



# Effect of Aluminum Toxicity on Root Growth and Morphology of Common Bean (*Phaseolus vulgaris* L.) Genotypes

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## Authors' contributions

*This work was carried out in collaboration between all authors. Author HL prepares the proposal, conduct the research and interpret the result and discussion. Authors RND, SG, GB and FM contribute in guiding, editing the manuscript to the final. All authors read and approved the final manuscript.*

## Article Information

DOI: 10.9734/IJPSS/2017/26045

Editor(s):

(1) Slawomir Borek, Faculty of Biology, Department of Plant Physiology, Adam Mickiewicz University, Poland.

Reviewers:

(1) Omena Bernard Ojuederie, Bells University of Technology, Ota, Nigeria.

(2) Raimundo Santos Barros, Universidade Federal de Viçosa, Depto de Biologia Vegetal, Universidade Federal de Viçosa, Viçosa, Brazil.

Complete Peer review History: <http://www.sciencedomain.org/review-history/17543>

**Original Research Article**

**Received 30<sup>th</sup> March 2016**  
**Accepted 20<sup>th</sup> June 2016**  
**Published 17<sup>th</sup> January 2017**

## ABSTRACT

**Aims:** To investigate the effect of aluminium on root growth, morphology and the concentration of aluminium in the root tissues of two common bean genotypes (new BILFA 58 and Roba 1) varying in soil acidity tolerance.

**Study Design:** Factorial combinations of five rates of aluminum (0.0, 12.5, 25.0, 50.0, and 100.0 mg Al kg<sup>-1</sup> soil) and two genotypes were laid out in a completely randomized design of three replications.

**Place and Duration of Study:** The experiment was conducted in the vegetation hall of Nekemte Soil Laboratory, western Ethiopia from July to October, 2012.

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**Methodology:** For each treatment, four plants were raised per pot, data related to root growth and aluminum content of the crop were collected at 25 and 35 days after seedling emergence (DAE).

**Results:** Aluminium and genotype interacted significantly ( $P=0.01$ ) to affect root growth parameters and aluminium contents of the roots. A difference in inhibition of tap root elongation was observed between the two genotypes at different levels of aluminum. As the applied aluminum level increased, the tap root length of both genotypes decreased under both lime-treated and -untreated soils. On average, application of aluminium led to 14.8, 9.9, 14.6 and 37.3, 22.3 and 16.2%, reduction in root biomass, total root length per volume soil, and root surface area at 25 and 35 DAE, respectively. In contrast lime application resulted in reduction of aluminium content of the roots by 56.3%.

**Conclusion:** Common bean production on strongly acidic soils with higher contents of exchangeable aluminium could be sustained through the integrated use of tolerant genotypes and application of modest rates of lime.

*Keywords: Acidity tolerance; lime; root biomass; root length; unlimed.*

## 1. INTRODUCTION

Acid soils are the most important cause of low yields of a number of crops. About 30% of the world's soils are acidic, and 60% of them are in tropical and subtropical areas associated with long periods of hot and moist weather [1]. It is generally accepted that Al toxicity is a primary factor limiting plant growth on acid soils [2]. Toxic effects of Al on plant growth have been attributed to several physiological pathways although the precise mechanism has not yet been understood [3]. Proposed mechanism of Al toxicity includes Al interactions with the root cell wall, Al disruption of plasma membrane and membrane transport processes and Al inhibition of mineral uptake and metabolism, especially that of Ca and P [4].

The easily observable symptom of Al toxicity is a rapid (minutes to a few hours) inhibition of root growth [5], resulting in a reduced and damaged root system that limits mineral nutrient and water uptake [3]. Ryan et al. [6] found that the root apex is the most Al-sensitive root zone, and Sivaguru and Horst [7] identified the distal transition zone (DTZ) as the specific site of Al injury in maize. However, in common bean, Rangel et al. [8] showed that both the transition zone (TZ, 1–2 mm) and the elongation zone (EZ) are targets of Al injury.

Great variability exists for root traits in common bean [9], and matching the root system to the environment will be a particular research challenge for the future. Different root systems are required for different soil environments. Shallow roots ramifying on the top soil are required to maximize P acquisition in a P-poor soil [10]; deep roots are required for water

acquisition under drought; greater numbers of root tips are needed for calcium absorption; exudation of organic acids is important as a defense mechanism against aluminum toxicity or as a mechanism of P- acquisition [11].

Common bean (*Phaseolus vulgaris* L.) is particularly sensitive to Al toxicity, and development of genotypes with better root growth in Al-toxic soils is a priority [12]. A preliminary field screening of common bean germplasm in western Ethiopia has demonstrated the presence of genetic variability among genotypes. Further insights into root characteristics of common bean genotypes with contrasting tolerance levels to low soil pH may serve as a foundation for selection and breeding of aluminum-tolerant common bean genotypes. The objective of this study was to test the hypothesis that differences exist in root growth and root aluminum concentration among common bean genotypes selected for soil acidity tolerance and the genotypes may differentially respond to aluminum rates on lime treated and lime-untreated acid soil.

