Aluminium Tolerance of Four Bean (*Phaseolus vulgaris* L.) Varieties

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ABSTRACT

Four bean (*Phaseolus vulgaris*) varieties ('Rosecoco'– GLP 2, 'Mwitemania'– GLP X 92, 'Mwezi Moja' – GLP 1004, and French bean – 'Amy') locally obtained from seed merchants in Kenya were investigated for their aluminium tolerance under two techniques of screening, namely root elongation and staining. Using hydroponic system, 3-day old seedlings were subjected to aluminium treatments of 0, 3, 5, 10, 20 and 50 μM, followed by subsequent root elongation studies and staining by Eriochrome cyanine R. The two techniques in combination produced the following increasing order of aluminium tolerance: French bean < Mwezi moja < Mwitemania < Rosecoco. Root elongation produced superior differential rating in assessing for aluminium toxicity in the beans. On the other hand, Eriochrome cyanine R staining lacked clear differentiation especially where there were marginal differences of Al tolerance. It follows that, screening for aluminium tolerance in common beans can preferably be accomplished through the staining technique procedure and only be followed by root elongation method under circumstances of ambiguity or where difference in tolerance are inseparable through the former.

KEYWORDS: Aluminium, toxicity, hydroponic, *Phaseolus vulgaris,* root, elongation, staining

1.0 INTRODUCTION

Aluminium (Al) toxicity is the main factor inhibiting crop growth in acid soils with pH below 5.0 through inhibition of root growth (Clarkson 1965, Foy *et al.* 1972 and 1978). This has also been observed in beans grown in unclassified acid soils of Uasin Gishu (Birech *et al.* 1999) and in *humic Nitisols* of Thika districts of Kenya (Mugai 2001). The inhibition of root growth reduces the plants' ability to take up nutrients and water (Foy *et al.* 1978). In Kenya, acid soils cover an area of about 5.5 million hectares

and are located mainly in the parts of Western, Nyanza, Southern Rift valley, Central southern Eastern and south western Coast provinces (Wokabi 1987, Mugai 2001). The common bean is grown in these soils because they are located in sufficient rainfall areas These soils are expected to acidify more due to continuous cropping and use of soi acidifying fertilizers as has already been demonstrated by many studies of acidification o acid soils of Australia (Porter *et al.* 1995). The reclamation of acid soils through liming may be economically not feasible in many developing countries (Foy *et al.* 1969, Furlan and Bastos 1990). Therefore selection and breeding for Al tolerance is a useful alternative approach in the utilization of acid soils. Aluminium tolerant crops are also adapted to the low phosphate conditions prevalent in these soils (Clark 1977).

Aluminium tolerance among common bean varieties grown in Kenya has not been investigated much except for the work reported by Mugai and Agong (1996) and Birech *et al.* (1999 and 2001). In the former report, Mugai and Agong (1996) established that the variety 'Rosecoco' experienced growth reduction from as low as 2 ppm in full nutrien sand culture experiment, although significant root growth differences from control were only observed from 20 ppm Al treatment. Birech *et al.* (2001) studied 13 bean East African bean genotypes in concentrated nutrient cultures. However, in all these previou studies, the Al-tolerance experiments were conducted under high concentrations o nutrient solution culture. This correspondingly reduced the sensitivity of the plants to lo Al treatments and in case of results of Birech *et al.* (2001) only the highest treatment c 200 µM gave significant growth reductions from control and was therefore not possibl to rate precisely the genotypes' response to varying Al levels.

