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Effect of Eco-Physiological Variability in *Leucaena leucocephala* Growth and Development

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Authors' contributions

This work was carried out in collaboration between both authors. Author VAP designed the study. Author ROK wrote the protocol and conducted the field study. Authors VAP and ROK managed the literature searches. Author VAP wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The effect of eco-physiological differences in two sites on growth and development of *Leucaena* was assessed. A 2-factor (provenance-site) experiment in a completely randomized design with three (3) replications was set up. Pot and field experiments were used to assess growth and development of three local *Leucaena* provenances in two regions, Maseno ICRAF/KEFRI centre (pH 4.8) and Chepkoilel Campus farm (pH 5.0). Seeds of three local *Leucaena* provenances K156 (Gede), K136 (Kibwezi) and KIT2724 (Kitale) were used in this study. Lime was applied at 0 and 33.3 kg per kg of soil (6.7 ton/ha) in each pot and aluminium at 0, 100, 200 and 300 μM. Field experiment was conducted at Chepkoilel Campus farm. Lime was applied at a rate of 7ton/ha. Number of leaves per plant, plant height, root length, root collar diameter, plant dry weight, selected nutrients (N, P and Ca) and Al were assessed from potted and field grown seedlings. Data were subjected to multivariate analysis of variance. There was relatively better seedlings growth at Maseno than at University of Eldoret. The differences in environmental factors, such as higher mean temperatures (25°C) at Maseno could promote growth, resulting in higher plant height, root

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length and plant dry weight than at Chepkoilel Campus, which had lower mean temperature 17°C, However, although the Maseno plants were taller, they had smaller root collar diameters, especially after 90 days. The comparatively higher levels of nitrogen in the Maseno soils could also be responsible for the better growth. This was also reflected in the shoot tissues where the seedlings grown at Maseno had higher nitrogen content than those same genotypes grown at Chepkoilel Campus site. Seedlings at Maseno ICRAF/KEFRI centre had significantly ($p < 0.05$) better growth than at University of Eldoret. Maseno soils had higher N and less Al than at University of Eldoret.

Keywords: Leucaena leucocephala; provenances; variability; growth; eco-physiology.

1. INTRODUCTION

Leucaena leucocephala (Leucaena) (Lam.) de Wit is an important tree in agroforestry systems in many parts of the world. It is currently being utilized for this purpose in Western and Central Kenya, including areas that have low soil pH, such as Maseno, in Kisumu district and Uasin Gishu district [1]. The plant is a shrubby leguminous multipurpose tree used in soil fertility improvement owing to its ability to fix atmospheric nitrogen. It is used as a source of fodder, browse, mulch, firewood, and poles [2]. However, most genotypes of *L. leucocephala* do not grow well in acid soils and under such conditions their full potential in biomass production is not realized [3,4].

Some researchers have observed significant variation in low pH tolerance among *L. leucocephala* germplasm grown in acid soils [5], [6]. Acid tolerant *Rhizobium* isolates that can nodulate *L. leucocephala* have also been isolated from Kenyan acid soils [7,8]. But, the selection of acid tolerant genotypes of *L. leucocephala* for use with the locally available acid tolerant *Rhizobia* has not been adequately accomplished in Kenya [7,8]. However, it is not known which of the *L. leucocephala* provenances that are currently grown in various localities in Kenya are acid tolerant. If some of the local germplasm of *L. leucocephala* in Kenya are tolerant to low pH, then such genotypes could be adopted for use in acidic soils. Likewise matching acid tolerant genotypes of *L. leucocephala* with tolerant *Rhizobium* could increase productivity of *L. leucocephala* in acid soils and realization of their potential in agroforestry systems [9].

In acid soils, the predominant ionic species of Al is Al^{3+}. Plants growing in acid soils often suffer Al stress, which can cause direct reduction of plant root growth [10]. Temporary cessation of root growth at high Al^{3+} concentrations may be caused by hormonal imbalance. It is normally difficult to separate primary or initial responses related to inhibition of root growth from secondary responses that arise as a result of damaged root system, such as inhibition of mineral and water uptake).

The accumulation of Al in plants is confined to the rhizodermal and root cap cells [11]. Root cap cells are major sites of phytohormone, such as abscicic acid (ABA), synthesis and action [12]. The plasticity and number of these cells regulate growth [13]. Auxins, such as indole acetic acid (IAA), increase the extension of this outermost layer of cells [14]. Reduced auxin levels in roots that grow in acid soils may be responsible for the inhibition of elongation of root cells at high concentrations of Al^{3+} as well as H^{+} [15].

