length) and grain yield in brassica. Foliar supplementation of nitrogen and potassium resulted in improved plant growth under salinity. Under saline condition the response of KNO_3 @ 30mM was better than @ 60mM. The values for the grain yield were comparatively high under (T1) treatment. This might be due to the provision of nutrients in adequate amount at a stage with maximum of their demand. Positive results of daily application of N & K were observed by Day *et al*., [23]; Rengel and Marschner, [24] in salt or drought stressed maiz and wheat.

Increased photosynthetic material also resulted in higher values of grain yield of Brassica under KNO_3 @ 30mM treatment. Siddique [13] reported that increased activity rubisco (a key enzyme for fixation of CO_2) may be responsible for higher utilization of leaf nitrogen. Further the higher portion of leaf –N in chloroplast, mostly invested in rubisco alone.

Accumulation of organic (i.e. proline and glycine betaine) and inorganic (sodium) solutes also increased in brassica plants due to salinity. (Marcum and Murdoch, [25]; Khan *et al*., [26] also observed increased proline and glycine betaine due to increasing salinity level. The accumulation of organic solutes is necessary to maintain the water potential equilibrium inside the cytosol (Tabatabaei and Fakhrzad, [27]. In the present studies there was an overall increase in proline irrespect to K and N application; while the accumulation of glycine betaine under KNO_3 treatments was encouraging. The accumulation of GB plant leaves in response to water and salt stress are also reported by Khafagy *et al.* [28]. They observed that pre-treatment with glycine betaine or ascorbic acid mitigates the effect of salinity on thickness of the midrib region and mesophyll tissue of leaf blade. The increase in Nitrogen Containing Compounds (NCC) might be due to foliar absorption of N by plants, as there was higher accumulation of N in KNO_3 treatments as compared to control. Siddique *et al*., [13] reported that foliar application of N plays a vital role in the synthesis of Nitrogen Containing Compounds (NCC), which are beneficial for several enzymes activities in plants (Mansour, [29]). According to Siddique et al., [13], the efficiency of N in alleviating the adverse effect of salt stress in plants may be attributed to their ameliorative effect on the primary growth potential, activities of NR and CA enzymes, membrane permeability and N-use efficiency.

Under salinity there was increase in Na and decrease in K in all the treated plants. The alleviating effects of KNO3 were quite significant under T1 treated plants, showing bit lesser increase in Na accumulation. The results of present investigation are in agreement with the findings of many workers in different plant species (Abdel-Rehman, [30]; Cha-um *et al*., [31]; Sultana *et al.,* [32]; Tabatabaei and Fakhrzad, [27] who observed better absorption of K in shoot when applied to foliage directly. This shows that under saline condition application of K in combination with N may be quite helpful in mitigating the toxic effects of sodium. It is therefore recommended that for better growth and yield response of brassica genotypes may be supplemented @ 30mM or 60mM KNO_3.

Table 1. Effect of N and K growth performance of Brassica genotype (cv: Agati sarheen)

Treatments	**Non saline**	**Saline**	**Rel. dec. (%)**
	plant height (cm)		
T0 (Distilled Water)	144 a	95 b	33.91
T1 (KNO_3 @ 30mM)	151 a	107 b	29.44
T2 (KNO_3 @ 60mM)	142 a	102 b	28.09
LSD (0.05) = 4.047			
	Slique length (cm)		
T0 (Distilled Water)	4.11	3.33	18.98
T1 (KNO_3 @ 30mM)	4.14	3.43	17.15
T2 (KNO_3 @ 60mM)	4.11	3.59	12.65
NS			
	Grains weight/ 5 slique (g)		
T0 (Distilled Water)	0.156	0.109	30.13
T1 (KNO_3 @ 30mM)	0.147	0.105	28.57
T2 (KNO_3 @ 60mM)	0.142	0.101	28.87
NS			
	Grain yield row^{-1} (15 plants)		
T0 (Distilled Water)	23.0 c	4.70 f	79.57
T1 (KNO_3 @ 30mM)	32.6 b	7.60 d	76.69
T2 (KNO_3 @ 60mM)	37.4 a	7.20 e	80.75
LSD (0.05) = 0.0813			

Figure 1. Effect of N and K application on solute (organic and inorganic) accumulation in brassica genotype (cv: Agati sarheen)

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