

The Effects of Essential Minerals in Different Cations and Anion Forms on Assimilation of Macro and Micronutrients in Soybean Leaves

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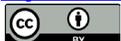
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Abstract

In our present study, we have investigated the acquisition of minerals into soybean leaves as affected by application of various minerals in different forms of positive and negative ions. A greenhouse experiment was conducted including the following 11 treatments applied to the soil: potassium nitrate (KNO_3), potassium iodide (KI), potassium chloride (KCl), sodium chloride (NaCl), calcium, magnesium and ammonium nitrates [$\text{Ca}(\text{NO}_3)_2$, $\text{Mg}(\text{NO}_3)_2$ and NH_4NO_3] and ammonium dihydrogen phosphate $\text{NH}_4(\text{H}_2\text{PO}_4)$, Na_2SO_4 , ammonium sulfate $(\text{NH}_4)_2\text{SO}_4$, and disodium phosphate $\text{Na}_2(\text{HPO}_4)$. Besides essential minerals, KI was also included as part of this comprehensive study, since according to some reports, molecular iodine (I^2) and KI may have positive effect on growth and increased stress tolerance in some species of plants. Soybean seeds were sown in pots arranged in twelve groups/3 replicates in each group including control. At V3 stage the plants were treated with 30 mmol/pot of the proper chemicals. Ten days after the treatments, leaves were sampled and were dried, analyzed for 5 macro (K, P, S, Ca & Mg) and micronutrients (B, Cu, Fe, Mn, Zn and Na). The results showed that of all the 11 treatments, KI (T4) application increased the maximum number of mineral nutrients in leaves, which increased K by 31.9, Mg by 8, P by 60.8, S by 38.7 respectively. KI treatment also reported increased micro minerals by B, Cu, and Zn were 53.6%, 36.3% and 10.8% respectively. Similarly, KNO_3 (T1) application increased several macro and micronutrients in leaves. We made an attempt to investigate the effects of various other cations like K^{1+} vs Na^{1+} , and NH_4^+ , Ca^{2+} vs Mg^{2+} ; and Anion comparisons I^{1-} vs NO_3^{1-} , Cl^{1-} , H_2PO_4^1 ; SO_4^{2-} vs $(\text{HPO}_4)^{2-}$ in the present investigation. After reviewing our results, of all the 11 (eleven), treatments studied in our present study, T1 (KNO_3), T4 (KI) and T10 $\text{Ca}(\text{NO}_3)_2$ applications proved to be the most efficient treatments by affecting the uptake of several minerals in soybean leaves.

Keywords

Cations, Anions, Macronutrients, Micronutrients, Essential Minerals

1. Introduction

Soybean is an important source of plant-based protein for humans and animal feed. The soybean seed composition consists of an enormous amount of nutritional substances, valuable for human consumption. Soybeans have the highest protein content and a significant amount of dietary minerals and vitamins. Soybean oil is another valuable product used in food industry accounting for 56% of global oilseed production; the United States accounts for 36.7% of world soybean crop production [1] [2]. The demand for soybean production stays strong as it is used in the formulation of various food and industrial products [2]. Soybean crop production could be greatly impacted by any mineral deficiency in the soil by natural or agricultural practices.

There are seventeen essentially important nutrients that plants must obtain from their environment to successfully complete their life cycle. Besides carbon, hydrogen and oxygen, which plants acquire from air and water, the other minerals the plants must acquire from the soil [3]. The soil in which the plants grow, generally contains all nutrients; however nutrients can become limiting or deficient by means of leaching or crop removal. Under certain conditions, nutrients especially in their cation form can be tightly bound to the soil which again can limit their availability to the plants. Applications of mineral fertilizer or biofortification are considered complementary approaches to the conventional breeding [4] [5] [6] [7]. Nutrient management and biofortification are great agricultural tools to combine high yield with high nutrient density (protein, minerals and vitamins) at low cost as adapted by farmers [8] [9] [10].

Understanding the nutrient movement in soil and uptake by plants either in anion or cation forms could help the crop growers choose the application of nutrients as per the demand of the crop to limit the environmental impact while increasing the effectiveness of the fertilizer. Besides, the 17 essential elements listed, there are other minerals regarded as beneficial elements which have been reported to have positive influence on plants but might have been neglected for a long time; those elements are iodine and sodium. Iodine is not an essential element for land plants, although some studies have reported beneficial effects including better growth, changes in tolerance to stress and its antioxidant capacity. Umaly and Poel (1970) have studied the effects of various concentrations of iodine as potassium iodide on the growth of barley, tomato and pea in nutrient solution cultures [11]. This study found enhancement in height, number of tillers and in fresh and dry weights of barley; however, the higher concentrations of 10.0 ppm had decreased growth and developed toxicity symptoms. In humans, iodine is essential for thyroid functioning, and in development of cogni-

tive abilities, hence use of iodine in agricultural practices for biofortification of crops seems to be an important area for research [12] [13].

