

**THE EFFECT OF LIMING AN ACID NITISOL WITH EITHER
CALCITE OR DOLOMITE ON TWO COMMON BEAN (*PHASEOLUS
VULGARIS* L.) VARIETIES DIFFERING IN ALUMINIUM TOLERANCE**

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ABSTRACT

Two common bean (*Phaseolus vulgaris*) varieties, Rosecoco (GLP 2) and French bean (cv 'Amy'), previously shown to differ in Al tolerance were tested for their response to liming with either calcite or dolomite in a potted, strongly acid and low fertility humic Nitisol. Dry matter yield, as a response to liming was highest at liming pH 5.6 for both varieties and lime types. However, 'Amy' responded more to dolomite liming than Rosecoco to attain whole plant growth above control to 108 % and 104% (calcite), and 127 % and 102 % (dolomite) respectively. Higher calcite liming (pH 6.8) significantly reduced growth and was attributed to magnesium deficiency through competition with calcium. Differential Al accumulation in the two varieties was higher in the shoots of 'Amy' French bean but lower in roots, an indication that one of the possible mechanisms of Rosecoco's Al tolerance is confinement of Al to roots rather than in shoots. However, Al uptake decreased in the roots and shoots of both varieties with increasing levels of liming. Calcium content in shoots increased with increasing liming levels but was higher in calcite than in dolomite treatments.

There were insignificant increases of Mg uptake with calcite liming, but the opposite was observed with dolomite liming. Rosecoco was more efficient in the uptake of Ca and Mg than the French bean. It is concluded that there exists a varietal difference in *Phaseolus vulgaris* response to soil acidity and associated hazards. Therefore, it is possible to select and breed/introduce Al tolerant cultivars for the acid soils of Kenya. Although both types of limes can be used to reduce the hazardous effect of acidity, dolomitic limes have an advantage due to the additional magnesium nutrition. To achieve the highest crop yield on acid Nitisols, it is therefore recommended that only dolomitic limes should be applied to a pH of around 5.6.

Key words: Al tolerance, humic nitisol, liming pH, lime type, *Phaseolus vulgaris*

1.0 INTRODUCTION

In Kenya, acid soils with a pH of 5.0 or less comprise some or all nitisols, acrisols, ferralsols, cambisols and Andisols (FAO, 1988; Wokabi, 1987). These soils are estimated to cover an area of about 5 million hectares (Mugai, 2000) They are located in the highlands where the climate is suitable for the intensive cultivation of many crops including the main source of protein to majority of Kenyans, the common bean. Continuous cultivation and use of soil acidifying fertilisers, especially those containing ammonium, are probable contributors to further acidification of these soils.

Although the hazards of soil acidity are obvious (e.g., inhibited root growth and consequent reduced water and nutrient uptake), the small-scale farmers have not adapted the culture of ameliorating it through liming, nor is information available regarding relevant soil properties and the liming requirements for a particular crop. In Kenya, two cheap types of lime are available - calcite (CaCO_3) and dolomite ($\text{CaMg}(\text{CO}_3)_2$). There are no comparative studies yet done to test the efficacy of the two lime types in the management of specific acid soils of Kenya. Moreover, the differential performance between bean varieties Rosecoco and French bean (cv Amy) that had been shown to differ in Al tolerance when grown in hydroponics (Mugai *et al.*, 2000; Mugai, 2000) needed to be confirmed in soil of high Al saturation, hence the justification for this research. The objectives of this research were to:

- (i) Characterise the acidity and related fertility properties of the acid humic nitisol.
- (ii) Determine the optimum liming requirement with either calcite or dolomite on Rosecoco and the 'Amy' French bean,
- (iii) Determine the differential effects of liming with either calcite or dolomite on the growth and nutrition aspects of the two common bean varieties, and,
- (iv) Evaluate the performance of Rosecoco and 'Amy' French bean in an acid, low fertility soil.