## 2. MATERIALS AND METHODS

### 2.1 Description of the Study Area

The experiment was conducted at Nekemte soil laboratory in western Ethiopia. The experimental site is located at 9°08' N latitude and 36°46' E longitude with an altitude of 2080 metres above sea level. According to the weather data recorded at the Nekemte Meteorological Station, the average annual rainfall of the study site is 1300 mm with 725 mm for the experimental period (July – October) and the monthly mean

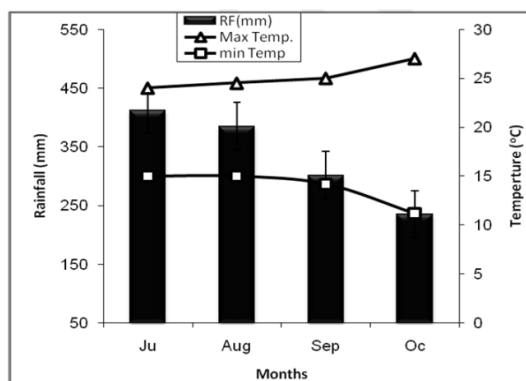
minimum and maximum temperatures are between 10-15°C and 24 to 28°C (Fig. 1). The soil used for the pot experiment had a pH (H<sub>2</sub>O) of 4.81, exchangeable acidity of 4.92 cmol (+)/kg soil, exchangeable Al of 3.1 cmol (+)/kg soils and acid saturation of 53.3% before applying the treatments.

## 2.2 Description of Planting Materials

From field (pH 4.5) and pot (pH 4.8) screening experiments conducted in 2009 and 2010, respectively, new BILFA 58 (NB58) and Roba1 were identified as the most tolerant and sensitive genotypes to soil acidity, respectively. New BILFA 58 is a genotype with type III growth habit and large seed size (53 g per 100 seed) whereas Roba 1 is a small-seeded (22 g per 100 seed) commercial cultivar in Ethiopia with type II growth habit.

## 2.3 Treatments and Experimental Design

The treatments consisted of factorial combinations of the two common bean genotypes (new BILFA 58 and Roba 1) and five rates of Al (0.0, 12.5, 25.0, 50.0, and 100.0 mg Al/kg soil). The different rates of Al were applied in the form of Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>. The ten treatments were laid in a completely randomized design in factorial arrangement with three replications. The experiment consisted two sets. The first set consisted of common bean plants of the two genotypes grown on lime-treated soil whereas in the second set comprised common bean plants of the two genotypes grown on unlimed soil.



**Fig. 1. Distribution and mean minimum and maximum temperatures of the experiment site (Nekemte) during the experimental period (June to October), 2012**

## 2.4 Experimental Procedure

Seeds of the two common bean genotypes were sown in pots (18 x18 cm) each filled with 10 kg soil. At the time of planting, the soil was fertilized with DAP at the rate of 92 kg P<sub>2</sub>O<sub>5</sub> per hectare (307 mg P<sub>2</sub>O<sub>5</sub> pot<sup>-1</sup>) considering the bulk density of 1.5 g cm<sup>-3</sup> and depth of 20 cm. Six seeds per pot were initially sown and later thinned to four plants when the first trifoliolate leaves unfolded. The different rates of Al and lime were applied four weeks prior to planting the seeds and worked into the soil. Lime was applied at the rate of 20 g pot<sup>-1</sup> (9 tonnes/hectare) after determining by the incubation method. Pots were watered periodically with tap water to the approximate field capacity to facilitate normal plant growth. All the recommended agronomic management practices including weeding were applied as required.

## 2.5 Data Collection

Plants in all experiments were harvested 25 and 35 days after emergence for the first and second harvests, respectively. After harvesting, the seedlings were separated into shoots, roots, and stems for determination of biomass and root parameters. For root separation, pots with the root containing ball of soil were soaked overnight in plastic buckets filled up with 10 liters of water. Then, the soaked soil root suspension in each pot was tipped into water in the bucket and stirred gently to detach soil lumps still attached to roots. Guarding against loss of roots, the soil water suspension was poured out onto 0.5 mm sieve and washed softly with a jet of tap water. Clean roots retained on the sieve were collected. Excess moisture was blotted from the roots with paper towels [13]. From each treatment, three seedlings were taken for measurement of root parameters.

All roots were collected, washed and scanned in EPSON Perfection V700/V750 Photo scanner, in gray scale to a resolution of 300 pixels per inch. The images were then analyzed, using WinRHIZOO 2008 software (Regent Instruments, Inc., Quebec, Canada), to determine total root length (TRL), average root diameter (ARD), root surface area, total root length per volume, and root volume. Subsequently, roots were dried in an oven at 65° C for 48 h and then weighed on an analytical scale balance to calculate root biomass (RB). The length of the primary root was measured by a ruler for the second harvest.

### **2.5.1 Relative root growth rate**

The relative root growth rate constant,  $K$ , ( $\text{day}^{-1}$ ), considering exponential growth rate was calculated by the following formula.

$$K = \frac{\ln(RL_2/RL_1)}{t_2 - t_1}$$

Where:  $k$  = relative root growth rate;  $RL$  = Root length;  $\ln$  = natural logarithm;  $t$  = harvest time, 1 and 2 refer to 25 and 35 days after emergence (DAE), respectively.

### **2.5.2 Aluminum content in the roots**

Aluminum concentration in the root tissue was analyzed using atomic absorption spectrophotometer as described in the official method of the Association of Official Analytical Chemists method 928.03 [14]. For determination of aluminum concentration in the root tissue, emission was measured at 396.15 nm and aluminum concentrations were estimated from a standard calibration curve (0.2-20  $\mu\text{g Al/mL}$ ) prepared from analytical grade Al metal.