The earlier studies done in our lab [14] in an attempt to investigate the effect of different chemical nutrient applications on soybean plants showed considerable modulation of leaf and seed mineral nutrient concentration by increasing several leaf and seed mineral content such as Cu, Zn, Mo B, K, N, P, Mg and S in several treatment groups. Based on our earlier promising results, our present investigation was extended to incorporate a more extensive approach in the study of essential minerals consisting of both cations and anion forms including the two beneficial elements KI and NaCl.

2. Material and Methods

2.1. Experimental Design

A greenhouse experiment was conducted at Mississippi Valley State University, Itta Bena, Mississippi, USA (latitude of N 33°28' and longitude W 90°20'). Soybean cultivar Bolivar (maturity group V) was planted in 34 pots (4 gallons). The pots were divided and labeled into 12 treatment groups, each group consisting of 3 replicates; Control (C), KNO₃ (T1), NH₄NO₃ (T2), NH₄H₂PO₄ (T3), KI (T4), KCl (T5), NaCl (T6), (NH₄)₂SO₄ (T7), Na₂SO₄ (T8), Na₂HPO₄ (T9), Ca(NO₃)₂ (T10), Mg(NO₃)₂ (T11). Pots with topsoil were arranged in a randomized complete block design. Eight seeds were planted in each pot.

2.2. Treatment and Statistical Analysis

The plants were treated with 300 mL of Hoagland solution and 300 mL of distilled water. This was to initiate germination and have healthy plants before chemical treatments. The plants germinated in ten days. Then pots were watered once a week. The temperature in the greenhouse throughout this experiment varied from 32.2°C to 35°C. The treatment included 30 mmol of chemicals application/pot at V3 stage to all 11 groups, while the control received the same amount of water. After 10 days of chemical treatments, leaves were sampled and dried in oven for 48 - 72 hours at 65°C. 2.0 grams of dry ground leaf samples were sent to Soil, Plant and Water testing laboratory, UGA, Athens, GA, for mineral analysis. Nutrients were analyzed by digesting 0.5 g of samples in a microwave digestion system. Briefly, dried samples were ground in a Wiley mill and passed through a 20 mesh screen. The samples were digested following EPA Method 3052 [15] as follows: 0.500 g samples were weighed out and placed in fluorocarbon polymer microwave vessels, 10 mL concentrated HNO₃ was added to each vessel. Vessels were sealed and placed in a microwave digester (CEM Mars 6 Microwave, Matthews, NC, USA) and heated at 200°C for 30 minutes. The digests (solutions) were transferred quantitatively into volumetric flasks and brought to 100 mL volume with deionized water. Finally, the solutions were analyzed for various elements (P, K, S, Ca, Mg, Fe, Mn, Al, B, Cu, Zn, Ni) following EPA Method 200.8 [16] by Inductively Coupled Plasma—Optical Emis-

sion Spectroscopy (ICP-OES). Means were separated using Fisher's Least Significant Difference test using 5% as level of significance.

3. Results

Leaf Mineral Composition

Results from leaf mineral analysis are illustrated in detail in **Table 1**, which contains mineral concentration, mean, and statistical significance compared to control treatments. According to our leaf mineral analysis, K concentration increased by 10% or more in the following treatments, T1, T2, T4, T5, T10 and T11, the highest increase found in T4 by 31.9%. P concentration increased in T3, T4 and T9, the highest increase again in T4 (60.8%), while Ca had increased only by two treatments T8 and T10 by 20%. Sulfur, another macronutrient showed an increase in the leaves by T2, T4 and T7. Among the micronutrients, B concentration was increased by several treatments (T1, T3, T4, T5, T6, T7 and T9), the highest increase observed in T3 and T4, which was 43.47% and 53.6% respectively. Cu concentration increased by T1, T4 and T10, the highest reported in T4 (36.3%). Zn adsorption also increased in T2, T4 and T7, while Fe increase was observed only in one treatment T8. Overall, several treatments (T1, T2, T4, T5, T8 and T10) increased K concentration in leaves, several treatments (T1, T2, T3, T4, T5, T7, and T9) increased B (**Table 2**). Out of all the above treatments, the four treatments T1, T2, T4 and T5 proved to be the most efficient in both K and B uptake. KI (T4) had the most positive outlook in acquisition of several mineral nutrients (Ca, K, Mg, B, Cu and Zn) by the plants (**Table 2**).