2.0 MATERIALS AND METHODS

2.1 Soil Sampling and Analyses

Top soil (0-15 cm) was obtained from a *humic Nitisol* (FAO, 1988; Sombroek *et al.*, 1980), in the tea-growing zone (altitude 2,150 m, Agro-climatic zone I-6) of Gatundu Division, Thika District, Kenya. The soil was air dried, passed in through 2 mm sieve and mixed to obtain a homogeneous mixture. A sub-sample of the soil was analysed for pH (1:2.5 soil/water; 1:2.5 soil/0.01 CaCl_2 solution); extractable Al (1N KCl) (Chapman and Platt, 1961); available phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) (Hinga *et al.*, 1980). Phosphorus was determined by the molybdate blue method and measured by spectrophotometry, K by flame photometry and Ca and Mg by the atomic absorption spectrophotometry (AAS). Organic carbon was determined by the wet oxidation method (Hinga *et al.*, (1980).

Cation exchange capacity (CEC) was determined by ammonium acetate method (Chapman and Platt, 1961) and effective cation exchange capacity (ECEC) as the sum of cations that had been extracted with un-buffered ammonium chloride. Al was determined by the colorimetric aluminon method (Chapman and Platt, 1961).

2.2 Neutralising Equivalence of Limes

The neutralizing equivalence of both calcite and dolomite was assessed and calculated as per Jackson (1958), as described by Chapman and Platt (1961). Briefly, 1 g of the lime were reacted with 1N HCl. After dilution, to 100 ml and boiling, the mixture was cooled and back-titrated with 1N NaOH using phenol-phthalein as indicator and calcium carbonate equivalence calculated.

2.3 Estimation of Liming Requirements and Plant Culture

Liming materials were procured from local merchants. Estimation of liming requirements was from a calibration curve of pH against lime after incubating wet soil (1: 2.5 soil: water) with varying amounts of lime (calcite or dolomite) for seven days (Alley and Zelazny, 1987). The liming levels were 0, 6.0, 16.0 and 32 and 0, 6.0, 17, and 34 g/4 kg soil of calcite and dolomite respectively, to attain pH levels of 3.95, 5.1, 5.6 and 6.8. The limes were thoroughly mixed with the soil before potting. 1.32 g of diammonium phosphate (DAP) fertiliser equivalent to 200 kg (DAP) per hectare was mixed with top 5 cm of potted soil in 3.5 litre pots before planting. Three uniform (2-true leaf) seedlings previously pre-germinated in sterile sand were planted per pot. After a week of growth, thinning was done to leave one seedling per pot. The liming treatments including the control (pH 3.95) were replicated three times in a completely randomised design and placed on raised benches in the greenhouse. Distilled water was applied for basal irrigation. After one month all the 48 plants were harvested, the soil washed off with running tap water, then rinsed with distilled water. The plants were partitioned into roots and shoots and then dried for 24 hours at 80 °C in a blow oven. The dry shoots and roots were weighed separately and then ground in a plant mill. A sub-sample of 0.5 g of either root or shoot per treatment was ashed in a furnace at 550 °C for 6 hours. The ash was dissolved in 5 ml of 6N HCl, dehydrated, then dissolved again in 2 ml of 6N HCl and diluted to 100 ml. Total Al, Ca and Mg in both shoots and roots and were then determined by same methods as described in 2.1 for soil.

3.0 RESULTS

3.1 Soil and Lime Analyses

The summary of the soil analyses data is presented in Table 1. The soil is strongly acidic. The Al content is high, reaching a saturation of about 50 %. The high organic carbon is an indication of a lower rate of humification due to high acidity of the soil and the cool climate of the area.

The chemical soil fertility is low except for K.

Calcite and dolomite were found to have neutralising equivalence values of 91.6 and 87 respectively.

