Characterization of Pearl Millet Genotypes for Aluminium Toxicity Tolerance Using Morphological Traits

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Abstract

Aluminum (Al) toxicity is one of the major limiting factors for wheat production on acid soils. It inhibits root cell division and elongation, thus reducing water and nutrient uptake resulting in poor plant growth and yield. Liming the soil is costly and lime takes long time to be effective. Nutrient solution screening method was used for evaluation process, where seven (7) stock solutions were prepared. Seeds were treated prior to germination with sodium hypochloride and the pre-treated pearl millet seeds were grown using completely randomized design (CRD) on shallow trays for 4 days thereafter stabilized in distilled water for a day. Initial seminal root length (ISRL) was taken and later the seed transferred to nutrient solution for 5 days (planting). After 5 days, Final Seminal Root Length (FSRL) for the controls and treatments taken. The roots were evaluated and used for phenotypic analysis to ascertain if there existed variability among the pearl millet genotypes. Phenotypic data was then subjected to analysis of variance (ANOVA). The means were separated using Duncan Multiple Range Test (DMRT) at 5% level of significance. After evaluation process, genotype 6 was moderately tolerant. The fact that Al treatment decreased root growth in a variable manner among some genotypes is an indication that Al toxicity could be one of the major factors limiting pearl millet production in Kenya. The differential reduction in root growth by Al treatments shows that the pearl millet germplasm contain useful genetic diversity for tolerance to Al toxicity which can be used to improve crop yield in low soil pH.

Key Words: Al Toxicity and Tolerance, Nutrient Solution, ISRL, RSRL, ANOVA, CRD

INTRODUCTION

Pearl Millet (*Pennisetumglaucum*) is a cereal crop in the grass family (Gramineae) that is cultivated for grain and fodder. It is mostly cultivated under hot, dry weather conditions on infertile soils of low water holding capacity where other crops generally fail (FAO, 1991). Pearl Millet as a food crop is limited to the developing countries in Asia and Africa. It is estimated that over 95% of pearl millet production is used as food, the remainder being animal and poultry feed (5%), other uses, (seed, bakery products, snacks etc.). It is traditionally used for food products like roti (flat bread, bhakri (stiff roti) and porridge or gruel. Pearl millet is a principal source of energy, proteins, vitamins and minerals for millions of the poorest people in the regions where it is cultivated.

Pearl millet yields best on fertile well drained soils. However, it also performs relatively well on sandy soils under acidic soil conditions of pH 6.0-6.5 and when

available soil moisture and soil fertility are low. This adaptation reflects pearl millet origin in the Sahel region of Africa (Ahlrichs *et al.,* 1991).

The greatest limitation to plant productivity on acid soils is Aluminium (Al) toxicity. The efflux of organic anions is an important mechanism for Al toxicity resistance in cereal and non-cereal species (Ryan *et al.,* 2009). Acid soils pH<5 increase the phytotoxic levels of trivalent Al (Al^{3+}) whereas at higher pH, other non-toxic forms such as $Al(OH)^{+}$ and $Al(OH)^{2+}$ are more prevalent (Delhaize *et al*., 1995). Al toxicity primarily affects the division and elongation of root meristems. When Al^{3+} penetrates into the root, it binds to the negative charges of phospholipid in the plasma membrane leading to rigidification and disruption of membranes function and enhancement of oxidative stress (Jones *et al.,* 2006). These physiological changes in the root cell results in the poor uptake of nutrients and water that ultimately affects Pearl millet crop yield. Al toxicity is mostly severe in soils with low base saturation, poor Ca and Mg. Acid soils (pH<5.5) are widely distributed constituting over 40% of arable land in the world (Hang, 1984). In Kenya, acid soils cover 13% (7.5 million hectares) of arable lands (Kanyanjua *et al.,* 2002). These soils are widely distributed in the highland areas and they cover significant parts of Mt Kenya region including Tharaka-Nithi in Meru County where pearl millet is grown (Kanyanjua *et al.,* 2002). Soil acidity causes chemical changes in the soil that result in fixation of essential plant nutrients (Adams, 1981). Phosphorus and molybdenum are fixed in the soils under acidic conditions. There is need to identify pearl millet genotypes that are most resistant/tolerant and more suitable to grow in acidic soils in order to sustain and increase world cereal production.

