Foliar and Soil Application of Potassium Nitrate Affects the Tolerance of Salinity and Canopy Growth of Perennial Ryegrass (*Lolium perenne* var Boulevard)

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**Abstract:** Two experiments were conducted to determine the effect of KNO3 application on perennial ryegrass salinity tolerance. Two NaCl levels (0 and 60 mM) factorially combined with four KNO3 (KN) levels (0, 5, 10 and 15 mM) as the treatments. The KN was applied either to the soil or foliarly sprayed. The results showed that in both experiments the increased salinity significantly reduced the vegetative characteristics of ryegrass. In the salinity treatment, supplying KN at 10 mM concentration increased the fresh and dry weight as well leaf area, however, the growth of the plants was lower at the 0 and 15 mM concentration of KN. The concentration of N, NO3, P and K was reduced in the salinity treatments, but the concentration of Na and Cl was increased. Salinity (NaCl60) significantly reduced the K/Na ratio, while it was increased when the KN was increased in the solution. The concentration of Cl was significantly reduced when KN increased in the solution so that its concentration in NaCl60 and 0 mM KN was 75% in soil and 66.6% in foliar sprays treatment, respectively, higher than that in NaCl60 and 15 mM treatment. Combination of both salinity and KN concentration remarkably increased the proline content. The concentration of chlorophyll was reduced in NaCl60 treatment however; additional supplying of KN increased the chlorophyll concentration. In NaCl60 treatment, the highest survival plants were observed at 10 mM KN as either foliar or soil application. It seems that the reduced Cl concentration, increased proline and K/Na ratio were the main reason for the improved tolerance for salinity of the perennial ryegrass in the 10 mM KN concentration.

**Key words:** Nitrogen, nutrition, perennial ryegrass, potassium, proline, sodium chloride

**INTRODUCTION**

Perennial ryegrass (*Lolium perenne* L.) is widely used for the lawns, athletic field, golf course, parks and other landscapes in many countries[27,34]. Perennial ryegrass is considered to be moderately tolerance of salinity (0-1000 mg L⁻¹) and can damage in salinity conditions[12]. The quality of irrigation water in arid and semi arid regions seems to be not suitable for the use of certain ornamental or horticultural crops. As irrigation water may often contain salts and ions, such as Na, B and Cl, that can have opposing impacts on the perennial ryegrass growth. It becomes more pronounced in summer when the evapo-transpiration rate increases, leaving salt in the root zone. Consequently, accumulation of salt in the root zone leads to the reduction of visual quality and survival of perennial ryegrass in these regions. Many arid regions of the world have demonstrated large amount of saline ground waters, which can be used to irrigate many crops like turf grasses[4].

Salinity adversely affects the plant growth. Taiz and Zeiger[39] stated that there are two processes involved in osmotic adjustment in response to changes in water potential. The first is an accumulation of ions in the vacuole. Ions, such as K, Ca and Na can accumulate in the cell to decrease water potential without adjusting turgor pressure during dry or saline conditions. At high levels this ions can be toxic to the cytosol, so they must be fix in the vacuole. Because these ions are compartmentalized in the vacuole, other solutes such as organic acid must accumulate in the cytoplasm as water potential equilibrium must be maintain in the cytosol. These organics acids, known as compatible solutes, include proline, glycin betaein, sucrose and sorbitol[30]. Compatible solutes are able to build up to high levels in the cytoplasm without interfering with cell metabolism[26].

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[14]. It is reasonable to believe that two or more of these processes may be occurring at the same time, but whether they ultimately affect crop yield or quality depends upon the salinity level, composition of salts, the crop species, the nutrient in question and a number of environmental factors.