3.2 Effect of Liming on Plant Growth

Table 2 presents the shoot and root dry weights of the two bean varieties against liming type and levels. The shoot and root dry biomasses of the two bean varieties were significantly improved under liming irrespective of the lime type. The highest growth in both shoots and roots was attained at pH 5.6 across the varieties. In the un-limed soil the French bean variety exhibited a significant suppression of growth as compared to Rosecoco. Liming with calcite produced significantly less growth compared to dolomite across the two varieties and at the various levels of liming. In both types of liming, growth increase declined for both varieties at pH 6.8, although the decrease was higher in 'Amy' than in Rosecoco.

Differential varietal response to lime was assessed by computing the relative whole plant growth against the un-limed control and expressed as a percentage (Figure 1). At maximum growth (pH 5.6), the growth response to calcite liming was similar in both varieties (102 %). However, dolomite liming gave higher growth response in 'Amy' than in Rosecoco showing a peak of 110 % for Rosecoco and 127 % for 'Amy' at liming pH 5.6.

3.3 Uptake of Al

Al contents in both shoots and roots are presented in Table 3. Both varieties accumulated less Al in shoots than in roots. French bean variety (cv 'Amy') concentrated a higher amount of Al in shoots than Rosecoco, while Rosecoco accumulated more Al in roots. As liming increased, the content of Al in roots and shoots of both varieties decreased. However, the amount of Al in the roots at calcite liming of pH 5.1 was highest - higher than even in the control plants. Generally, the Al uptake in the dolomite-limed plants contained less Al than in calcite limed ones in both shoots and roots of both varieties.

3.4 Uptake of Ca and Mg

The contents of Ca and Mg in shoots and roots in both varieties are presented in Table 4. The Ca concentration in both shoots and roots was higher in calcite-limed plants than in dolomite limed ones. The Ca concentration in both shoots and roots of control plants was higher in Rosecoco than in 'Amy' French bean. The calcite-limed plants had the highest Ca concentration in both shoots and roots at pH 5.6 while dolomite limed plants had their peak concentration at pH 6.8. However, there was no significant difference in Ca contents between the two varieties in the Ca treatments. The Ca concentration in both shoots and roots in the calcite and dolomite-limed

plants was higher in Rosecoco than in 'Amy'.

Mg accumulation in shoots of control plants was also higher in Rosecoco than in 'Amy' French bean. It increased with liming with calcite to reach a peak at pH 5.6. In the dolomite-limed plants, Mg increased with liming to reach a peak at pH 6.8. Mg accumulation in the roots of control plants was higher in Rosecoco than in 'Amy' French bean. In calcite limed plants the Mg accumulation in the roots increased with increasing liming to reach a peak at pH 5.6 in both varieties. Generally the Mg accumulation in the roots was higher in Rosecoco than in 'Amy' for both types of limes. Mg accumulation in both shoots and roots of the dolomite-limed plants was much higher than in calcite treatments.

The control plants in both varieties showed clear symptoms of Ca deficiency. These were severe stunted growth, crinkled, curled and abnormally green young leaves, and some patchy interveinal chlorosis in the middle leaves (plate not presented). In the plants limed with calcite to pH 6.8, symptoms of Mg deficiency mainly interveinal chlorosis occurred in older leaves, while those treated at same pH level with dolomite were normal (plate not presented).

4.0 DISCUSSION, CONCLUSIONS AND RECOMMENDATION

The humic nitisol (FAO, 1988) on which the experiment was conducted is strongly acid and low available nutrients (Table1). The poor fertility of acid soils is a combination of many factors (Uexküll and Mutert, 1995). The main ones are high Al toxicity and low levels of P, Ca and Mg. Liming soil has the primary objective of raising the pH in order to decrease the soluble amounts of Al, Mn and Fe, which may not only cause toxicity to plants, but also immobilise P (Tisdale *et al.*, 1993). The solubility of Mn and Fe is more controlled by soil redox than by pH and therefore in well drained soils like in this case in Kenya highlands where acid soils dominate, Al toxicity and soil infertility are the main hindrance to high crop yields (Mugai *et al.*, 2001).