Aluminium reacts strongly with orthophosphate to form aluminium phosphate, which is highly cytotoxic. It inhibits several enzymes, such as hexokinase [16], [17] and ATPase [18]. It also inhibits DNA synthesis [19] and calmodulin function [20,21]. If it reaches chloroplasts and mitochondria, Al^{3+} inhibit photosynthesis and respiration [22]. Some acidic soils may also contain high levels of Mn^{2+}, which may compete with Mg^{2+} for the binding sites at the roots surface, causing magnesium deficiency [23]. Excessive levels of Mn increase IAA oxidase activity [24], lowering auxin levels and increasing shoot branching [25]. Toxic levels of Mn *in* soil solution also inhibit the translocation of Ca to the shoot apex, causing an induced Ca deficiency in the roots [26]. Root growth requires a high level of available Ca [15]. However, Ca availability decreases with increasing soil acidity [27]. The root growth of many trees species, including *Leucaena* is significantly reduced in very acidic soils (pH 3.0 - 3.5) [28]. Nitrogen fixing symbiotic systems is usually more adversely affected by soil acidity than other physiological aspects of plants. This has been reported for some legumes [29], in which reduced nodulation was also observed [30]. The reduced nodulation has been attributed to high levels of Al^{3+}, Mn^{2+} and H^{+} [31].

Soil acidity adversely reduces nodulation by changing vigour and morphology of lateral roots and root hairs. It may also directly reduce

rhizobium adsorption to the root surface impairing root exudation [32,33]. And lastly, the *Rhizobium* population may be adversely affected leading to their reduced vigour in the soil. Some relatively acid tolerant *Leucaena* species, such as *L. diversifolia* and *L shannoni*, characteristically absorb high levels of Ca [34]. Analysis of just expanded stem tip leaflets showed <170 ppm Al, normal N, P, Mg, and K, but 0.16-0.31 percent Ca in *L. leucocephala* and 0.54 – 0.61 percent Ca in *L. diversifolia* [35]. This suggests that the basis of the latter's resistance to Al, could be in its ability to absorb more Ca as compared to the former. This tolerance to acidity may be transferred to their hybrids with *L. leucocephala* [36]. The root growth of different varieties of *L. Leucocephala* shows significant variations among varieties in sand culture [5]. Significant reduction in growth was reported for Al concentrations of over 4 ppm, which could not be alleviated by Ca application [37]. This study assessed the effect of eco-physiological differences in two sites with differing soil structure and properties (Maseno and Chepkoilel) on growth and development of *Leucaena*.

2. MATERIALS AND METHODS

2.1 Experimental Site

The study was carried out in both greenhouse and fields at University of Eldoret, in Uasin Gishu District, and at the International Center for Research in Agroforestry (ICRAF)/ Kenya Forestry Research Institute (KEFRI) sub-station at Maseno in Kisumu District.

Chepkoilel Campus site is situated in Uasin Gishu District of Rift Valley province. It is located on longitude 36° E and latitude 30° N and at an altitude of 2180 m. The annual temperatures lie between a mean maximum of 23°C and a mean minimum of 10°C. The annual rainfall ranges between 900 mm and 1100 mm and has bimodal distribution, with the first peak in April and second peak in August [38]. The soils at this site are acidic (pH <5), dark red, friable, rhodic ferralsols [38].

Maseno ICRAF/KEFRI sub-station is situated at the boundary between Western and Nyanza provinces. The site is located on longitude 34° 35' and latitude 0° N and at an altitude of about 1500 m. It receives an average rainfall of about 1736 mm, which is bimodally distributed, with the first peak (long rains) between March/April and June/July, and the second peak (short rains) between September and November. The annual temperatures lie between a mean maximum of 29°C and a mean minimum of 21°C. Soils in this region are classified as acidic nitisols pH< 5 [39].

2.2 Soil Sampling and Analysis

Soil analysis for selected attributes was done for each experimental site before the experiment. Soil samples were collected at a depth of 20-cm (using a soil auger) from the fields at Chepkoilel Campus and Maseno ICRAF/KEFRI centre. Five soil samples were collected from each of the 30 sub-plots and bulked forming a composite sample, and then five representative sub-samples were withdrawn from the composite sample after thorough mixing. The sub-samples were air-dried in the laboratory and ground to pass through the 2 mm sieve. The samples were then analyzed for pH, cation exchange capacity (CEC), organic carbon (C), exchangeable Ca and Al, Olsen phosphors (P), and total nitrogen (N) as described by [40].