Aluminium (Al) toxicity is one of the limiting factors for crop production on strongly acidic soils. It inhibits root cell division and elongation, thus reducing water and nutrient uptake consequently resulting in poor plant growth and yields. It has been estimated that over 50% of the world‘s potentially arable lands are acidic (Von *et al.,* 1995; Bot *et al.,* 2000) hence Al toxicity is a very important worldwide limitation to crop production. Pearl millet is a crop mostly grown in arid and semi-arid areas where the soils are sandy. Sandy soils occur throughout arid regions primarily as a result of weathering processes common in dry environment (salt, chemical, biochemical, mechanical, pressure release or exfoliation, wetting and drying and isolation or thermal expansion) (cals.arizone.edu//arid soils.html). Sandy soils allow water to pass through quickly hence allowing calcium minerals to leach hence becoming acidic. Furthermore, up to 60% of the acid soils in the world occur in developing countries where food production is critically low. Therefore, identifying crops with increased Al toxicity tolerance has been an active area of research.

Physiological mechanisms of Aluminium toxicity in soils are present as insoluble alumino-silicates and oxides. As the soil pH drops below 5, the octahedral hexahydrate $Al(H_2O)_6^{3+}$ more commonly referred to as Al^{3+} is solubilized in to the soil solution.This form of Al appears to be the most phytotoxic (Kinraide *et al.,* 1991; Parker *et al.,* 1989). Al^{3+} interferes with a wide range of physical and cellular processes. Potentially, toxicity could result from complex Al interactions with apoplastic (cell wall), plasma membrane, and symplastic (cytosol) targets. Since the

acidity of the soils are increasing due to the use of inorganic fertilizers and generally poor crop production methods e.g. poor crop rotation programs which have allowed buildup of nitrates in the soil consequently lowering soil pH, There is need to identify Al toxicity tolerate genotypes that can produce good yields in acidic soils.

The use of Al toxicity tolerant genotypes reduces liming practices that are aimed at neutralizing the acidity. However in many agricultural systems, the application of lime as the only way of managing acid soils is either costly or takes many years for the lime to be effective. Thus there is need for Al toxicity tolerant species to be used in place of less tolerant to sustain and improve production. Furthermore, heavy application of lime may have adverse effects on some crops in the rotation or causes deficiencies of certain nutrients (Whitten *et al.,* 1987). Thus, with the need of consumers for both quality and varying pearl millet products, there is need to extensively collect, exploit and evaluate unknown pearl millet germplasm in order to provide the sustainable varieties to the developing world characterized by low food production due to poor soils (acidic soils). Systematic study and characterization of pearl millet germplasm that tolerate high levels of Al is of great importance to current and future agronomic and genetic improvement of the crop. In this context, this study aimed at characterization of pearl millet genotypes for Aluminium toxicity tolerance using morphological characteristics.

Field evaluation, though necessary for assessing tolerance to acid, is very expensive and is frequently affected by large spatial variability in these soils. Several bioassays have been developed for more rapid evaluation for example haematoxylin staining technique, in –vitro tissue culture and molecular markers (Reiter *et al*., 1991). However, the result of these procedures apart from that of nutrient cultures, generally, did not correlate well with field performance. Thus nutrient solution screening is found to be a cheap and fast and has been routinely used (Magnavaca *et al*., 1987).

MATERIALS AND METHODS

Plant Material

A total of seven pearl millet genotypes were evaluated/screened for Aluminium toxicity tolerance. Obtained the materials (seeds) from a member of biotechnology department

Experiment

The phenotypic evaluation of the pearl millet genotypes for Al toxicity tolerance was carried out at Botany laboratory (Botany lab 2) University of Eldoret.