In this study, phosphorus and nitrogen were supplied to the plants through diammonium phosphate fertilizer, as is the custom in the small-scale cultivation of beans. The higher growth exhibited in limed plants as compared with control plants is an indication of either lower toxicity of Al and increased Ca uptake (calcite liming) or increased Ca and Mg nutrition (dolomite liming). These soils are low in Ca and Mg and therefore a liming material like dolomite, which contains both of the elements, was bound to increase the yield of crops more than under calcite liming, which contain only Ca.

The differences in acidity tolerance of the two bean varieties agree fairly well with the results earlier obtained (Mugai *et al.*, 2001; Mugai *et al.*, 2002) where root elongation in three day-old seedlings of Rosecoco and 'Amy' French bean was depressed by 38 % and 78 % respectively in nutrient solution containing 5 μ M Al. Earlier work (Ma *et al.*, 1997) showed that Al-sensitive plants' root elongation

responded more to low Al levels than Al-tolerant ones. The results of this study more or less agree with this phenomenon (Figure 1). Consequently, the stimulation of root growth upon liming was more pronounced in Al-sensitive 'Amy' than in the relatively Al-tolerant Rosecoco albeit insignificantly ($P = 0.05$). However the depression of growth upon over-liming (pH 6.8) in French bean cultivar was greater than in Rosecoco (Table 4 and Fig. 1.) Under excessive calcite liming, Rosecoco was able to take up more Mg from the soil probably because of:

(i) Its more developed root system, and,

(ii) Higher availability of Mg through chelation by excreted citric acid (Mugai, 2001) while under excessive dolomite liming it was able to take up more nutrients including trace elements which might have been unavailable through immobilisation (by high pH) or competition for uptake by Ca. The explanation for higher Mg uptake in dolomite-limed plants is because of (ii) above, higher soil supply through dolomite and less competition by Ca because of near 1:1 Ca:Mg ratio in dolomite. The higher growth response to liming with dolomite than with calcite is easily explained by the presence of Mg in the former. The higher response to liming in 'Amy' was not well correlated with either Ca or Mg concentration. This may probably be that Al toxicity was more critical than Ca or Mg nutrition in this particular soil.

In control plants, the shoots of Rosecoco contained less Al than the Al-sensitive 'Amy'. Therefore, the sensitivity of 'Amy' to Al is not only its reduced ability to exclude Al from roots through its reduced capacity to excrete citric acid (Mugai, 2001; Mugai *et al.*, 2002), but also its inability to restrict the Al translocation to the shoot. There is therefore a need to also study the toxicity effects of Al in shoots, which has hitherto been neglected. However, un-limed Rosecoco contained more Al in roots than in 'Amy'. This agrees with earlier results (Mugai *et al.*, 2000), where 3 day-old seedlings of Rosecoco had more Al in roots than 'Amy' at more than 30 μM Al treatment. One probable reason for this phenomenon may be that the high Al suppressed root surface area in the un-limed French bean to an extent of even reducing the total uptake of Al uptake. Thus, the root suppressing capacity in un-limed soil was equivalent to that induced by more than 30 μM Al in CaCl_2 culture solution (Mugai *et al.*, 2000).

The Al concentration in both varieties and in both types of liming declined as liming level was increased. This is expected because as pH is increased the toxic monomeric species of Al also decreases. However, concentration of Al in calcite treatment at pH 5.1 was higher than that in controls in the roots of the two varieties. This may be explained by the very poor growth in the controls, which reduced the uptake of all elements including Al (Tables 3 and 4).

The lower concentration of Al in dolomite treated plants at pH 5.1 and above implies that Mg just like Ca plays an Al-toxicity ameliorating role (Huang *et al.*, 1992).