Leucaena leucocephala, being a tropical plant species, requires warm temperatures of between 20° and 30°C [41,42]. It can grow well in humid to semi-arid ecological zones [43]. It also grows in a variety of soils, including heavy clay, coral, sandy neutral and alkaline soils. The plant grows at altitudes of about 1600 m above sea level, and requires an annual rainfall of between 500 mm and 1700 mm.

2.3 Data Analysis

The data were subjected to multivariate analysis of variance (MANOVA; [44]), using a computer programme (SPSS version 7.5; SPSS® Inc). Means that were significantly different were separated using Tukey HSD test. Differences were accepted as significant at $p \leq 0.05$. The fixed factors were aluminium, lime, site and provenances, and dependent factors included plant height, root collar diameter, root length, number of leaves per plant and plant dry weight.

3. RESULTS

3.1 Soil Chemical Composition at Chepkoilel Campusand Maseno Experimental Sites

The selected soil chemical properties of the Chepkoilel Campus and Maseno ICRAF/KEFRI

experimental sites at the beginning of the experiment are presented in Table 1. The soils were acidic with pH < 5 although Chepkoilel Campus soils had slightly higher pH than Maseno soils. The concentration of organic carbon was similar in both sites. The soils at Chepkoilel Campus had significantly ($p < 0.05$) higher CEC than Maseno soils. The concentration of calcium was the same in both sites. Maseno soils had slightly lower concentration of aluminium compared to that of University of Eldoret, however % Al saturation in Maseno was more than double that at Chepkoilel Campus. The soils at Chepkoilel Campus had higher phosphorus and lower nitrogen but Maseno soils had significantly ($p < 0.05$) lower phosphorus and higher nitrogen.

3.1.1 Concentrations of some elements in shoot tissues of potted *L. leucocephala*

The concentration of Ca, exchangeable Al, available P and N potted seedlings is presented in Table 2. K136 had the highest whole plant tissue calcium concentration at both Chepkoilel Campus and Maseno ICRAF/KEFRI sites. The tissue concentration of Al was higher in seedlings grown at Maseno ICRAF/KEFRI compared to Chepkoilel Campus. In addition, K156 had higher concentration of tissue Al than the other two provenances at both sites. The concentration of tissue phosphorus was higher in seedlings grown at Chepkoilel Campus than Maseno ICRAF/KEFRI. KIT2724 and K156 had the highest tissue concentration of P at Chepkoilel Campusand Maseno ICRAF/KEFRI respectively. The percentage of N was significantly higher in K156 seedlings than the other two provenances at both sites. Percentage tissue N was also significantly ($P < 0.05$) higher in Maseno ICRAF/KEFRI seedlings compared to seedlings at Chepkoilel Campus.

3.1.2 Concentrations of Some Elements in the Shoots of Field Grown *L. leucocephala*

The concentration of mineral elements in shoots of field grown plants is presented in Table 3. K136 had significantly ($P < 0.05$) higher concentration of tissue calcium than the other two provenances. However, KIT2724 had significantly lower tissue aluminium concentration than K156. The percentage of P and N were significantly different in all the provenances.

3.1.3 Effect of site on growth response of *L. leucocephala* genotypes at maseno ICRAF/KEFRI and Chepkoilel campus

There was relatively better seedling growth at Maseno ICRAF/KEFRI (Fig. 2) than at Chepkoilel Campus (Fig. 3). The differences in environmental factors, such as higher mean temperatures (25°C) at Maseno ICRAF/KEFRI could promote growth, resulting in higher plant height, root length and plant dry weight than at Chepkoilel Campus, which had lower mean temperature 17°C, However, although the Maseno ICRAF/KEFRI plants were taller, they had smaller root collar diameters, especially after 90 days. The comparatively higher levels of nitrogen in the Maseno ICRAF/KEFRI soils could also be responsible for the better growth. This was also reflected in the shoot tissues where the seedlings grown at Maseno ICRAF/KEFRI site had higher nitrogen content than those same genotypes grown at Chepkoilel Campus site.