Since beans are a very important source of protein in Kenya (Wabule *et al.* 1991), is economically relevant to identify and avail Al-toxicity tolerant varieties to farmer Secondly identification of the Al-tolerant varieties would facilitate genotype developme for the world's 3950 million ha of acid soils under the severe influence of Al toxici (Meyers and De Pauw 1995, von Uexküll and Mutert 1995). Information on inter-varie Al tolerance is not only essential in fitting crops to acid soils but is also a prerequisite the studies of the mechanisms of Al tolerance. The common bean is genetically variab for soil acidity or Al tolerance as described by Ryan *et al.* (1993). Consequent screening the beans for Al tolerance should provide a basis for improvement of th

germplasm for greater economic yields. Hence the objectives of this study were to establish the degree of Al tolerance of four popular Kenyan bean varieties at various concentrations and also test the appropriateness of Eriochrome cyanine R root staining and the root elongation techniques as tools for screening the *Phaseolus vulgaris* germplasm for Al tolerance.

2.0 MATERIALS AND METHODS

The study was conducted at Jomo Kenyatta University of Agriculture and Technology (JKUAT). The experimental design was a three replicated completely randomised design. The experiments were performed in a growth chamber with continuous aeration for the hydroponic system. In each replicate, ten seedlings were grown and used for the data collection.

2.1 The bean germplasm

Four bean varieties: namely 'Rosecoco' (GLP 2), 'Mwezi Moja' (GLP 1004), 'Mwitemania' (GLP X 92) sourced from Kenya Seed Company and an imported French bean, cultivar 'Amy' obtained from the local outlet of Royal Sluis Company of Holland, were studied. The first three varieties are grown as dry beans and horticultural crops whereas the exotic one is mainly cultivated as horticultural crop for export market.

2.2 Seedling culture

Seeds of each bean variety were sterilised against fungal infection with 1% sodium hypochlorite for 15 min, soaked in water for 6 h and then germinated in petri-dishes under darkness at 25^0 C for 3 days. The germinated seeds were then transferred to floats and cultured in aerated solutions containing 100 µM $CaCl_2$ at pH 4.5 and grown for 2 days under 120 W M^{-2} fluorescent lamps for 12 h photoperiod with temperature maintained at 25^0C and relative humidity of 80% in a growth chamber. The growth solution was renewed every 24 h. After pre-treatment, a new solution containing 100 µM $CaCl_2$ at pH 4.5 with varying Al concentrations of 0, 3, 5, 10, 20, and 50 µM substituted the earlier one and the seedlings were allowed to grow under the same environmental conditions as in the pre-treatment period for an additional one day. The nutritional

requirements of the seedlings over the whole growth period were entirely met from the seed reserves except the extra calcium provided from the added $CaCl_2$ to enhance cell division through its role in cell extension (Schmit 1981).

2.3 Root elongation measurements

Root lengths of seedlings for each treatment was measured just before treatment application and after 24 h following the commencement of treatments. The length of the root constituted the distance of the root apex from the lower surface of the basal end of cotyledon which was equivalent to the styrofoam holding point of the seedling. Root elongation within each variety was expressed as:

Root elongation (mm) = $L_t - L_0$; where: L_t = Root length (mm) after 24 h of Al treatment; L_0 = Root length (mm) before Al treatment.

The percentage root elongation (Relative root elongation) within each variety was calculated using the formula:

% Root elongation = $(L_t - L_0 / L'_t - L'_0) \times 100$; where: L_t = Root length (mm) after Al treatment; L_0 = Root length (mm) before Al treatment; L'_0 = Root length (mm) before control treatment (0 μM Al); L'_t = Root length (mm) of the control treatment (0 μM Al) after 24 h.

The data was subjected to statistical analysis using the COSTAT software package.

2.4 Staining procedure

The Al localisation in the roots as a measure of Al uptake by the plants was determined by staining the roots with Eriochrome cyanine R (Sigma Chem. Co. St L.) as per the procedure outlined by Ma *et al.* (1997). Roots were washed with distilled water 3 times before soaking them in distilled water for 10 minutes. The roots were then stained with 0.1% Eriochrome cyanine R for 10 minutes and afterwards rinsed with distilled water. The staining patterns were then observed under a light microscope. The tolerance classes were assigned according to the Al levels at which staining occurred in the root apex (Takagi *et al.* 1981).