Many studies have shown the role played by Ca in reduction to Al toxicity in plants. Al reduced Ca uptake in Al-sensitive wheat cultivars (Huang *et al.*, 1992) while Al-tolerant Dade Snap bean cultivar contained more Ca in its exudate than that of Al-sensitive Romano cultivar (Foy *et al.*, 1972). Higher Ca concentrations in nutrient solution decreased Al-tolerance differences among maize inbred lines (Rhue and Grogan, 1977). In this study (Table 4), the Ca content in shoots agrees very well with the work of Foy *et al.* (1973) where the tolerant Dayton barley cultivar accumulated more Ca than the Al-sensitive Kearney when Al was added into the nutrient solution. On the other hand, Rosecoco plants had a higher Ca concentration than the 'Amy' irrespective of liming level and type, indicating its higher capacity to take Ca due to its better root development even under acid soil conditions.

The peak Ca uptake to both roots and shoots was arrived at pH 5.6 in calcite limed plants and at pH 6.8 in dolomite limed ones. The reason may be that in calcite limed plants, the unequal Ca and Mg content (wide Ca: Mg ratio) ratio in soil at pH 6.8 liming led to a reduction in Mg uptake and consequent reduced growth while in dolomite limed plants both Ca and Mg were more or less equally supplied (Ca/Mg = 1) and offered equal competition against each other. The content of Ca in shoots of control plants is less than 1.3 % the minimum for optimal growth for dicotyledons (Marschner, 1995)] and this explains the Ca deficiency symptoms mentioned earlier.

In controls and calcite-limed plants the Mg concentration in shoots was higher in the more Al-tolerant Rosecoco than in the 'Amy' French bean, probably due to its better-developed root system and higher exudation of citric acid which was able to avail the deficient Mg through chelation process.. The Mg concentration in the shoots and roots of calcite limed plants rose with liming levels to reach a peak at liming pH 5.6 and declined at pH 6.8 due to reduced growth associated with reduced Mg uptake. In dolomite-limed plants, the peak Mg content was reached at liming pH 6.8 (Table 4). This was due to the ample supply of Mg through dolomite.

The content of Mg in control plants and in all calcite limed plants is less than the critical range 0.15 - 0.35 % of dry weight (Marschner, 1995), (Table 4) thus the cause of the Mg deficiency symptoms mentioned above.

The study has conclusively shown the benefits of liming a low fertility, acid soil. Dolomite liming gave better growth than calcite and therefore the former should be recommended as the type of liming material for acid soils of the tea zones of Kenya in the cultivation beans in. The increase in the dry weight of the whole plant is well related to Al-tolerance - the more the response to liming the more Al-sensitivity of the bean variety.

Al accumulation in the shoots of un-limed plants is a good indicator of acidity tolerance just like in young plants (Mugai *et al.*, 2002) but not so for Al in roots which seem to accumulate more in Al-tolerant *Phaseolus vulgaris*. The accumulation of Al in roots of *Phaseolus vulgaris* grown in soil to near maturity gives contradictory

result to that found in young plants grown in hydroponics and therefore use of Al contents in screening plants in soil grown to near maturity can only be done in combination with other techniques. Both Ca and Mg accumulation can be used to assess Al-tolerance in the common bean (*Phaseolus vulgaris*) grown in soil.

The most ideal liming pH level, in this strongly acid soil irrespective of the liming material, is around 5.6.

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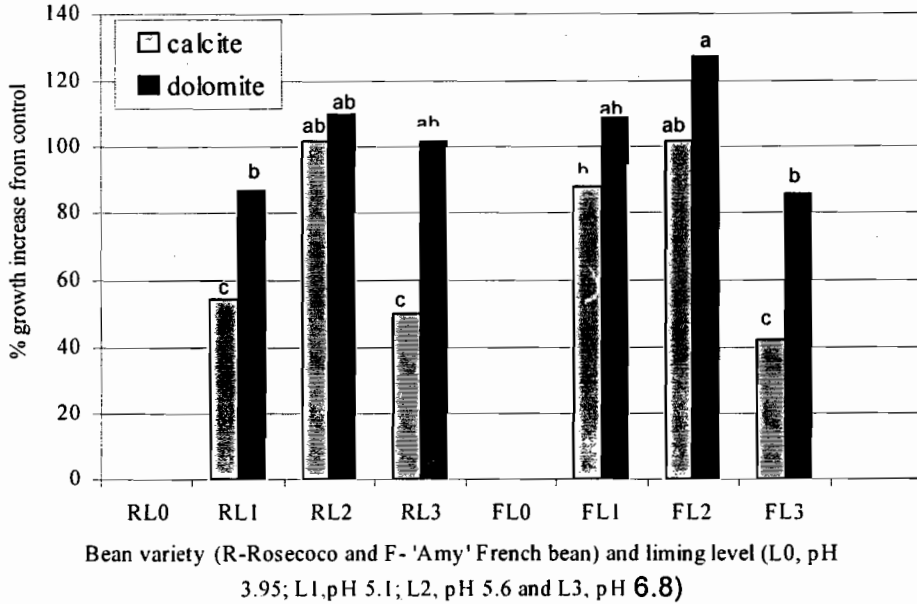