## **2.6 Data Analysis**

Data were subjected to analysis of variance (ANOVA) using statistical programme SAS version SAS 9.1 [15]. Mean separations were done using the least significant difference (LSD) test at  $P = 0.05$

## **3. RESULTS**

### **3.1 Root Growth Parameters**

The result obtained indicated restriction of root growth by aluminum toxicity as compared to the control. Shorter roots with absence of normal branching pattern were observed at higher levels of aluminum (50 and 100 mg Al/kg soil) application compared to the control treatment (0 mg Al/kg soil). Considerable variability existed between the two common bean genotypes for the different root parameters considered. Increasing the aluminum concentrations both under lime-untreated and treated soils affected the growth of roots of both genotypes. However, the reduction was higher for lime-untreated soil (Fig. 2). In addition, the tolerant genotype, new BILFA 58 was less affected than the sensitive genotype Roba1 for almost all traits except for average root diameter. Highly significant ( $P =$

0.01) differences were found for root biomass, total root length, root surface area, total root length per soil volume due to the main effects aluminum and genotypes as well as their interaction at both harvesting times and under both soil liming regimes. Furthermore, the differences due to Al level and genotype were significant for root volume; tap root length and relative root growth rate under lime-treated and untreated soils (Table 1).

Root biomass was significantly reduced as the applied aluminum level increased relative to the control treatment (Fig. 2). On average, the genotypes produced higher root biomass at both harvest times when grown under lime-treated soil. Root biomass of the genotypes was reduced by 14.8 and 37.3% when grown under lime-untreated soil as compared to lime-treated soil for the first and second harvests, respectively (Fig. 2). At the highest Al rate (100 mg Al  $\text{kg}^{-1}$  soil), root biomass yields of the two genotypes under both limed and unlimed soils were comparable (Fig. 2).

A difference in inhibition of root elongation due to varying rates of aluminum was observed between the two genotypes (Fig. 3). Roots of New BILFA were relatively more vigorous than those of Roba 1 under both soil liming regimes. In addition, the tap root of new BILFA 58 (47.9, 48.6 cm) was longer than that of Roba 1 (24.9, 34.5 cm) under both liming regimes, respectively. Similarly, lime application increased the tap root length of both genotypes except at the highest aluminum levels applied (100 mg Al  $\text{kg}^{-1}$  soil) (Table 2). Relative to lime-untreated soil, roots of both genotypes grew longer under lime-treated soil condition. Higher root length reduction was observed for Roba 1 (27.8%) compared to new BILFA 58 (1.44%) under lime-untreated soil relative to lime-treated soil.

At the different rates of aluminum, root surface area of the genotypes were relatively higher when grown in limed than in the unlimed soils (Fig. 4). On average 14.8 and 16.2% root surface area reductions were observed in response to growing the genotypes under unlimed soil as compared to growing them under limed soil for the first and second harvests, respectively. Increasing the rate of applied aluminum decreased root surface area of the genotypes under both lime-treated and lime-untreated soils. Under both lime-treated and -untreated soils, New BILFA 58 had higher root surface area than Roba 1 at both harvest times. Higher reduction in

root surface area was recorded for Roba 1 as compared to new BILFA 58 with values corresponding to 20.8 and 27.3% and 9.5 and 7.6% of 25 DAE and 35 DAE, respectively.

Aluminum application reduced root length per volume and root volume under both lime-treated and untreated soils, with the effect more pronounced under the latter condition. Under both soil liming regimes, New BILFA 58 had higher root length per volume and root volume than Roba 1 (Table 3). Relative to the total root length per volume obtained under the lime-treated soil, the total root length per volume recorded for the lime-untreated soil decreased by 9.92 and 22.3% at the first and second harvests, respectively. When the two genotypes are

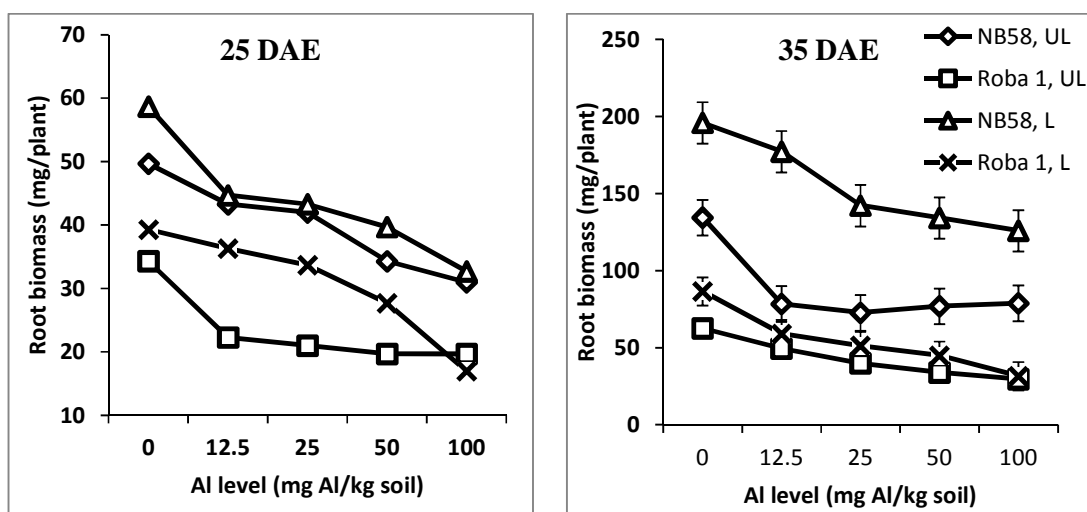
compared, a higher reduction in total root length per volume soil was recorded for Roba 1 (20.2 and 42.5%) than new BILFA 58 (1.7 and 6.7%) for the first and second harvests, respectively.