3.0 RESULTS

3.1 Root elongation

In all the four varieties, the root elongation decreased with increasing Al concentrations attaining minimum root length at 50 μM Al treatment for all the varieties (Fig.1 and Table 1). The root inhibition relative to control was significant (P=0.05) from 3 μM Al treatment for all varieties (Table 1).

Table 1: The effect of varying Al treatment on root elongation of the bean varieties

	Bean variety			
Al treatment (μM)	Rosecoco	Mwitemania	Mwezi moja	French bean
0	36.8a[1]	36.3a	38.0a	35.6a
3	30.8b	31.2b	29.4b	23.5b
5	19.6c	20.6c	16.0c	9.0c
10	14.7c	12.4d	10.9d	7.1cd
20	10.7d	7.1e	7.3de	4.0de
50	4.5d	4.4e	2.9e	2.1e
Lsd,0.05	5.1	4.3	4.5	4.4
CV %	65.2	69.6	74.2	92.6

[1] Means with similar letters within a column are not significantly different based on DMRT at P=0.05.

The differential root elongation of the varieties is given in Fig. 1. There were no significant (P=0.05) root growth differences among varieties at 0 and 3 μM Al treatments. At Al treatments of 5 and 10 μM only French bean showed significant root growth inhibition against the control treatment (0 μM Al). At 20 and 50 μM Al all the varieties showed significant root growth reduction relative to control treatment. The data of root elongation as a percentage of control (The relative root elongation) correlated well with root elongation results (Fig.1 and 2).