Figure 1: Relative growth (% change from control) of Rosecoco (R) and French bean (cv Amy) (F) varieties in response to liming at different levels with either calcite or dolomite. Means separation by Lsd test at $P = 0.05$ ($n = 3$)

Table 1: Selected chemical properties of the test soil

| Mapping unit (FAO) | pH Water | pH CaCl ₂ | CEC _s (cmole ⁽⁺⁾ /kg) | ECBC (cmole ⁽⁺⁾ /kg) | Organic Carbon % | Available nutrients (cmole ⁽⁺⁾ /Kg) | Extractable Al (cmole ⁽⁺⁾ /Kg) | |
|-----------------------|-------------|-------------------------|--|------------------------------------|---------------------|---|--|---------|
| | | | | | K | Ca | Mg | P (ppm) |
| Humic Nitisol | 3.95 | 3.4 | 24.8 | 11.4 | 1.9 | 0.67 | 0.25 | 11.0 |
| | | | | | | | | 5.11 |

Table 2: The effect of liming levels with either calcite or dolomite on the growth of two Phaseolus vulgaris varieties, Rosecoco (R) and French bean (cv Amy) (F)

| Variety and Liming level (pH) | Calcite | | Dolomite | |
|-------------------------------|-------------------------------|---------------------|----------------------|---------------------|
| | Shoot dry weight (g) | Root dry weight (g) | Shoot dry weight (g) | Root dry weight (g) |
| R 3.95 | 0.4142 ± 0.027 c ¹ | 0.1713 ± 0.023 b | 0.4142 ± 0.027de | 0.1713 ± 0.023 c |
| R 5.1 | 0.6854 ± 0.043 b | 0.2222 ± 0.017 a | 0.855 ± 0.021 b | 0.2433 ± 0.029 b |
| R 5.6 | 0.9011 ± 0.041 a | 0.2817 ± 0.0021 a | 0.995 ± 0.052 a | 0.2967 ± 0.012 a |
| R 6.8 | 0.6185 ± 0.14 b | 0.2583 ± 0.0164a | 0.9133 ± 0.025 b | 0.225 ± 0.0071 b |
| F 3.95 | 0.1917 ± 0.016 e | 0.0783 ± 0.0153 d | 0.1917 ± 0.016 e | 0.0783 ± 0.0153 e |
| F 5.1 | 0.3733 ± 0.038 c | 0.095 ± 0.00 d | 0.4325 ± 0.062 e | 0.1405 ± 0.0076 d |
| F 5.6 | 0.4433 ± 0.034 c | 0.1033 ± 0.0153 d | 0.4583 ± 0.04 d | 0.159 ± 0.025 cd |
| F 6.8 | 0.2867 ± 0.0058 d | 0.0783 ± 0.0153 e | 0.3925 ± 0.0035 e | 0.0875 ± 0.035 e |
| Significance ² | | | | |
| Liming | *** | *** | *** | *** |
| Variety | *** | ** | *** | *** |
| Liming x variety | ** | *** | ** | *** |

Means within columns (plus/minus standard deviation) with similar letters do not differ significantly (p < 0.05) by Lsd (n = 3)