Lime application generally increased root length per volume soil as well as root volume of the common bean genotypes. Higher root volume was recorded for the control treatment (0 Al) at both harvest times and soil liming regimes (Table 3). Root volume decreased as the amount of aluminum applied increased on both lime-treated and -untreated soils. On average, 24.4% reduction in root volume was observed 35 days after emergence (DAE) when the two genotypes were grown under lime-untreated soil as compared to lime-treated soil.

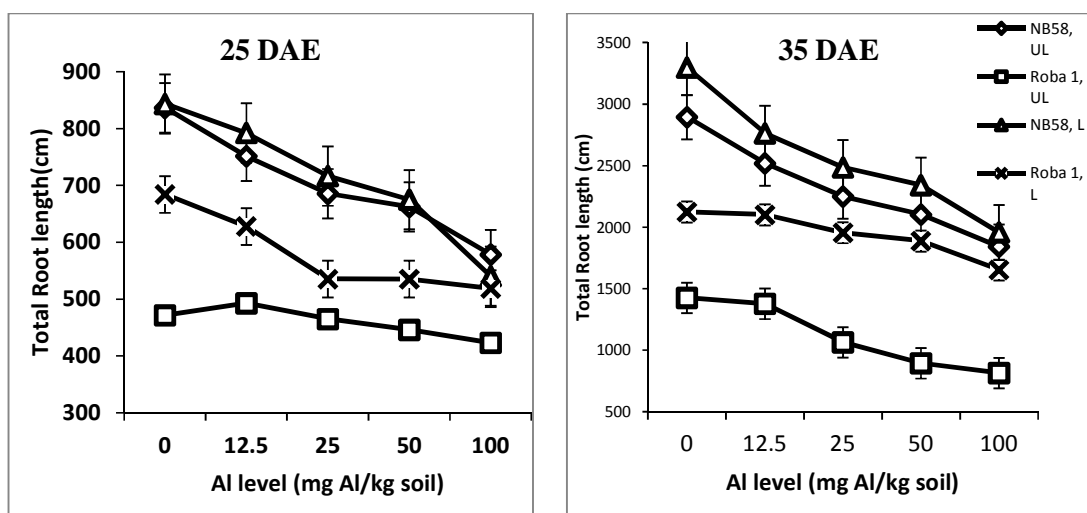
**Table 1. ANOVA table showing mean squares of root growth parameters of common bean genotypes as affected by aluminum levels and genotypes under unlimed (UL) and limed (L) soil, and mean values for the combined data for unlimed and limed soils**

Parameters	Lime (mean)	Al	Genotype	Al*G	
Total root length (25)	UL	581.5 <sup>b</sup>	21297 <sup>***</sup>	443827 <sup>***</sup>	9028 <sup>**</sup>
	L	645.3 <sup>a</sup>	45390 <sup>***</sup>	144511 <sup>***</sup>	7030 <sup>*</sup>
Total root length (35)	UL	1719.0 <sup>b</sup>	677632 <sup>***</sup>	10918188 <sup>***</sup>	39811 <sup>***</sup>
	L	2213.2 <sup>a</sup>	468782 <sup>***</sup>	2260069 <sup>***</sup>	45847 <sup>ns</sup>
Root Length(cm) (35)	UL	36.4 <sup>b</sup>	144.34 <sup>***</sup>	3979 <sup>***</sup>	9.41 <sup>ns</sup>
	L	41.6 <sup>a</sup>	181.96 <sup>***</sup>	1491.1 <sup>***</sup>	91.48 <sup>*</sup>
Root biomass (25)	UL	31.7 <sup>b</sup>	255.1 <sup>***</sup>	2083.3 <sup>***</sup>	26.9 <sup>ns</sup>
	L	37.3 <sup>a</sup>	475.8 <sup>ns</sup>	1267.5 <sup>***</sup>	30.42 <sup>***</sup>
Root biomass (35)	UL	65.8 <sup>b</sup>	2108.5 <sup>***</sup>	15300.2 <sup>***</sup>	429.8 <sup>**</sup>
	L	104.9 <sup>a</sup>	3719.9 <sup>***</sup>	75701.6 <sup>***</sup>	243.4 <sup>**</sup>
Root volume (25)	UL	1.04 <sup>a</sup>	0.382 <sup>***</sup>	1.133 <sup>***</sup>	0.068 <sup>*</sup>
	L	1.01 <sup>a</sup>	0.134 <sup>***</sup>	0.0598 <sup>*</sup>	0.002 <sup>ns</sup>
Root volume (35)	UL	4.4 <sup>b</sup>	4.91 <sup>***</sup>	30.1 <sup>***</sup>	0.656 <sup>ns</sup>
	L	5.8 <sup>a</sup>	4.99 <sup>***</sup>	9.75 <sup>***</sup>	0.695 <sup>ns</sup>
Root surface Area (25)	UL	44.4 <sup>b</sup>	249.26 <sup>***</sup>	655.39 <sup>***</sup>	166.66 <sup>***</sup>
	L	52.2 <sup>a</sup>	186.98 <sup>***</sup>	591.14 <sup>***</sup>	19.98 <sup>ns</sup>
Root surface area (35)	UL	415.5 <sup>b</sup>	68280.2 <sup>***</sup>	106803.3 <sup>***</sup>	49267.4 <sup>***</sup>
	L	478.8 <sup>b</sup>	54328.9 <sup>***</sup>	92559.6 <sup>***</sup>	17987.5 <sup>*</sup>
Average Root Diameter (25)	UL	0.37 <sup>a</sup>	0.017881 <sup>**</sup>	0.046 <sup>**</sup>	0.0019 <sup>ns</sup>
	L	0.36 <sup>a</sup>	0.006456 <sup>*</sup>	0.0004 <sup>ns</sup>	0.012241 <sup>**</sup>
Average Root diameter (35)	UL	0.66 <sup>a</sup>	0.023824 <sup>**</sup>	0.038 <sup>**</sup>	0.0008 <sup>ns</sup>
	L	0.57 <sup>b</sup>	0.034399 <sup>**</sup>	0.0109 <sup>ns</sup>	0.0274 <sup>**</sup>
Root length per volume soil (25)	UL	205.0 <sup>b</sup>	2647.7 <sup>***</sup>	55179.5 <sup>***</sup>	1122.4 <sup>**</sup>
	L	277.6 <sup>a</sup>	5643.1 <sup>***</sup>	17966.6 <sup>***</sup>	874.1 <sup>*</sup>
Root length per volume soil (35)	UL	606.1 <sup>b</sup>	84248 <sup>***</sup>	1357423 <sup>***</sup>	4950 <sup>*</sup>
	L	780.4 <sup>a</sup>	58282 <sup>***</sup>	280987 <sup>***</sup>	5700 <sup>ns</sup>
Relative root growth rate	UL	1.03 <sup>b</sup>	0.0834 <sup>***</sup>	0.81 <sup>***</sup>	0.0407 <sup>***</sup>
	L	1.23 <sup>a</sup>	0.0026 <sup>ns</sup>	0.0102 <sup>*</sup>	0.0057 <sup>*</sup>
Al content in the roots	UL	49.8 <sup>a</sup>	83.21 <sup>***</sup>	12875.4 <sup>**8</sup>	1.61 <sup>***</sup>
	L	21.8 <sup>b</sup>	149.8 <sup>***</sup>	2363.5 <sup>***</sup>	49.5 <sup>***</sup>