Alleviating the opposing effects of salinity on the plant growth could be possible by modification of nutrients supply. Adverse salinity effects on plant growth and tolerance to salinity can be altered by mineral nutrition[8,13,15]. It is well known that addition supply of Ca or NO_3 to salt stressed plants improves the tolerance to salinity[13,22,31]. However, there is little information available about the effect of the increased KNO_3 (KN) at the saline conditions on the salinity tolerance of perennial ryegrass. Therefore, the aim was the investigation of the combined effect of K and NO_3 on perennial ryegrass to elucidate responses to NaCl at different rates of supplemental KN.

**MATERIALS AND METHODS**

**Plant growth conditions:** Two glasshouse experiments were carried out between May and October 2005. Pots of 50 cm in diameter were filled with the mix of perlite and sand (1:1 V) and then the perennial ryegrass (Lolium perenne var Boulevar) seeds were sown at rate of 30 g m^{-2}. The pots were arranged in a randomized complete block with four replicates and placed on an open plastic mist bench. For two weeks, all pots received half strength of Hoagland's solution[18] according to filed capacity moisture. Enough solution was supplied to the pots to reduce the increased salt concentration in the growing media. During the experiments, plants were under natural sunlight. Daily and nightly temperature of the glasshouse was set up to 25±3 and 20±3°C, respectively.

**NaCl salinity and KNO_3 treatments:** Upon sufficient plant growth when they reached approximately 5 cm height, both salinity and KN treatments began by irrigating with solutions. Each experiment consisted of 8 treatments with two salinity concentration as NaCl (0 and 60 mM) factorially combined with 4 KN concentrations (0, 5, 10 and 15 mM), each treatment being replicates four times. The basic solutions were prepared by adding either NaCl or KN to the half strength of Hoagland's solution. The solution pH was adjusted to 6.5 by adding H_2SO_4. In foliar application experiment, salinity was applied by irrigating with NaCl treatments and KN was sprayed on the canopy of plants following salinity treatments. Foliar application was repeated at the beginning of new growth. In soil experiment, both salinity and KN treatments were applied by irrigation of plants two or three times per week.

**Data collections:** The plants were cut when they reached 10 cm and their fresh and dry weight, as well leaf area were measured. The leaf area was measured via leaf area-meter (Li-Cor, model Li-1300, USA). After weighing the leaves, they were dried at 80°C in an air forced oven for 48 h. During the period of the plants growth the visual symptoms of salinity toxicity in the leaves (such as either necrosis or chlorosis) were assessed. The plants were harvested six times at the period of growth. The sum of all the harvested plant materials was considered as total fresh and dry weights. All plants grown in the pots were taken for determining vegetative characteristics.

For the chlorophyll analysis, 1 g of leaves was ground and the chlorophylls were extracted in acetone. The color density was measured via spectrophotometer (Motic, CL-45240-00, China) at 642.4 nm (chlorophyll b) and 660 nm (chlorophyll a). Nitrate in tissue samples was determined by nitrification of salicylic acid[31]. Approximately 0.2 g of dried tissue powder was placed in 125 mL container and 25 mL hot water was added. The samples were shaken for 30 min on a Wristaction shaker and filtered through Whatman No 42 filter paper. Nitrate in the filtered solution was determined by adding a 0.2 mL sample aliquot containing 0.8 mL of 5% (w/v) salicylic acid H_2SO_4 mixture and 19 mL 2 N NaOH. Samples were allowed to cool at room temperature for 1 h and developing color was measured at 410 nm by spectrophotometer. For determining proline concentration, approximately 0.5 g fresh plant material was ground and homogenized in 10 mL of 3% sulfosalicylic acid and the homogenate filtered through a Whatman No. 2 filter paper. Two ml acide ninhydrin and 2 mL glacial acetic acid were added to 2 mL of the filtrate and incubated at 100°C for 1 h, the reaction terminated by placing test tubes in water bath. The mix was extracted with 4 mL toluene and vortexed for 15-20 s. The absorbance of chromophore containing toluene was read at 520 nm using toluene as the blank. The concentration of proline was determined from a standard curve[37].