4. Discussion

4.1. Comparing Anion Effects

a) Comparing T1 (KNO_3) to T4 (KI) Treatment: I^- to NO_3^-

Iodine is not considered as an essential element for land plants, however, KI treatment in our study shows iodine to have beneficial effect on uptake of several minerals in the leaves for example K, Mg, P, S, B, Cu and Zn by 32.0%, 8.0%, 61.0%, 38.0%, 53.6%, 36.3% respectively compared to control, which was similar to the findings reported in other studies [12] [17]. While comparing the two treatments T1 vs T4, T1 increased K, B, Cu concentration in the leaves by 11.9%, 23.0% and 11.5%, but iodine anion impacted more positively by increasing uptake and accumulation of several other minerals in the leaves as listed above. Iodine has been studied to have significant impact on the redox state of the system that absorbs the element, therefore, it interacts with other chemical components of the system, such as organic compounds and metal ions, modifying the oxidation state and bioavailability [18] [19]. The results from our experiments showed synergistic effect on other elements as evidenced by their increased accumulation in the soybean leaves. However, results widely vary in different plant

Table 1. Mean values of macro and micronutrients, and significant differences of treatments with respect to control.

Treatments	Macronutrients (%)					Trace elements (Micronutrients) (mg/kg)					
	Ca	K	Mg	P	S	B	Cu	Fe	Mn	Zn	Na
Control	0.64 BCD	4.26 E	0.49 B	0.23 CDE	0.31 DE	71.26 F	6.97 C	70.67 B	596.5 BCD	134.60 F	299.63 BC
T1-KNO ₃	0.51 F (-10%)	4.77 BC (11.9%)	0.47 BCD	0.22 DE	0.30 DEF	87.70 BC (23%)	7.77 B (11.5%)	76.27 B	525.9 D	140.87 DEF	210.3 G (-29.81%)
T2-NH ₄ NO ₃	0.64 B	4.87 B (14.0%)	0.49 B	0.24 C	0.39 C (26.0%)	90.75 B	6.75 CD	76.40 B	549.7 D	156.50 B (16.2%)	233.10 EFG (-22.0%)
T3-(NH ₄) ₂ HPO ₄	0.58 CE	4.39 E	0.48 BCD	0.33 B (43.47%)	0.31 DEF	104.03 A (46%)	6.83 C	67.77 B	581.0 BCD	145.63 CD (8%)	218.63 FG (-27.2%)
T4-KI	0.57 DEF	5.62 A (31.9%)	0.53 A (8%)	0.37 A (60.8%)	0.43 B (38.7%)	109.50 A (53.6%)	9.50 A (36.3%)	92.40 B	388.5 E (-34.9%)	149.20 BC (10.8%)	465.2 A (55.25%)
T5-KCl	0.65 BC	4.72 C (10.8%)	0.48 BC	0.22 DE	0.24 G (-22.6%)	82.73 CD (15.9%)	6.53 CD	71.80 B	565.0 CD	138.50 DEF	255.43 DEF (-14.75%)
T6-NaCl	0.61 CDE	4.56 D (7%)	0.46 CDE (-6.1%)	0.23 CDE	0.27 FG (-12.9%)	79.67 DE (11.7%)	6.80 C	83.53 B	559.4 CD	140.90 DEF	256.90 CDEF
T7-(NH ₄) ₂ SO ₄	0.68 B	4.0 F (-6.1)	0.47 BCDE	0.24 CD	0.52 A (67.8%)	79.13 DE (11%)	6.23 D (-11%)	104.50 B	614.1 BC	167.40 A (24.4%)	295.70 BCD
T8-Na ₂ SO ₄	0.77 A (20.3%)	4.54 D (5.6%)	0.48 BCD	0.22 CDE	0.33 D	71.36 F	6.83 C	197.00 A (179%)	555.6 CD	138.83 DEF	272.57 CDE
T9 -Na ₂ (HPO ₄) ₄	0.61 BCE	4.35 E	0.45 E (-8.2%)	0.32 B (39.13%)	0.29 EF	77.80 DE (9%)	6.47 CD	79.20 B	648.4 B	143.23 CDE	219.53 FG (-26.7%)
T10-Ca(NO ₃) ₂	0.81 A (20%)	4.76 BC (11.7%)	0.54 A (10.2%)	0.20 E (-13.0%)	0.27 FG (-12.9%)	76.13 EF	5.50 E (14.3%)	76.67 B	726.3 A (21.8%)	135.00 F	189.47 G (-36.77%)
T11-Mg(NO ₃) ₂	0.56 EF (-12%)	4.75 BC (11.5%)	0.46 DE (-6.1%)	0.21 E (-8.7%)	0.33 D	74.93 EF	6.63 CD	71.90 B	400.6 E (-32.8%)	135.63 EF	321.93 B