The experimental set up of the solution culture screening was used i.e. the screening process used the nutrient solution of magnavaca (1987). It consisted of growth chamber, germination trays and air tubes served with an electric air pump. The solution culture used for screening the pearl millet seedlings was comprised of seven stock solutions prepared prior to commencement of screening process. Solution seven was Aluminium source (AlK $(SO_4)_2.7H_2O$), solution one calcium source

($Ca(NO_3)_2.4H_2O$) and NH_4NO_3; solution 2, 3, 4 were potassium, magnesium and phosphorus sources respectively. Solution 5 was Iron chelate (FeHEDTA) for two litres of stock solution. Iron nitrate as Fe_3 $(NO_3)_3.9H_2O$ M.W 404.0g/mol and solution 6 micronutrients (to make 1 litre) solution of sodium hypochloride was prepared by mixing hypochloride and distilled water at ratio 1:10. 0.1M HCl and 0.1M NaOH prepared to adjust pH. Ruler to measure root length. The solution culture contained 73.92ml calcium source, 55.44ml potassium, 36.96ml magnesium, 8.10ml phosphorous, 36.96ml iron and 18.48ml micronutrients. For the Aluminium 5.3ml was added to the tray designated to have concentrations of 148µM and 19.2ml added to the tray with the highest combination of 222µM. 24 litres of distilled water was used hence each tray took 8 litres.

Experimental Design

The experimental design was a completely randomized design, 5 replicates of each genotype carried out in each treatment. There were 3 treatments which amounted to 3 trays. These were OAl, 148µM and 222µM Al.

The model of the design is:

$Y_{ij} = \mu + t_i + e_{ij}$

Where:

Y_{ij} = overall observation

T_i= treatment effect

E_{ij} = error term

Procedure

Seeds of each genotype were washed with distilled water and sterilized in 5.6% solution of sodium hypochloride prepared by mixing sodium hypochlorideand distilled water at a ratio of 1:10. The seeds were agitated for 10 minutes on the mechanical shakers and then washed 8 times with distilled water. They were then wrapped in paper towels in sterilized germination trays and put in the growth chamber (incubator) to germinate at a constant temperature of 26^oc. Relative humidity of 60%and low density illumination for a period of 4 days. After germination, the seedlings were stabilized for a day in a nutrient solution culture (magnavaca *et al.*, 1987) without Aluminium and on the second day, Aluminium was introduced to 2 trays. One tray received 148µM while another received 222µM Al. Initial seminal root length (ISRL) measurements and visual observations were made before addition of Aluminium to the trays. The seeds were selected and only those that had uniform germination allowed growing for 5 days in the nutrient culture. The screening trial was set up using 3 Al concentration treatments (AlK $(SO_4)_2.7H_2O$) replicated two times in a completely randomized design (CRD). The pH was adjusted to 4.2 using 0.1 M HCl and 0.1 M NaOH. After 5 days measurements of theseminal root length of the seedlings growth in the nutrient solution containing 148µM and 222µM concentrations of Al and nutrient culture without Al taken and designated Final Seminal Root Length (FSRL). Measurements were done by lifting the cup from the nutrient solution and roots of the seedlings placed along a ruler and measurements

taken. Other parameters included root tolerance index (RTI) and %response to Al treatments. These were determined as described in the equations below.

a) Root Tolerant Index RTI = $\frac{Final\ root\ length\ of\ Al\ treated\ roots}{Final\ root\ length\ of\ control\ plant}$

b) % Response = $\frac{Rootlength\ of Altreatedplants - Rootlength\ ofzeroAl}{Rootlength\ ofzeroAl\ (controlplant)} X\ 100$

c) Net seminal root length (NSRL)= Final seminal root length (FSRL) – Initial seminal root length (ISRL)

d) Relative Seminal Root Length (RSRL)= $\frac{Net\ seminal\ root\ length\ (NSRL)}{Initial\ seminal\ root\ length\ (ISRL)} X\ 100$

The selection criteria for Al tolerance was based on relative seminal root length (RSRL) as per the following scales ≥70% categorized as tolerant (scale 2), 50-59 moderately tolerant (scale 3) and ≤49 categorized as sensitive (scale 4) or Net seminal root length (NSRL). More often the NSRL gives more reliable results (magnavaca *et al.*, 1987)

Data collected was analyzed using GENSTAT software package version 12.1. It was also subjected to analysis of variance (ANOVA) at 5% level of significance and means separated by Duncan's Multiple Range Tests.

RESULTS AND DISCUSSION

Seminal Root Length (NSRL) of genotypes in each treatment was calculated. The table 1 shows the performance of each genotype under different treatments.