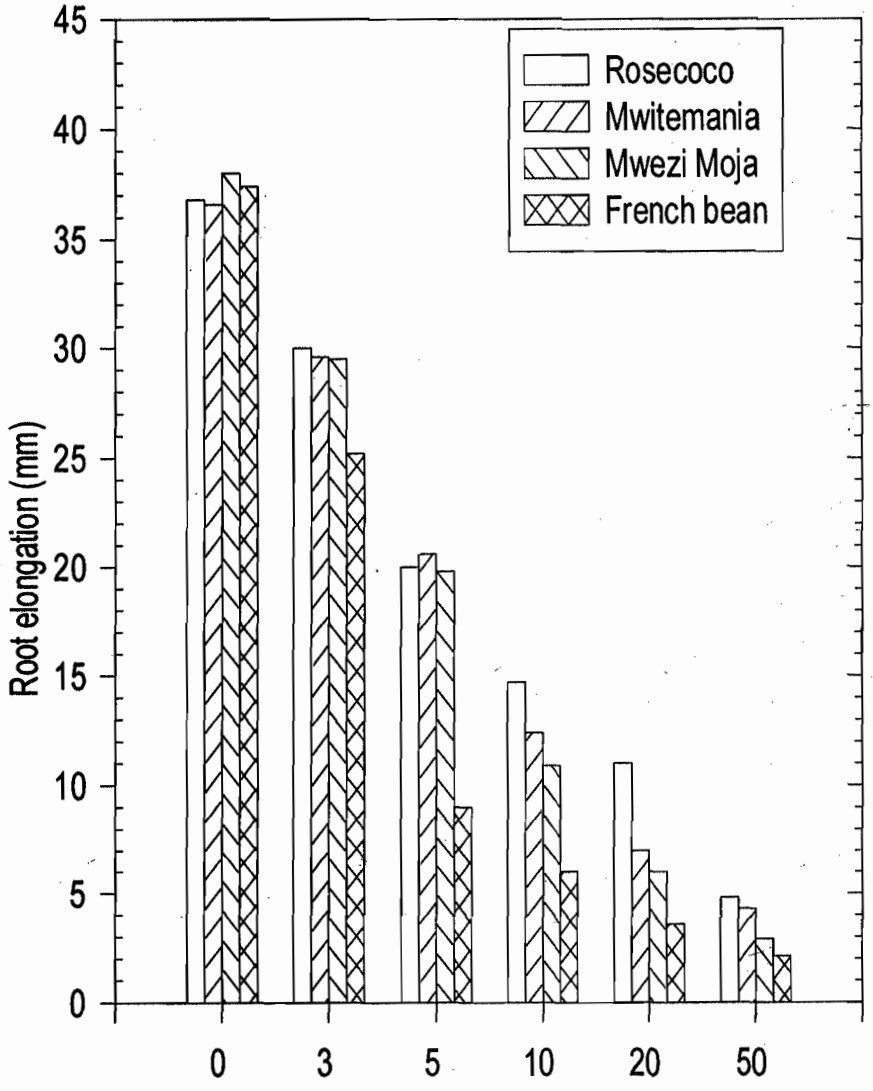

Figure 1. The differential effect of Al on root elongation in the four bean varieties

Means with similar letters within a treatment are not significantly different, as separated by Duncan's Multiple Range test (P = 0.05). Vertical bars show the Least Significance Differences (P = 0.05) for varieties within a treatment.

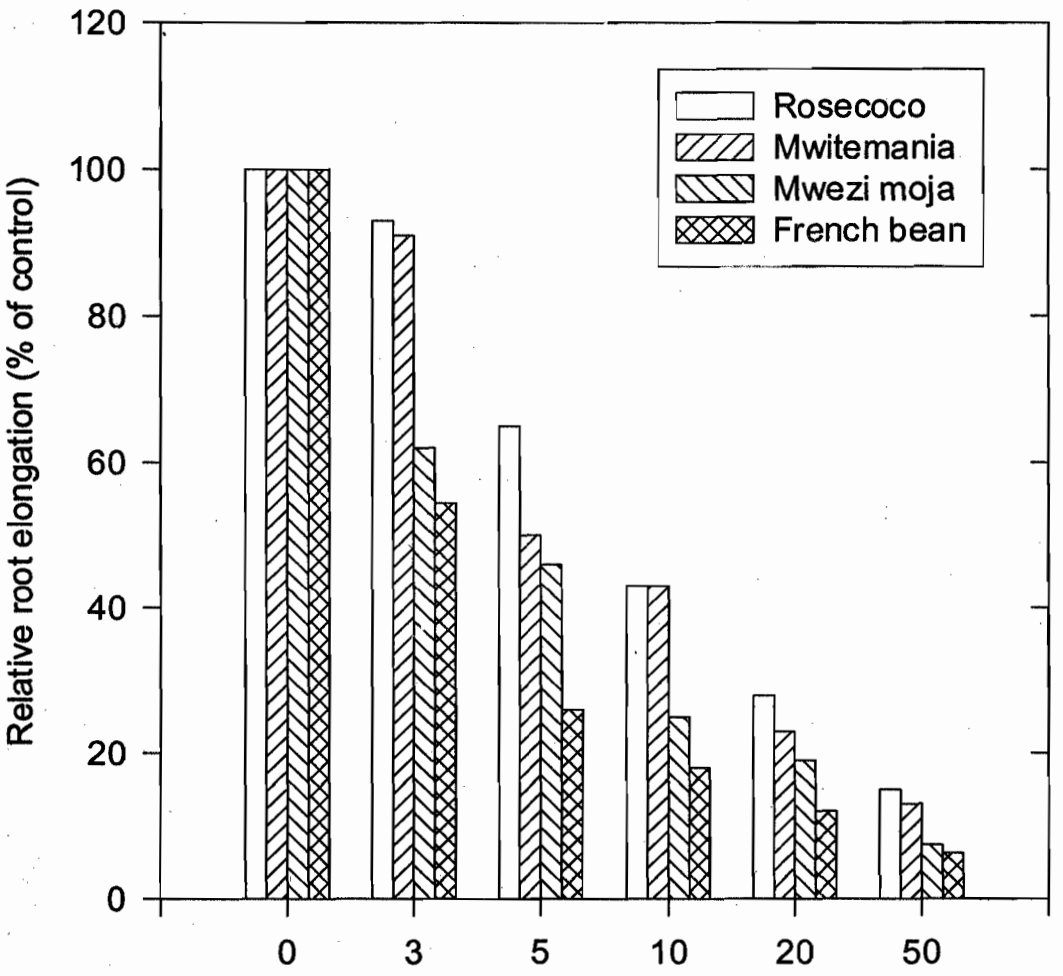

Figure 2. The relative root elongation of the four bean varieties, expressed as a percentage of untreated control