Where, UL- unlimed, L- limed, 25 and 35 days after emergence, respectively, Al- aluminum, G- genotype, NS- non-significant, \*P(0.01-0.05), \*\* P(0.001-0.01), \*\*\*P=0.001)



**Fig. 2. Root biomass (RB, mg/plant) harvested at 25 and 35 days after emergence (DAE) of two common bean genotypes as influenced by different levels of aluminum applied under unlimed and limed acid soils**



**Fig. 3. Total root length (TRL, cm) of two common bean genotypes as influenced by different levels of aluminum applied under unlimed and limed soils measured at 25 and 35 days after emergence**

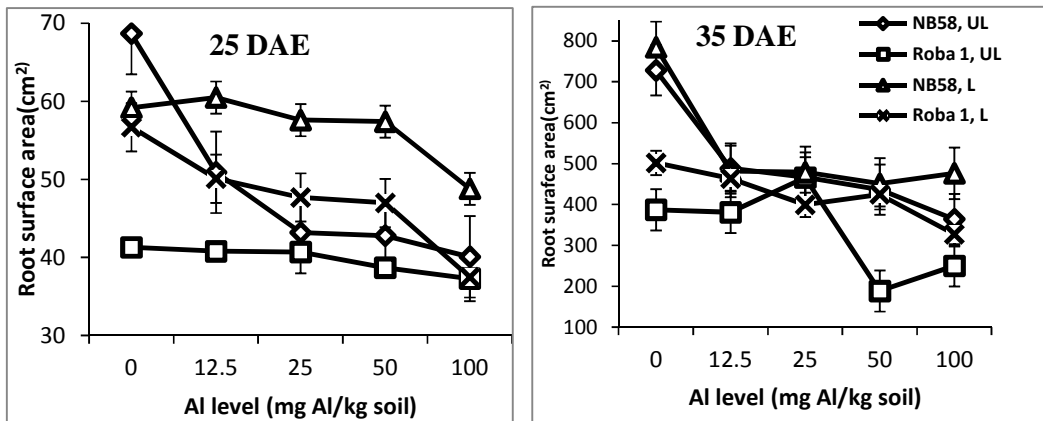
Root volume reduction due to soil acidity under lime-untreated soil was higher for Roba 1 than for New BILFA 58 at 25 DAE and 35 DAE (Fig. 5). At both harvests, in response to increasing the level of applied aluminum, the root volume decreased under both lime-untreated and -treated soils, with a higher reduction observed for the lime-untreated soil. Average root diameter of the common bean genotypes increased in response to the applied aluminum levels (Fig. 6).

Highly significant differences were observed for root diameter among the different rates of aluminum treatment and between the two genotypes both under unlimed and limed acidic soils. On average, the root diameter of the genotypes recorded for the lime-treated soil was less than the root diameter recorded for the lime-untreated acidic soil with the smallest value observed for the control treatment under both soil liming regimes (Fig. 6).

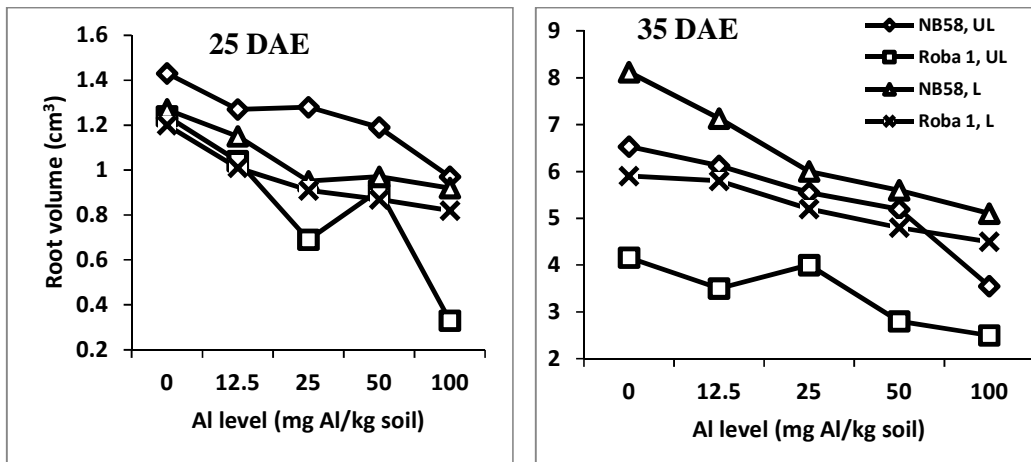
**Table 2. Mean tap root length (35 days after emergence)(cm) common bean genotypes as influenced by aluminum levels on unlimed and limed acid soils**