Notes: Means given within a column bearing the same letter as the control are not significantly different at $p < 0.05$. The numbers in the brackets with % are the percentage changes increase or decrease in seed composition.

species and often have inconsistent result [20] [21]. Further, plants can accumulate iodine in the leaves but this effect does not necessarily translate into fruits and vegetables. The agricultural application of iodine is not fully studied as very little study exists, however, since few studies undertaken have shown promising role of iodine in enhancement of plant growth, antioxidant capacity and increased stress tolerance, hence, there is a critical need to expand research in the area of iodine biofortification. The concentration of the KI treatment used in our study although increased the uptake of several minerals (Table 2) in the leaves but eventually caused toxicity to the plants in which most plants died soon after the leaf sampling, similarly, as evidenced by other studies [11]. The toxicity could be due to volatilization of iodine and changes in the mineral composition of plants due to redox phenomenon [19].

b) Comparing T1 (KNO₃) to T5 (KCl) Treatment: NO₃¹⁻ to Cl¹⁻

T1 increased K, B, Cu, in the leaves by 11.9%, 23.0% and 11.5% and decreased Ca and Na by 10.0% and 29.8% respectively, which is consistent with other studies [16] which demonstrates nutrient cation exchange is possible up to a certain concentration without causing any injury to the plants. Earlier studies [22] have

Table 2. Overall effect of the application of each treatment according to the significant differences.

Chemical Treatment	Main Results	Main Nutrients Affected
KNO ₃	+	K, B, Cu
KNO ₃	-	Ca, Na
NH ₄ NO ₃	+	K, S, Zn
NH ₄ NO ₃	-	Na
(NH ₄)HPO ₄	+	P, B, Zn
(NH ₄)HPO ₄	-	Na
KI	+	K, Mg, P, S, B, Cu, Zn, Na
KI	-	Mn
KCl	+	K, B
KCl	-	S, Na
NaCl	+	K, B
NaCl	-	Mg, S
(NH ₄) ₂ SO ₄	+	S, B, Zn
(NH ₄) ₂ SO ₄	-	K, Cu
Na ₂ SO ₄	+	Ca, K, Fe,
Na ₂ (HPO) ₄	+	P, B
Na ₂ (HPO) ₄	-	Mg, Na
Ca(NO ₃) ₂	+	Ca, K Mg, Cu, Mn
Ca(NO ₃) ₂	-	P, S, Na
Mg(NO ₃) ₂	+	K
Mg(NO ₃) ₂	-	Ca, Mg, P, Mn

Main Results: (+), positive; (-), negative.

also reported that the plants tend to be healthier where KNO₃ is the source for K compared to KCl. T5 treatment increased K and B by 10.8% and 15.9% respectively and decreased S and Na by 22.6% and 14.8% respectively. Both treatments NO₃¹⁻ and Cl¹⁻ increase K, B while both decreasing Na. Previous studies have shown similar results, when Cl is applied to soils in KCl form, it tends to substitute for N, S, P in plants resulting in lower protein content [23]. Hence, KCl will not be a good treatment where plants are grown for protein value for example soybean.

c) Comparing T2 (NH₄NO₃) to T3 ((NH₄)H₂PO₄) Treatment: NO₃¹⁻ to H₂PO₄¹⁻
 T2 treatment increased K, S, B, Zn by 14.0%, 26.0%, 27.3% and 16.0% respectively while decreasing Na by 22.0%. T3 increased P, B, Zn by 43.5%, 46.0% and 8.0% respectively and decreased Na by 27.0%. Common in both are B and Zn. H₂PO₄¹⁻ increased P by 43.5% and S by 46.0%. In comparison, ammonium nitrate is better suited to controlling acidic soil, however, application of ammo-

nium phosphate with the two basic components of the fertilizer upon dissolution will separate to release ammonium (NH_4^+) and phosphate (H_2PO_4^-), both of which plants rely on for healthy, sustained growth [24]. Both treatments had positive effect on mineral uptake, however, will depend on the soil mineral test and crop preference.

d) Comparing T8 (Na_2SO_4) to T9 ($\text{Na}_2(\text{HPO})_4$) Treatment: SO_4^{2-} to HPO_4^{2-}