Table 1. Mean net seminal root length (NSRL, cm) of each genotype in each treatment

GENOTYPE	TREATMENT(Al)		
	0 µM	148 µM	222µM
1	3.160ab	2.740a	2.380a
2	3.240ab	2.340a	3.040ab
3	4.060bc	2.940ab	2.580a
4	4.380c	2.600a	3.160ab
5	2.960ab	2.960ab	3.060ab
6	3.200ab	3.040ab	4.660c
7	4.400c	2.320a	2.680a

S.E 0.3678; S.E.D 0.5201; L.S.D 1.0344; %C.V 26.2

Genotype 1 and 3 had decreased net seminal root length with increasing levels of Aluminium (table 1). Genotypes 2, 4, 6 and 7 showed differing trends in that at 0 Al, the means were high, decreased at 148µM and increased at 222µM. Genotype 5 had equal means at 0 and 148µM but increased at 222µM.

Overall mean performance of each genotype in the treatments are shown in table 2. Generally, genotype 6 had a greater mean although it had no significant difference with genotype7.

Table 2. Overall yield performance of seven pearl millet genotypes

GENOTYPE	MEAN
1	2.760a
2	2.873a
3	3.193ab
4	3.380ab
5	2.993ab
6	3.633b
7	3.133ab

S.E 0.2123; S.E.D 0.3003; L.S.D 0.5972; %C.V 26.2

Response to different aluminum concentrations of each genotype is provided (tables 1 and 2). It was expected that for the genotypes that are sensitive to Aluminum toxicity, the root length could decrease as Aluminum concentration increased and vice versa. However, some sensitive genotypes showed increased root lengths with increased Aluminum toxicity.

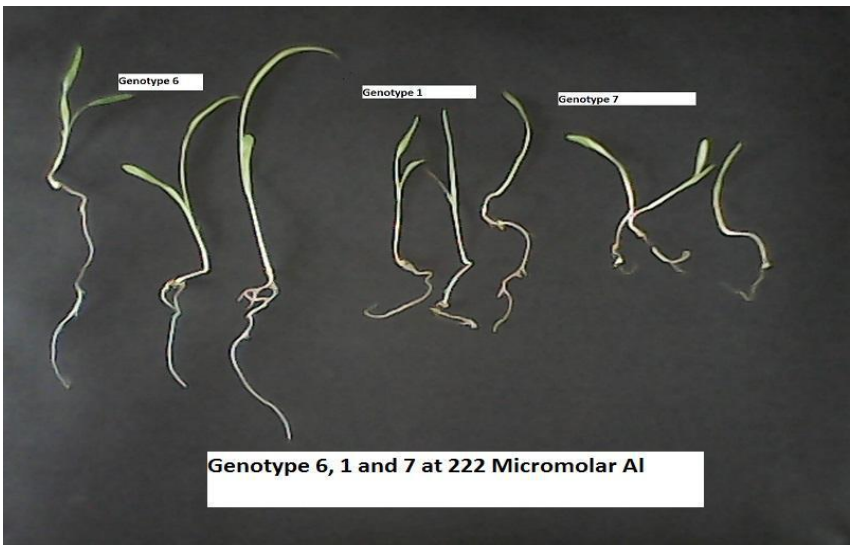

Figure 1: Performance of genotypes 6, 1 and 7 at 222µM respectively

Table 1. and figure 1 show that genotypes 2, 4, 6 and 7 had longer seminal root length at 0 µM Al as compared to at 148µM AI and a slight increase at 222 µM Al. Figure 2

shows the effect of different aluminum on root lengths of genotype 7. This indicates that Al has an influence on root performance in pearl millet. Ahlrichs *et al*. (1991) indicate that there was differential tolerance to Al toxicity in pearl millet.

Figure 2: Performance of genotype 7 at 0, 148µM Al and 222µM Al respectively

Genotypes 1and 3 (table 1 & 2, Figure 3) showed decreasing trends in net seminal root length with increasing levels of Aluminum concentrations. Those are the Aluminum sensitive genotypes whereby with high Aluminum concentrations at low pH (< 5), there is decreased mitotic activity as a result of Aluminum exposure in root tips leading to reduced root growth. Aluminum induced oxidative stress and changes in cell wall properties have also been suggested as two major factors leading to Aluminum toxicity. Oxidative stress occurs when any conditions disrupts the cellular redox homeostasis. The reactive oxygen species (ROS) have the capacity to oxidize cellular components such as lipids, proteins, enzymes and nucleic acids leading to cell death. Cell wall proteins and outer surface of plasma membrane seem to be major targets of Aluminum toxicity. Aluminum binding to biomembranes leads to rigidification which seems to facilitate the radical chain reactions by (Fe) ions and enhance the peroxidation of lipids (Delheize *et al.,* 1995).