Al level (mg Al/kg soil)	Taproot length (cm)			
	Unlimed		Limed	
	NB58	Roba 1	NB58	Roba 1
0.0	55.7±2.7	33.7±3.2	59.2±2.4	36.0±0.9
12.5	48.7±3.2	25.0±2.1	56.5±6.2	36.2±2.1
25	47±1.2	21.9±1.4	46.8±2.6	33.7±2.0
50	45.6±3.0	20.3±1.4	44.7±4.7	34.7±2.1
100	42.6±2.4	23.4±2.3	35.9±1.5	32.1±1.4
Mean	36.4		41.6	
CV (%)	11.5		11.4	
LSD Al	5.1		5.8	
G	3.2		3.6	
Al*G	NS		3.1	

Where, NB58- new BILFA 58, CV- coefficient of variation, LSD- least significant difference, Al- aluminum, G- genotype, NS- non significant at 5 % (  $P>0.05$  )



**Fig. 4. Root surface area (cm<sup>2</sup>) of two common bean genotypes as influenced by different aluminum levels under unlimed and limed acid soils measured at 25 and 35 days after emergence**



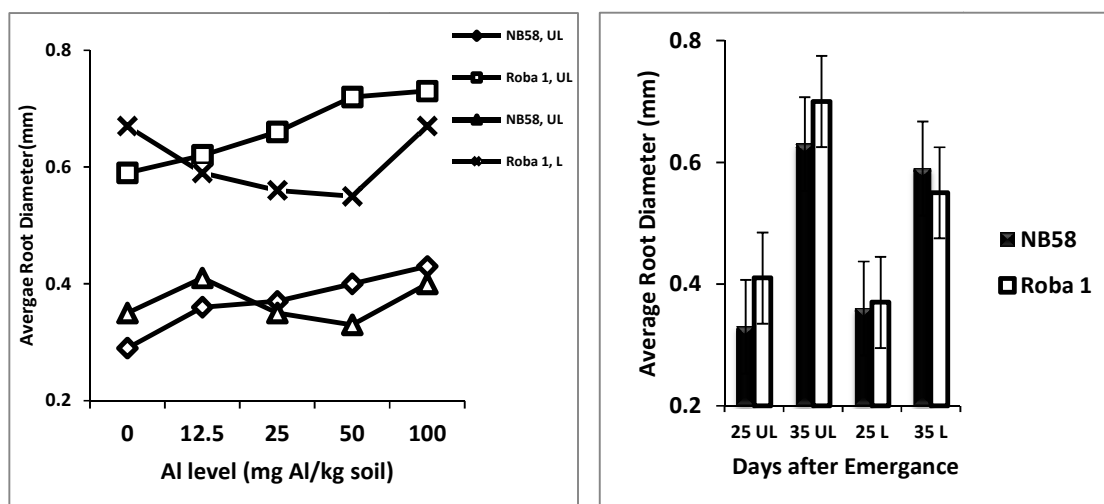
**Fig. 5. Root volume (cm<sup>3</sup>) of two common bean genotypes as influenced by different aluminum levels under unlimed and limed acid soils measured at 25 and 35 days after emergence**

**Table 3. Mean values for total root length per volume soil (TRLpV, m/m<sup>3</sup>) of two common bean genotypes as influenced by aluminum levels for unlimed and limed acid soils**

Al Level (mg Al/kg soil)	TRLpV , 25 DAE		TRLpV , 35 DAE		TRLpV, 25 DAE		TRLpV, 35 DAE	
	Unlimed				Limed			
	NB58	Roba 1	NB58	Roba 1	NB58	Roba 1	NB58	Roba 1
0	295.1±10.3	166.3±10.1	1020.9±20.3	502.6±23.8	299±2.9	229.7±11.7	1018±13.1	749.1±33.9
12.5	264.9±11.7	173.8±6.7	888.4±19.6	486±13.4	279.5±15.4	221.5±6.9	974.4±35.9	741±40.7
25	241.9±7.1	164±7.1	793.3±37.7	375.5±16.8	252.8±1.22	192.4±6.1	876.8±38.7	689.7±28.9
50	233.7±4.3	157.3±2.5	741.8±15.5	315.4±12.8	238.1±5.2	188.8±5.6	825.9±13.4	655.2±30.3
100	204±9.4	149.3±0.6	649.9±13.2	287.6±5.6	190.7±8.8	183±1.5	690.6±16.1	582.8±14.8
Means	205		606.1		227.5		780.4	
CV(%)	6.8		5.9		6.2		6.6	
LSD (5%)								
Al	16.8		42.9		16.9		62.6	
G	10.6		27.2		10.7		39.6	
Al*G	23.8		60.8		24.0		NS	

Where, TRLpVUI- total root length per volume soil(m/m<sup>3</sup>), 25 and 35 days after emergence (DAE), NB58- new BILFA 58, CV- coefficient of variation, LSD- least significant difference, Al- aluminum, G- genotype, NS- non significant at 5 % ( P>0.05),