Table 1. Selected chemical properties of soils at Maseno ICRAF/KEFRI and Chepkoilel Campus experimental sites at the time of planting

Soil properties	Mean values	
	Maseno site	Chepkoilel site
pH (1 soil: 2.5 water)	4.8[b]	5.0[a]
CEC (Cmol/kg)	8.9[b]	11.6[a]
Organic Carbon (%)	1.9[a]	2.0[a]
Calcium (me/100 g)	2.0[a]	2.0[a]
Exch. aluminium (me/100 g)	0.2[b]	0.9[a]
Aluminium saturation (%)	16.7[a]	7.1[b]
Available phosphorus (ppm)	2.6[b]	4.9[a]
Nitrogen (%)	1.1[a]	0.2[b]

Key: - Means followed by the same letter in each row are not significantly different ($p < 0.05$) from each other according to Tukey HSD test

Table 2. The concentrations of mineral elements of whole plant tissues of potted *L. leucocephala* at Chepkoilel campus and Maseno ICRAF/KEFRI

Mineral elements	Provenances	Conc. at Chepkoilel	Conc. at Maseno
Calcium (mg/kg)	K156	0.38^{a}	0.86^{b}
	K136	1.52^{b}	1.22^{b}
	KIT2724	1.26^{b}	1.18^{b}
Aluminium (mg/kg)	K156	6.51^{a}	6.90^{a}
	K136	4.05^{a}	6.57^{a}
	KIT2724	3.03^{a}	3.24^{a}
Phosphorus (%)	K156	0.27^{b}	0.20^{a}
	K136	0.23^{b}	0.17^{a}
	KIT2724	0.33^{b}	0.16^{a}
Nitrogen (%)	K156	2.78^{c}	4.42^{a}
	K136	2.43^{d}	3.43^{b}
	KIT2724	2.64^{c}	4.33^{a}

Key: - Means within a given row for each mineral element followed by the same letters are not significantly different at P<0.05 according to Tukey HSD test

Table 3. The concentrations of mineral elements of plant shoot tissues of field grown *L. leucocephala* at Chepkoilel campus

Mineral elements	Provenances	Concentrations
Calcium (mg/kg)	K156	0.80^{b}
	K136	0.98^{a}
	KIT2724	0.68^{b}
Aluminium (mg/kg)	K156	1.17^{b}
	K136	1.77^{ab}
	KIT2724	2.55^{a}
Phosphorus (%)	K156	0.13^{a}
	K136	0.12^{a}
	KIT2724	0.14^{a}
Nitrogen (%)	K156	2.88^{a}
	K136	2.77^{a}
	KIT2724	2.68^{a}

Key: - Means within each row for each mineral element that are followed by the same letters are not significantly different from each other at P < 0.05 according to Tukey HSD test

4. DISCUSSION

4.1 Soil Chemical Composition in Maseno and University of Eldoret

The soils in the two sites are acidic, pH < 5. However soils at Maseno ICRAF/KEFRI were slightly more acidic (pH 4.8) than the Chepkoilel Campussoils (pH 5.0). The pH value in these two sites is far below the critical value for optimal growth and development of *L. leucocephala*, which has been suggested as 6.8 [45,3,36]. It is therefore anticipated to affect the growth and establishment of *L. leucocephala* directly through aluminium and H^{+} stress and phosphorus and calcium deficiency, as has been stated by other workers [34,46,47]. The values of CEC obtained in this study, Maseno ICRAF/KEFRI (8.9 Cmol/kg soil) and Chepkoilel Campus (11.6 Cmol/kg soil) were quite low [48,49]. This is an indication that the soils are highly leached. The CEC of nitisols and ferralsols is significantly influenced by pH because of the nature of major clay particles in them [50,51].The low pH in the soils may indirectly affect the growth and development of *L. leucocephala* by affecting the biological nitrogen fixation (BNF) process. For most plant species, the effect of low soil pH is manifested in reduction of root growth, which leads to increased shoot to root ratio, and reduced mineral ion and water absorption [29,52,53]. It interferes with the growth of *L. leucocephala,* among other tree legumes, either directly by reducing its root volume in the soil, or indirectly by suppressing BNF [2]. Photosynthesis is also affected, resulting in reduced biomass and general poor plant growth [54].