T8 increased Ca, K, and Fe by 20.3%, 5.6% and 179.0% respectively, while T9 treatment increased P and B by 39.1% and 9.0% respectively and decreased Mg and Na by 8.2% and 26.7% respectively. In comparison, Na_2SO_4 offers significant increase in Fe. Though Na (T8 and T9) is considered as beneficial element by some agricultural crops [25] and as evidenced in the present study Na_2SO_4 (T8) application considerably increased the transport and uptake of Fe into the plants, however, there is need to further study the concentration of the chemical applied more carefully since higher dosage might cause plant injury

4.2. Comparing Cation Effects

a) Comparing T1 (KNO_3) to T2 (NH_4NO_3) Treatment: K^{1+} to NH_4^+

T1 treatment increased K, B, Cu, in the leaves by 11.9%, 23.0% and 11.5% increased respectively, while decreasing Ca and Na by 10.0% and 29.8% respectively. T2 treatment increased K, S, B, Zn by 14.0%, 26.0%, 27.3% and 16.0% respectively and decreased Na by 22.0%. However, KNO_3 is a soluble source of two major essential plant nutrients, commonly used of high value crops that could benefit from nitrate (NO_3) and a source of potassium (K) free of chloride [26]. Ammonium (NH_4) is again a cation which competes with other cations like potassium, calcium and magnesium which may cause Ca and Mg deficiency at high concentrations [27]. Hence, it is important to test the capacity of plant species to utilize ammonium at acidic as well as near neutral pH to dissociate ammonium and pH effects. However, the concentrations used in our present study had similar positive effect in increasing K, B while significantly decreasing Na.

b) Comparing T5 (KCl) to T6 (NaCl) Treatment, K^{1+} to Na^{1+}

T5 treatments increased K and B by 10.8% and 15.9% respectively and decreased S and Na by 22.6% and 14.8% respectively. T6 treatment increased K and B by 7.0% and 11.8 % respectively while decreasing Mg and S by 6.1 and 12.9% respectively. It seems both cations (K^{1+} vs Na^{1+}) increase K and B, however KCl treatment decreased S and Na, and NaCl decreased S and Mg, As studied earlier, when Cl is applied to soils in KCl, it tends to substitute for N+S+P in the plants growing on the soil and lower their protein content [23]. High Cl concentrations reduce the photosynthetic capacity due to chlorophyll degradation [28]. Furthermore, sulfur being an important constituent of protein and magnesium having an important role in chlorophyll production, the two treatments do not offer positive outcome.

c) Comparing T10 ($\text{Ca}(\text{NO}_3)_2$) to T11 ($\text{Mg}(\text{NO}_3)_2$) Treatment: Ca^{2+} to Mg^{2+}

T10 treatment increased Ca, K, Mg, P, Cu, and Mn by 20.0%, 11.7%, 10.2%,

13.0%, 14.3% and 21.8% respectively and decreased S and Na by 12.9% and 36.8% respectively. Similarly, previous studies have reported an increase in K content while decreasing Na in plants in the presence of supplemental Ca, which is in part due to Ca, K and Mg which are known to be involved in cation exchange. T11 treatment, similarly, increased Ca, K, P, but in contrast to T10, increased Na by 7.4%, while decreasing Mg and Mn by 6.1% and 32.8% respectively. Although Both Ca^{2+} and Mg^{2+} had a positive role in increasing Ca, K, and P in leaves, in comparison, T10 increased several other minerals like Mg, Cu and Mn, hence, T10 with additional benefits and at the same time less expensive to treat could be the mineral of choice in many instances depending upon mineral nutrient index in the soil [29] [30].

5. Conclusion

In comparison, T1 (KNO_3), T2 (NH_4NO_3), T4 (KI) and T5 (KCl) treatments were most effective in increasing K and B both. Iodine is not considered as an essential element for land plants; however, KI treatment (T4) in our study shows iodine to have very promising effect on uptake of several minerals in the leaves like K, Mg, P, S, B, Cu and Zn. Another chemical was $\text{Ca}(\text{NO}_3)_2$ which offered positive results in the uptake of number of minerals like Mg, Cu and Mn. Both Ca^{2+} and Mg^{2+} have a role in increasing Ca, K, and P in leaves. Although every treatment had an impact in increasing one or two of the essential nutrient elements required by the plants, we concluded the three treatment groups T1 (KNO_3), T4 (KI) and T10 $\text{Ca}(\text{NO}_3)_2$ to be the most efficient treatments among all applications in increasing several minerals in soybean leaves (Table 2). The choice of treating the crops will ultimately depend on crop preference, soil pH, and nutrient deficiency.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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