The relative root elongation of the varieties at all Al treatments was in the order Rosecoco > Mwitemania > Mwezi Moja > French bean. However, significant differences (P=0.05) between relative root elongations between Rosecoco and Mwitemania were noted at 20 μM Al and above. Notably, French bean was most affected with elevated Al concentrations in the nutrient solution thereby suggesting relatively greater susceptibility to the Al toxicity. Based on the results of the root elongation, the Al tolerance of the four

bean varieties was established to be in the following decreasing order: Rosecoco > Mwitemania > Mwezi Moja > French bean.

Staining by Eriochrome cyanine R in the roots

Al tolerance classification schemes of Takagi *et al.* (1981) have been adopted in rating the Al-tolerance of the four bean varieties. Staining procedure provided clear distinction among the Al-tolerant and Al-sensitive bean genotypes with increasing Al levels in the nutrient solution (Fig. 3).

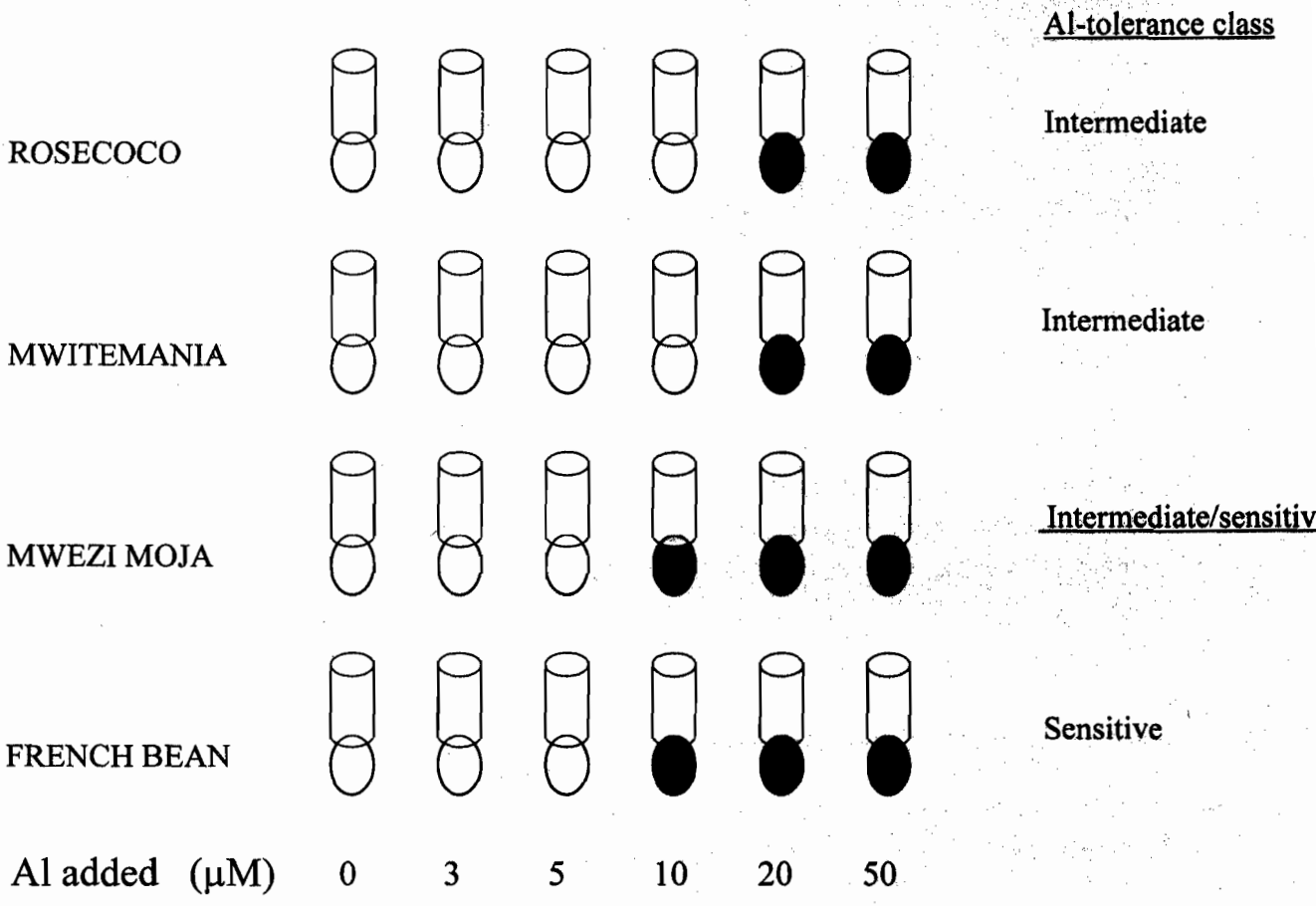

Figure 3. Al staining patterns (white - no staining; grey - slight staining; black - strong staining) by Eriochrome cyanine R in the root tips and subsequent tolerance classes of the four bean varieties.

Rosecoco and Mwitemania showed no staining in root apex at 10 μM Al treatment suggesting no uptake of the Al into the root apex. At higher levels of Al (20 and 50 μM)