*** ** Significant different at p = 0.01 and 0.001 respectively

Table 3: Aluminium content (mg/g) in dry matter of shoots and roots of Rosecoco and French bean (cv Amy) in response to liming with either calcite or dolomite

| | Calcite | | | Dolomite | | |
|--------------------|----------------------|--------|--------|----------|--------|-------|
| | 3.95 ⁺ | 5.1 | 5.6 | 3.95 | 5.1 | 5.6 |
| Liming pH | | | | | | |
| | | | 6.8 | | | 6.8 |
| Rosecoco | | | | | | |
| Shoot | 0.45a ¹ * | 0.36b* | 0.29c* | 0.46a* | 0.36b* | 0.18e |
| Root | 6.1c* | 12.0a* | 8.8b* | 6.1c* | 6.1c* | 2.9d |
| French bean | | | | | | |
| Shoot | 0.82a* | 0.45b* | 0.33c* | 0.82a* | 0.29d* | 0.19e |
| Root | 3.9c* | 5.8a* | 5.0b* | 3.9c* | 3.2e* | 2.5f |

+ pH (1:2.5 soil :water, w/w ratio)

¹Means within liming treatments of either shoot or root separated by Lsd at P<0.05 (n=3). Means within columns and similar plant part (Root or shoot) across the two varieties and indicated with * are significantly different (P = 0.05; n =3)

Table 4 : Ca and Mg content (mg/g) in the dry matter of shoots and roots of Rosecoco (R) and French bean (cv Amy) (F) upon liming at various levels with either calcite or dolomite

| Liming pH | 3.95 | | | 5.1 | | | 5.6 | | | 6.8 | | |
|-----------|--------------------------|--------|--------|--------------------------|-------|-------|--------------------------|--------|-------|--------------------------|-------|--------|
| | Rosecoco Shoot | | | Rosecoco Shoot | | | Rosecoco Shoot | | | Rosecoco Shoot | | |
| Ca | 2.7c | 16.2b | 24.2a | 18.7ab | 2.7c | 11.8b | 14.4b | 18.5ab | 2.7c | 11.8b | 14.4b | 18.5ab |
| Mg | 1.04c | 1.2c | 1.4c | 1.2c* | 1.04c | 4.9b | 6.0ab | 7.5a | 1.04c | 4.9b | 6.0ab | 7.5a |
| | Root | | | Root | | | Root | | | Root | | |
| Ca | 2.2c* | 7.8b* | 18.5a* | 17.1a* | 2.2c | 7.4b* | 8.8b* | 9.3b | 2.2c | 7.4b* | 8.8b* | 9.3b |
| Mg | 0.94c | 1.6e* | 3.3 d | 2.4de | 0.94c | 5.3c* | 8.5b* | 11.1a | 0.94c | 5.3c* | 8.5b* | 11.1a |
| | French bean cv Amy Shoot | | | French bean cv Amy Shoot | | | French bean cv Amy Shoot | | | French bean cv Amy Shoot | | |
| Ca | 2.7d | 18.7ab | 19.3a | 18.8ab | 2.7d | 8.8c | 11.0c | 15.5b | 2.7d | 8.8c | 11.0c | 15.5b |
| Mg | 1.0d | 1.24cd | 1.74c | 1.65cd* | 1.0d | 5.1b | 5.7b | 6.9a | 1.0d | 5.1b | 5.7b | 6.9a |
| | Root | | | Root | | | Root | | | Root | | |
| Ca | 1.3d* | 4.3c* | 10.3a* | 8.7ab* | 1.3d | 4.0c* | 7.5b* | 8.3b | 1.3d | 4.0c* | 7.5b* | 8.3b |
| Mg | 0.8f | 1.2e* | 2.5c | 1.65b | 0.8f | 3.0b* | 6.0a* | 5.9a | 0.8f | 3.0b* | 6.0a* | 5.9a |

*Means within rows with similar letters are insignificantly different at P = 0.05 by Lsd test (n=3)

(P = 0.05; n = 3)