Chlorine concentration was measured according to MAFF[23]. The concentration of total N, Na and K in the youngest fully expanded leaves was determined by Kjeldahl method and atomic absorption spectrophotometer, respectively (Perkin-Elmerse, Model 110, USA). A statistical analysis was carried out
using analysis of variance in the SAS 8.2 software and the means were separated by LSD test at the 5% level.

**RESULTS AND DISCUSSION**

The vegetative characteristics (leaf area, fresh and dry weight of leaves) of perennial ryegrass plants at the various concentration of salinity as function of KN concentrations are given in Table 1. Leaf area, fresh and dry weight of leaves was significantly reduced by the increasing of salinity. Interactive effect of salinity and KN on leaf area and fresh weight showed that the leaf growth of perennial ryegrass in saline conditions depends on KN level in the solution so that the increased KN level up to 5 and 10 mM promote the leaf growth at NaClO₃ and NaClO₄, respectively. However, more increasing of KN level (15 mM) significantly inhibited both the fresh weight and leaf growth. In NaClO₃ treatment, plants supplied with KN, however, showed less reduction leaf area, fresh and dry weight compared with plants growing without KN. The lowest growth in terms of leaf area and fresh weight was observed in the treatment in NaClO₃KN₁₅ treatment. In general, in NaClO₃ treatments 5 mM KN caused optimal growth of the aerial parts, whereas in NaClO₄ treatment it was observed in 10 mM KN.

The opposing effects of salinity on the plants are a serious problem reported by many researchers[13,16,40]. Ion toxicity and imbalanced nutrition in saline conditions are the main constraints for plant growth as described by Greenway and Munns[16]. Addition supplying of KN to the salt treated plants may reduce toxic ions uptake as well improve K and N status of salt treated plants. Ebert et al.[13] found that supplying of Ca(NO₃)₂ at 10 mM had a beneficial effect on growth and metabolism of NaCl treated guava seedlings.

The decline in leaf growth is an earliest response of the plants to salinity[28]. Both the reduction in leaf growth and increasing of dead leaves at NaClO₄KN₁₅ led to further reduction in leaf area. This may be caused by ions accumulation in the leaves, particularly old leaves[16]. The reduction in plant growth was due to the reduced leaf growth, which agrees with finding of Cramer[10]. At NaClO₃, however, KN at 15 mM concentration not only had no effects on growth promotion, but reduced the plants growth. It implies that other factors (such as osmotic pressure) have some effects on plant growth. The direct factors might be salinity (such as osmotic effect, Cl or Na toxicity) as reported by researchers[15,41]. As a result of higher leaf area in NaClO₃ with 10 mM KN application fresh and dry weight of the plants were higher, suggesting that the optimal growth of the salt treated plants is obtained by supplying further KN. Salinity reduced the visual quality of perennial ryegrass and it became more sever at high level of KN. It has been reported a reduction in perennial ryegrass color and burning of Bermuda grass with high salinity[29].

The data of leaf ion concentration of the plants in relation to salinity and KN levels are presented in Table 2. Salinity in the root zone led to a significant decrease in both K concentration and K/Na ratio in the plant tissue regardless of the KN levels. From the data given in Table 2-3 on both N and NO₃ concentrations, it is clear that raising the NaCl Concentration in the solution reduced N and NO₃ concentration of the leaves. As shown in Table 2-3, both nitrate and total N concentration was the highest in NaClO₃KN₁₅ treatment and decreased as the salinity increased. In general, the results suggest that high salinity causes a depression in NO₃ and N concentration and K/Na ratio. Raising of KN from 0 to 10 mM in the solution increased NO₃ concentration in the leaves, however it was ineffective at 15 mM KN in restoring the decreased in the leaf NO₃ caused by the increased salinity. In contrast to Na concentration in NaClO₄ treatment, the