strong staining was observed. Mwezi Moja had some slight staining in the root at 10 μM Al and strong staining at 20 and 50 μM Al. French bean was most severely affected through conspicuous absorption of Al from as low as 10 μM Al. Based on the presence, degree, or absence of Eriochrome cyanine R staining (no stain, faint stain, or strongly stained) in the root tips, and the level of Al treatments at which the staining occurred, we could place the bean varieties studied in the following Al-tolerance classes: Intermediate (Rosecoco and Mwitemania), Intermediate-sensitive (Mwezi Moja), and Sensitive (French bean).

4.0 DISCUSSION AND CONCLUSION

Both root elongation and staining with Eriochrome cyanine R procedures consistently offered clear characterisation of the four bean varieties as relates to their reaction to Al toxicity stress. Reduction in the elongation of root has been used previously to characterise bean response to Al treatment (Foy *et al.* 1969 and 1972, Mugai and Agong 1996, Birech *et al.* 1999 and 2000, Mugai 2001). Similarly, several workers have demonstrated the usefulness of the procedure for underscoring for Al tolerance in other species (Furlan and Bastos 1990, Ricon and Gonzale 1992, Sasaki *et al.* 1994). On the other hand only limited work has been reported regarding alternative procedures for rapid and precise determination of tolerance to Al toxicity especially in beans whereas crops like wheat have received greater concern (Takagi *et al.* 1981, Ricon and Gonzales 1992, Kochian 1995, Ma *et al.* 1997). Undoubtedly, similar rapid and effective screening procedures would be definitely necessary for crops like beans which are being grown in more Al toxic prone zones of Western, Nyanza, Southern Rift valley, Central, southern Eastern and south western Coast provinces of Kenya (Wokabi 1987, Mugai 2001).