**Fig. 6. Average root diameter (mm) of common bean genotypes as influenced by aluminum levels under unlimed and limed acid soils, measured at 25 and 35 days after emergence**

### 3.2 Root Growth Rate

Relative root growth rate (RRGR) of the genotypes was higher for lime-treated soil than the lime-untreated soil (Table 4). Aluminum application adversely affected the root growth rate of both genotypes under unlimed soil with a more pronounced effect on Roba 1 than new BILFA 58. Moreover, New BILFA 58 had higher RRGR than Roba 1 under both soil liming regimes. New BILFA 58 had higher RRGR on lime-untreated soil whereas Roba 1 had higher RRGR on lime-treated soil (Table 4). Relative to the limed soil condition, RRGR of the genotypes was reduced by 16.3% under the unlimed soil condition. For Roba 1, the decrease in RRGR under similar situation was 28.9%.

### 3.3 Aluminum Concentration in Root Tissue

Highly significant differences were observed among aluminum levels, between genotypes, and the interaction terms for root aluminum content under both soil liming regimes (Table 1). Aluminum concentrations in the root tissue were significantly lower for the tolerant genotype (new BILFA 58) than for the sensitive genotype (Roba 1) at all levels of application of the element under both soil liming regimes (Fig. 7). As the applied aluminum levels increased, aluminum concentrations in the root tissue also increased with the highest values found for the sensitive genotype (Roba 1). Root aluminum contents of the genotypes decreased under lime-treated soil

as compared to lime-untreated soils. On average, lime application reduced aluminum concentrations of the root by 56.3%.

## 4. DISCUSSION

Root growth characteristics of the two genotypes were differentially affected across the range of aluminum levels applied under both lime-treated and -untreated soils. Genotypes varied in terms of growth of roots and aluminum concentrations in the root tissue, the potential of which could be exploited by breeders to develop cultivars tolerant to soil acidity. Poor root growth of the genotypes in response to aluminum application was a manifestation of the detrimental effect of aluminum on root growth. Similar inhibitory effects of aluminum on root growth of other crops grown on acidic soils were reported by Reynolds et al. [16].

New BILFA 58 had higher root biomass, total root length, root surface area, and root volume than Roba 1 at all levels of Al applied under lime-treated and -untreated soil conditions. The significant Al level by genotype interaction indicates that the genotypes produced different root dry weights and total root lengths in response to different rates of Al applied. These traits are helpful for measuring levels of Al resistance, as they do differentiate resistant genotype from susceptible ones. Large root systems are known to have a greater capacity for absorbing water and minerals, as they are able to explore a larger rhizosphere soil volume [17].

Corroborating the results of this study, Alamgir and Akhter [18] also found that dry weight of root and shoot were affected by aluminum at varietal and treatment levels, and generally decreased with the rise in Al<sup>3+</sup> concentrations.

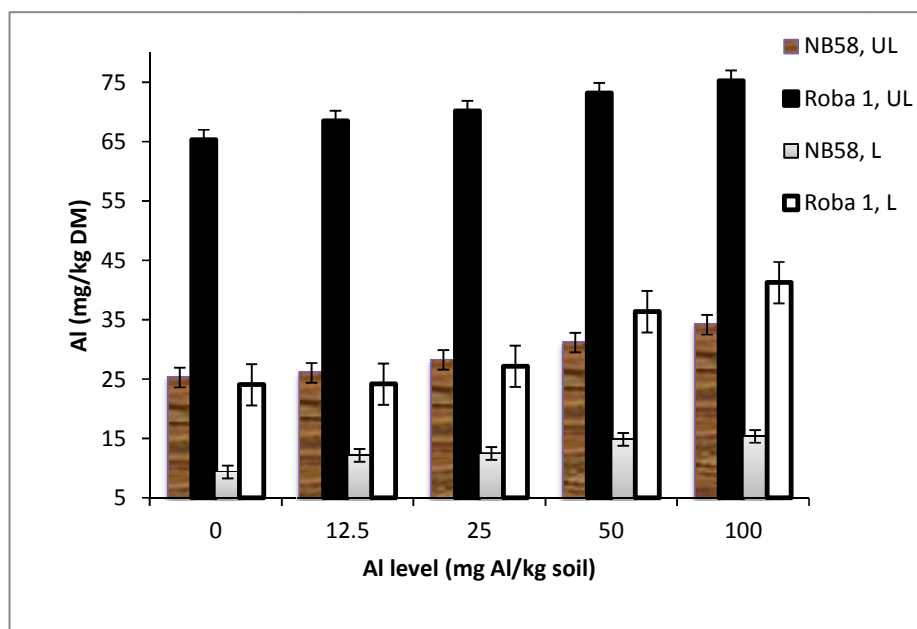
A difference in inhibition of tap root elongation was observed between the two genotypes at different levels of aluminum. As the applied aluminum level increased, the tap root length of both genotypes decreased under both lime-treated and -untreated soils. However, it was

noted that the inhibition was much higher for the untreated soil and for Roba 1. In line with this finding, several researchers reported that the first and most recognized effect of Al-toxicity in plants is the inhibition of the division and elongation of meristematic cells and thereby the reduction in root growth [19]. The initial symptom of Al toxicity is the inhibition of root elongation, which has been proposed to be caused by a number of different mechanisms, including Al interactions within the cell wall [20], the plasma membrane [21], or the symplast [2].