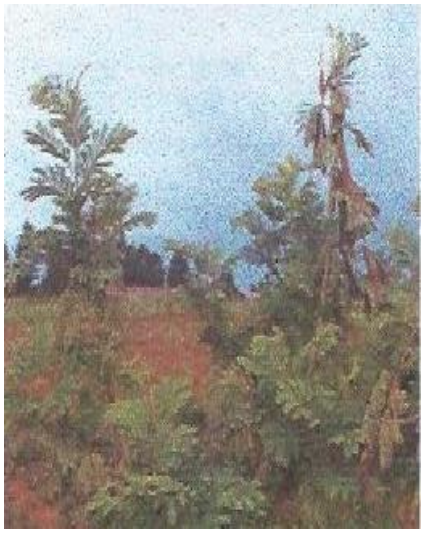

Fig. 1. *Leucaena* plant with pods at Chepkoilel Campus site

The low concentration of exchangeable aluminium in the soils (Maseno ICRAF/KEFRI, 0.2 me/100 g soil and Chepkoilel Campus, 0.9 me/100 g soil), was not expected. This result contrasts with the findings of [55] who worked in the same site (Chepkoilel Campus) and obtained Al concentration of 4 me/100 g soil. [55] used titration method to determine the level of exchangeable Al as opposed to the atomic absorption spectrophotometer (AAS) analysis used in this study. The differences in these results might also reflect heterogeneity of the soils in this site (Chepkoilel Campus) because the two experiments [55] and this study) were conducted in two different plots that are 800 m apart. The difference in Al concentration could also be due to other intrinsic chemical soil properties. Percentage aluminium saturation was equally low, 7.1% in this study compared to 44% reported by [55]. The low exchangeable Al or percentage saturation in the soils could explain why addition of 100 μM Al level still promoted growth of *L. leucocephala* in the pot experiment. Such wide differences observed in % Al saturation suggest that more study consisting of several samples from this site should be undertaken to resolve the discrepancy.

The available phosphorus in the soils (Maseno ICRAF/KEFRI 2.6 ppm, Chepkoilel Campus 5.0 ppm) was extremely low. Interpretation of [56] and [57] indicates that less than 15 ppm P is too low for proper plant growth and development. These soils have been reported to be generally low in Olsen P [58,1,55]. For purposes of this experiment and future considerations, these two sites are low in available P and this element should be applied to the soil to avoid P-related problems in the interpretation of the results.

Fig. 2. Response of 120 days seedlings (K136) to lime and aluminium application at Maseno. Q1 is limed pot with 300μM Al added to the pot, R1 is unlimed pot with 300 μM Al added to the pot

Fig. 3. Response of 120 days seedlings (K136) to lime and aluminium application at Chepkoilel Campus University of Eldoret. I_3 limed pot with 300μM Al added to the pot and P_3 Unlimed pot with 300 μM Al added to the pot

The amount of nitrogen in Chepkoilel Campus soils (0.2%) was considered to be low according to [59] who rated 0.1% – 0.2% total N as being low. The low nitrogen content of the Chepkoilel Campus soils may have been due to reduced microbial activity in the soil caused by low pH, which in turn minimized the breakdown of organic matter [48] to release nitrogen. However, Maseno ICRAF/KEFRI soils had moderately higher level of nitrogen (1.1%). Thus, explains

why the seedlings at Maseno ICRAF/KEFRI site had better growth and establishment compared to the ones at Chepkoilel Campus.

The soils in the two sites had similar concentrations of calcium and organic carbon. The concentration of Ca (2 me/100 g soil) in both sites is regarded to be low according to the description of [60] and [61] who considered Ca levels of 0.2 me/100 g to be very low for optimal growth of crops. The percentage organic carbon in the Maseno ICRAF/KEFRI soils (1.9) and Chepkoilel Campus soils (2.0) was also low according to the broad rating by [59], in which soils with $<2\%$ was considered to be very low in carbon content. Overall, the nutrient status of the soils in these two sites can be regarded as low and for optimal growth of plants including *L. leucocephala*, application of organic fertilizers or manure shall be mandatory.

Plant species and genotypes within species therefore differ significantly in tolerance to acid soil stress [62,63]. [36] made some progress in breeding for acid soil tolerance in *L. leucocephala* in Hawaii. The adverse effect of soil acidity on plant growth can be alleviated by application of lime or organic matter [9]. However, this is not economical especially on small scale farming, because of the high cost and huge quantities of lime and/or organic matter normally required to ameliorate acidity. For example, the recommended rate of lime and organic matter that can alleviate low pH problem is about 5-6 t/ha for tropical soils [64]. Moreover, lime is normally transported long distances from the site of mining and processing to farmers' fields, which often makes it too expensive, especially for small-scale farmers. Excessive lime could also change soil structure and cause deficiency of some mineral nutrients, such as P, N and Mg [65]. Therefore, use of *L. leucocephala* genotypes that are naturally tolerant to soil acidity could greatly improve biomass production and, consequently lead to high rate of adoption by farmers living in the regions affected by such soils [58]. More research effort should therefore be devoted to screening tree legumes for tolerance to soil acidity [2,8].

5. CONCLUSION

Seedlings at Maseno ICRAF/KEFRI centre had significantly ($p \leq 0.05$) better growth than at University of Eldoret. Maseno ICRAF/KEFRI soils had higher N and less Al than at Chepkoilel Campus. Production of *Leucaena* for commercial purposes could be done in Maseno region better than at in Uasin Gishu.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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