| Treatments | Leaf fwt (g m⁻²) | Leaf dwt (g m⁻²) | Leaf area index | Root volume (ml) |
|------------|-----------------|-----------------|-----------------|-----------------|
|            | foliar soil     | foliar soil     | foliar soil     | foliar soil     |
| NaClO₃     | KN₀ 115.5ᵃ 103.4ᵇ 15.8ᵇ 18.7ᵇ 3033.7ᵇ 3054.9ᵇ 6.3³ 8.3ᵇ | | | |
| KN₁ 117.7ᵃ 118.3ᵇ 16.0ᵇ 18.4ᵇ 2983.4ᵇ 3184.8ᵇ 6.0³ 6.0ᵇ | | | | |
| KN₁₀ 119.2ᵇ 102.9ᵇ 16.0ᵇ 18.4ᵇ 2983.4ᵇ 3184.8ᵇ 6.0³ 6.0ᵇ | | | | |
| KN₁₅ 97.8ᵃ 92.4ᵇ 15.0ᵇ 19.1ᵇ 2632.7ᵇ 2895.4ᵇ 6.3³ 7.0ᵇ | | | | |
| NaClO₄     | KN₀ 63.4ᵃ 63.1ᵇ 6.7ᵇ 13.9ᵇ 1408.5ᵇ 2044.4ᵇ 5.3³ 3.0ᵇ | | | |
| KN₁ 75.0ᵇ 64.7ᵇ 8.8ᵇ 14.3ᵇ 1584.3ᵇ 2093.2ᵇ 5.3³ 2.3ᵇ | | | | |
| KN₁₀ 85.2ᵇ 77.3ᵇ 12.3ᵇ 18.5ᵇ 1651.6ᵇ 2584.7ᵇ 4.5³ 2.0ᵇ | | | | |
| KN₁₅ 58.0ᵇ 36.2ᵇ 6.3ᵇ 9.7ᵇ 1286.0ᵇ 1534.6ᵇ 3.5³ 1.0ᵇ | | | | |

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reduced when KN increased in the solution so that its F values

Fig. 1: Effect of salinity and KN (foliar, A and soil, B) application of on CI concentration in the leaf tissues

reduction of CI concentration became more severe in response to KN concentration in NaCl0 treatment (Fig. 1). The concentration of CI was significantly reduced when KN increased in the solution so that its concentration in NaCl0KN0 treatment was 75% in soil and 66.6% in foliar, respectively higher than that in NaCl0KN15 treatment. There was a negative correlation \( R^2 = 0.71^{**} \) between NO3 and CI concentration (Fig. 2). Present data clearly demonstrated that negative relationship between NO3 and CI as it has been previously reported\[21,32\]. It implies that addition to the growth media of KN above what would be considered sufficient for optimal growth in the absence of salinity, decreased CI concentration to the extent that canopy injury was reduced, thereby lessening growth inhibition\[3\].

![Table 2: Effect of salinity and KN on the nutrient concentration in ryegrass](image)

![Table 3: Effect of salinity and KN on the chlorophyll content and NO3 concentration](image)

![Fig. 2: Regression between NO3 and CI concentration in the leaf tissues](image)
Survival plant (%)

NaCl0
NaCl60

Survival plant (%)

KNO3 (mM)

Proline concentration (mmol kg⁻¹)

Proline concentration (mol kg⁻¹)

Fig. 3: Effect of salinity and KN (foliar, A and soil, B) on canopy quality, as percentage of survival plants

Fig. 4: Effect of salinity and KN (foliar, A and soil, B) on the proline concentration in the leaf tissues

Toxicity symptoms such as dead leaf edge and drop were recorded at NaCl₆₀ treatment, which was the highest at 15 mM KN concentration in both soil and foliar applications. Most of the plants treated with NaCl₆₀ and 15 mM KN were died at the end of the experiment (Fig. 3). Reduction of plant density caused by salinity led to the reduction in visual quality.

In regard nutrients status of plant, addition supplying of KN to the salt treated plants either by foliar or soil application increased the K, N, NO₃ and K/Na while reduced Na and Cl concentration.