**Table 4. Relative root growth rate (mm/day) common bean genotypes as influenced by aluminum levels on unlimed and limed acid soils.**

Al level (mg Al/kg soil)	RRGR (mm/day)			
	Unlimed		Limed	
	NB58	Roba 1	NB58	Roba 1
0	1.24±0.04	1.11±0.03	1.23±0.02	1.18±0.05
12.5	1.21±0.02	1.03±0.05	1.25±0.04	1.21±0.02
25	1.19±0.06	0.83±0.03	1.24±0.01	1.28±0.03
50	1.16±0.03	0.69±0.03	1.24±0.01	1.24±0.02
100	1.16±0.03	0.66±0.02	1.29±0.03	1.16±0.03
Mean	1.03		1.23	
CV (%)	6.2		4.7	
LSD Al	0.08		NS	
G	0.05		NS	
Al*G	0.11		NS	

Where, RRGR- relative root growth rate(mm/day), NB58- new BILFA 58, CV- coefficient of variation, LSD- least significant difference, Al- aluminum, G- genotype, NS- non significant at 5 % ( P>0.05),



**Fig. 7. Aluminum content of the root of two common bean genotypes as influenced by aluminum levels under lime-treated and untreated acid soils**

The Al resistance in new BILFA 58 observed in this study could be due to various mechanisms which indicate more detailed studies are demanded in the future. For example, variability for resistance to Al toxicity between common bean genotypes may partly be related to differences in the extension of the root elongation zone. Rangel et al. [8] found that aluminum toxicity resistant common bean genotype such as ICA Quimbaya presented a larger elongation zone than did a susceptible genotype such as VAX1. Consistent with the results of this study, Al toxicity caused inhibition of root growth by injuring primarily the root apex of the growing plant [8].

Reductions in the root surface area were observed when the genotypes were grown under different rates of applied aluminium on lime-untreated soil. However, New BILFA 58 genotype had a higher root surface area as compared to Roba 1 under both soil liming regimes. Corroborating the results of this study, Zahng, et al. [22] reported that root surface areas of soybean decreased in response to increasing the concentration of Al<sup>3+</sup> from beyond 200 mg kg<sup>-1</sup> in the medium of growth. The decrease in root surface area due to high Al concentration can be explained by deficiency of calcium and magnesium as reported by Ridolfi and Garrec [23] and Godbold et al. [24].

Average root diameter of the genotypes increased as the applied aluminum increased from 0 to 100 mg Al /kg soil and average root diameter was lower for the limed than the unlimed acid soil. The result implies that aluminum toxicity resulted in growth of thicker roots which are less efficient for nutrient absorption. Application of aluminum induced the roots to swell causing an increase in average root diameter more under the unlimed soil than under the limed soil at both harvests. The increase in average root diameter was associated with a decrease in root surface area. This result is consistent with the report of Pietraszewska [25] that symptoms of Al toxicity in beans included the production of shortened roots with the presence of thickened, but fragile roots that undergo browning.

The two genotypes did not have significant differences in root diameter (root swelling) for the lime-treated soil except at the highest rate applied (100 Al mg/kg soil). The increase in average root diameter was higher for the sensitive genotype, Roba 1 compared with New

BILFA 58. The result of this study agrees with that of Blair et al. [26] who reported that root swelling is a typical symptom of susceptibility to aluminium toxicity. The root growth inhibition and subsequent increase in root diameter is commonly observed in roots exposed to Al [27]. Al-induced inhibition of root elongation results in curved, swollen, cracked, brownish, stubby, and stiff root apices [28].

Relative root growth rate of both genotypes was reduced regardless of the soil liming regime, with New BILFA 58 having maintained higher rate compared to the sensitive genotype, Roba 1. Genotypic differences in relative root growth rate in response to Al present in soils were earlier reported for many economically important crops, including maize [29] and mung bean [30]. Root aluminum content varied with the rate of aluminium applied and between the two genotypes under both soil liming regimes. Lower Al accumulation and thus the detoxification of Al in the apoplast through root exudates, such as Al-activated exudation of citrate from root tips, play a key role in Al resistance in common bean [31,32]. However, although shoot aluminum concentrations were considerably lower for the tolerant genotypes (new BILFA 58) than for the sensitive genotypes (Roba 1), evidence for mechanism of internal detoxification of aluminum cannot be substantiated in this study. Nevertheless, soil acidity tolerant genotype (New BILFA 58) contained less aluminum in its root tissue compared to Roba 1. It is, therefore, reasonable to assume that the aluminum tolerance of new BILFA 58 may have involved aluminum exclusion. Consistent with this suggestion, Rangel et al. [8] reported that Al resistance in common bean is related to lower Al accumulation in the root tips.

## 5. CONCLUSION

The results of this study demonstrated that differences existed between the common bean genotypes for traits related to root growth and morphology when subjected to different rates of aluminum on lime-treated and lime-untreated acidic soils. The parameters considered were useful in differentiating between Al toxicity resistance and sensitive genotypes. Thus, the parameters can help in selecting cultivars suitable for acid soil conditions. Lower aluminum concentration in the roots of tolerant genotype compared with the sensitive one implies that exclusion aluminum from uptake by the root is the mechanism of tolerance involved for the

tolerant common bean genotype. Lime application improved all root growth related parameters of both tolerant and sensitive genotypes presumably through lowering the detrimental effect of aluminum on root growth and increasing the uptake of nutrients and water. Therefore, common bean production and productivity on strongly acidic soils with higher exchangeable or soluble aluminum and higher acid saturation can be sustained through integrated use of tolerant genotype and application of modest amounts of lime. This may enhance food production thereby ensuring food security and protein nutrition of the people in the study region.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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