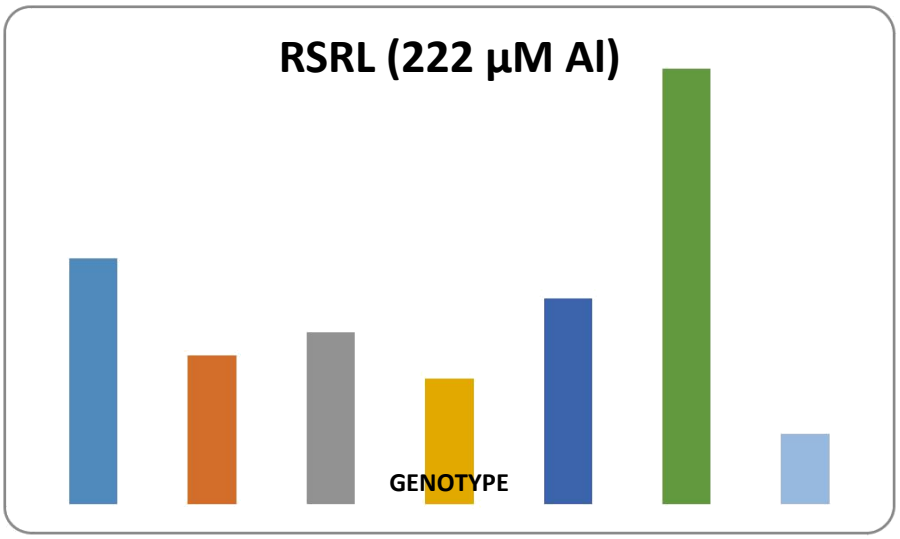

Figure 3. Relative seminal root lengths of genotypes at 222µM Al

Genotype 5 showed interesting results in that the net seminal root length was increasing with increasing levels of Aluminum concentrations and genotype 6 had a greater root length mean at higher Al concentration. This was attributed to the fact some genotypes have physiological mechanisms to tolerate high levels of Aluminum toxicity by releasing some organic acids from the root apices that are able to complex Al^{3+} into forms that are not toxic to plants. It has been found that organic acids with hydroxyl and carboxyl groups are able to form stable ring structures with Al^{3+} that conferred the greatest protection from Al toxicity (Hue *et al.*, 1986). Organic acids commonly found in plants that fit this criterion are citric, oxalic and malic acids. A range of techniques have shown that Aluminum tolerant genotypes accumulate less Al in root apices than Al sensitive genotypes. It is now known that tolerant genotypes of many species exude organic acids in response to Al (Ryan *et al.*, 2001; Me *et al.*, 2001). These organic acids chelate that Al and therefore protect the root from Al toxicity. In several of the examples, the efflux of organic acids occurs primarily from the root apices and this makes good sense since this is the part of the root system most susceptible to Al toxicity.

The selection criteria for Aluminum tolerance was based on relative seminal root lengths. Results indicate that genotype 6 was moderately tolerant (scale 3) with relative seminal root length of 55%. The remaining 6 genotypes were categorized as sensitive since their relative seminal root lengths were less than 49% (scale 4). The moderate tolerance and sensitivity are attributed to the above discussed mechanisms.

CONCLUSION

The fact that Al treatment decreased root growth in a variable manner among some genotypes is an indication that Al toxicity could be one of the major factors limiting pearl millet production in Kenya, particularly in acid soils where Al concentration could be high enough to hinder root growth and development.

Secondly, the differential reduction in root growth by Al treatments shows that the Kenyan pearl millet germplasm contain useful genetic diversity for tolerance to Al toxicity which can be used to improve the crop yields in low soil pH. Thirdly, solution culture is efficient method of discriminating pearl millet genotypes for Aluminum tolerance.

RECOMMENDATIONS

It is recommended that screening of more genotypes is done in order to identify those that are tolerant to Aluminum toxicity in acidic soils of different ecological zones. Further analysis should be done at molecular level in order to appreciate the variability for Aluminum toxicity at molecular level.

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BIO-DATA

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