This study presents a strong and consistent correlation between root elongation and staining in ranking the four bean varieties for Al tolerance. This is because both techniques are very clear in separating Rosecoco and Mwitemania as the most Al tolerant followed by Mwezi Moja and French bean in that order. French bean showed strong staining at only 10 μM Al, while Mwezi Moja had slight staining at 10 μM Al, and, Rosecoco and Mwitemania had the staining initiating at 20 μM Al. Root elongation

results gave similar Al-tolerance separations at less than 20 μM Al treatments (P = 0.05). Considering the lack of differences in root staining between Rosecoco and Mwitemania, staining is unable to separate the two varieties. However, root elongation, and more particularly the relative root elongation, was quite precise in separating all the four varieties on basis of their Al-toxicity tolerance especially at 20 μM Al treatment (P = 0.05). Thus based on relative root elongation results, Rosecoco is most tolerant, closely followed by Mwitemania; French bean the most Al sensitive and Mwezi Moja falling in between Mwitemania and French bean. Thus both staining and inhibition of root elongation are of practical benefit especially where classification of varieties for Al tolerance produces ambiguous results on the basis of only one screening procedure. Nonetheless, use of a combination of both procedures may be inevitable given that Rosecoco and Mwitemania were not separable based on staining in the root apex alone.

As would be expected, the most obvious sign of Al toxicity in plants is inhibition of root elongation (Clarkson 1965, Foy *et al.* 1972 and 1978). The Al-affected roots are also stubby, brittle and brownish in colour (Thawornwong and Van Diest 1974). The decrease in root growth corresponded to Al uptake levels as manifested by Eriochrome cyanine R staining thus confirming its toxicity effects as reported by Foy *et al.* (1978), Kochian (1995). In general, young seedlings are more susceptible to Al toxicity than older plants (Sivasuramania and Talibudeen 1972) and hence necessitating our confinement to this critical plant development stage in screening the bean genotypes for Al tolerance. On the other hand, staining by Eriochrome cyanine R is a direct indication of Al uptake, accumulation, localisation and subsequent tissue damage as has been shown for other Al^{3+} chelators like aluminon or hematoxylin (Aimi and Murakami 1964, Poll *et al.* 1978, Ryan *et al.* 1993). Thus, staining in the root apex corresponds to Al absorption by the meristematic cells through which inhibition of root growth is initiated (Yamamoto *et al.* 1997). Consequently, this technique is suitable in addition to the already documented simple and quick staining procedures for screening plants for Al tolerance, namely, aluminon, hematoxylin and Evans blue staining (Aimi and Murakami 1964, Poll *et al.* 1978, Mugai 2001). Chief advantage of the staining procedure is that it is fast, simple and highly reproducible (Ma *et al.* 1997).

Mwezi Moja variety has been grown successfully in less acid soils (pH >5.5) of arid and semi-arid areas principally because of its more efficient utilisation of the little available soil moisture over the short rain seasons, while Rosecoco and Mwitemania varieties are commonly grown in higher rainfall regions with more acidic soils. French bean is imported from Holland and has been selected for the neutral to alkaline soils of temperate regions, implying its lack of adaptation to acid soils/Al toxicity. The results of this work confirm that Al-tolerance mechanisms of the bean must have evolved with ecological adaptations.

If Rosecoco is most tolerant of the four varieties through adaptational processes, for having been grown in soils of a relatively high Al content (Mugai and Agong 1996) then certain physiological mechanisms must be responsible. Some of these mechanisms have now been elucidated as less Al uptake into the roots and higher organic acid exudation (Mugai *et al.* 2000, Mugai 2001). Important to note from this study is also the fact that root elongation offers precise classification and can only be supplemented with cost effective procedures like staining technique.

In conclusion, the study has shown that differences associated with Al tolerance exist among the four *P. vulgaris* genotypes. This is a pertinent step in studies of Al-tolerance physiology and in improvement of this germplasm for acid soils of Kenya. It is suggested that further studies include identification of genes responsible for the relative Al-tolerance of Rosecoco and Mwitemania as relates to the less Al-tolerant French bean. It is recommended that for high yields of beans to be achieved, acid soils should be limed to appropriate pH levels (Birech *et al.* 1999, Mugai 2001). This is even more critical for the Al-sensitive French bean as was shown by Mugai (2001).

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