Reduction in both K and K/Na at high salinity is another opposing effect of salinity, which impairs the function of K in the salinity treated plants. A reduction in K concentration and K/Na ratio in saline conditions was reported. The decline of K concentration under salinity conditions has been found. This was also the case for perennial ryegrass. Hence, maintaining of relatively high K concentration or K/Na ratio in the leaves acts an important role in regulation monovalent cationic osmoticum and physiological function of K or N assimilation. Our data showed that while increases in salinity in the root zone, K demand also increases. The demand of K in spinach leaves grown in solution cultures containing 250 mM NaCl was twice as high as in non-saline substrates. On the other hand, the presence of adequate K in the root zone affects K/Na ratio (Table 2) by shifting the uptake ratio in favor of K at the expense of Na³⁻. Taking into account all published information on the increased in external K/Na ratio reveals that the deleterious effect of salinity can be alleviated by the addition of K to the solution.

Increasing KN from 0-10 mM in the solution increased NO₃ concentration in the leaves, however it was ineffective at 15 mM in restoring the decreased in the leaf NO₃ caused by the increased salinity. This indicates that the effect of salinity could be a dependent factor according to KN. The results clearly demonstrated that under salinity conditions increasing of KN is effective in counteracting the adverse effects of salinity, which may build up during the growing period or high salinity conditions.

Leaf chlorophyll content was lower in the NaCl₆₀ treatment compared to NaCl₀ treatment (Table 3). Supplement KN to the plants grown in NaCl₀ and NaCl₆₀, had a beneficial effect on the chlorophyll concentration, which was the highest in the 10 mM KN.

In both experiments, there was a large increase in proline concentration in salinity treatment (Fig. 4). Supplying of high level of KN, even in NaCl₀ treatments, significantly increased proline concentration. The highest concentration of proline was observed in NaCl₀ KN₁₀ treatment. The increased of proline caused by salinity has been found by many researchers. Marcum and Murdoch looked at the influence of salinity on the perennial ryegrass and found that with increased salinity, levels of proline and glycine betaine also increased. Proline is known to
contribute to membrane stability\(^{[17]}\) and mitigates the effect of NaCl on cell membrane disruption\(^{[24]}\). The results reported here for proline can be explained in view of some earlier reports that proline accumulation is one of the common characteristics in many plants under saline conditions\(^{[1,2]}\).

The results of the experiments clearly indicated the ameliorating effect of KN application on the growth, mineral nutrients (K and N) concentration, chlorophyll and proline content in saline conditions. Furthermore, the reduction of Cl and increase of K/Na ratio due to the increased KN in the solution is most likely played an important role in the reduction of deleterious effect of salinity. A significant reduction in plant growth in terms of fresh weight and leaf area with higher salinity and KN was expected because of the increase in both osmotic stress and ion toxicity. In NaCl\(_{60}\) treatments, applying 5 mM KN had no remarkable effect on the plant growth, but at 10 mM KN concentration plant growth significantly improved. The higher concentration both NaCl and KN impaired the growth of the plants. These imply that under salinity conditions plant demands higher K and N in order to cope with salinity stress as it has been reported\(^{[2,3,18]}\).

In these experiments, foliar application of KN was proven to be more effective way compared to soil application to improve perennial ryegrass growth in salinity conditions. The possible explanation is that supplying of NaCl along with KN to the salt treated perennial ryegrass increases both osmotic potential and ion toxicity while, the adverse effects of salinity on the increased osmotic potential should be lower when KN is supplied by foliar application.

**CONCLUSION**

The experiments clearly demonstrated the beneficial effects of KN application on perennial ryegrass growth in saline conditions. It should be possible to ameliorate the deleterious effect of salinity by supplying further KN to the either irrigation water or soil. It also appeared that foliar application of KN in saline condition is more effective than the soil application. Moreover, further increase of KN concentration at the increased salinity concentration is unlikely to improve the growth and nutritional status of ryegrass.

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