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Screening of Wheat (*Triticum aesativum* L.) Genotypes at Germination and Seedling Stage for Aluminum Toxicity Tolerance

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Abstract

In Lesotho, wheat productivity is low because of the negative impact of various biotic and abiotic stresses. Aluminum toxicity is one of the major causes of low productivity necessitating corrective measures. To address this challenge, wheat genotypes tolerant under these conditions could be a remedy. The objectives of this study were to (1) identify wheat genotypes tolerant to Aluminum toxicity, (2) rank wheat genotypes according to their tolerance to Aluminum toxicity. The laboratory experiment was undertaken at the National University of Lesotho, Department of Crop Science. Factorial design was applied with factor A genotypes and factor B Aluminum chloride concentrations. Completely Randomized Design was used to set up experiment. There were 41 treatments with four replications. Three Aluminum concentrations (0.000, 0.014, and 0.027 gL^{-1} Alcl) were used to irrigate specific set of wheat genotypes placed in petri dishes incubated in the growth chamber. Haematoxylin stain was prepared a day before measuring initial root length which was in day 4, after which seedlings were re-grown in a plastic bowl in a growth chamber. Root re-growth was re-measured after day 6 of germination. Microscope was used to observe the intensity of stain. To score the intensity of staining, arbitrary scale of 0 to 4 was used. The absence of color was assigned 0 and maximum staining was 4. Hematoxylins staining of root apices were performed after 48h exposure to Alcl. Data generated were analyzed using Genstat 16 software to perform analysis of variance and mean separation. Results of analysis of variance revealed that there was a highly significant difference ($P < 0.01$) among the genotypes, concentrations and their interactions. Wheat genotypes most tolerant to Aluminum toxicity were SST 843 and SST 317.

***Key words*:** Aluminum toxicity, hematoxylin, Lesotho, wheat genotypes

Soil is the unconsolidated outer layer of the Earth's crust and serves as a medium for plant growth [1]. Healthy soils are able to store and regulate water flow and mitigate climate change [2]. But when extreme weather occurs, the scale of floods and droughts increases and the soil becomes acidic. The hydrogen ions (H^+) get dissolved in soil solution and are held on the clay and humus particles [3]. In order to know the status of the soil, pH needs to be determined. pH measures the available hydrogen ions in a solution, and describes the relative level of acidity or alkalinity [4]. A value below 7.0 is acid, 7.0 is neutral and above 7.0 is alkaline. As the soil pH decreases below 7.0, soils become more and more acidic. The main problem associated with such soils is that, they have high content of exchangeable Al (Al^{3+}) [5], which could be detrimental to crop growth. In acidic soils, toxicities such as hydrogen and aluminium ion, and deficiencies in phosphorus, calcium, magnesium and the micronutrients may suppress the microbial activity which may definitely limit agricultural productivity. Among the agricultural impairments, Al is the most abundant metal on the earth [6]. However, it has no essentiality known to man [7]. The importance of Al toxicity in acidic soils is recognized by the "alic" subgroup/qualifier (N_2.0 cmol (+) kg^{-1} KCl-extractable Al) in soil taxonomy [8]. An increase in soil acidity typically causes higher solubility of monomeric aluminum [9]. The more the concentration of Al^{3+} in the soil increases, the more it impacts on plant roots. Root growth is severely reduced when exposed to Al^{3+} in solution [10]. Al^{3+} also has a number of secondary effects on plant roots, including reduced water and nutrient uptake [11]. Al^{3+} exposure can reduce NO_3-uptake by plants [12. The soluble soil Al^{3+} can also interfere with cell membrane H^+- ATPase activity, reducing the cell's capacity to pump out H^+ [13]). Consequently, the plants which are sensitive to acidity will decline in growth and productivity allowing weeds to increase or reduce soil cover which can lead to soil erosion [14].

The acidic soil degrades the agricultural land [15]. The issue of soil acidification is of principal concern when considering sustainable agricultural crop production system. The acid soils need to be ameliorated for plant growth. After improving soil acidity, the parameters of critical soil pH and soil Al concentration will be determined. Soil acidification may be accelerated by applying excessive Nitrate ion [16]. Liming of acid soils can increase soil pH, alleviate Al toxicity and maintain a suitable pH for the growth of a variety of crops including wheat [17-18]. Neutral soil pH condition may be considered as the best for crop growth. However, optimum pH

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condition for individual crops varies. Some crop varieties are being developed to tolerate lower pH and higher aluminum levels [19]. Researchers identify the genotypes that are able to tolerate the changing environmental conditions. For instance, Sadic *et al.* [20] studied the root malate efflux and expression of *TaALMT1* in Serbian winter wheat genotypes that differed in Al tolerance. They were evaluated on relative root length (RRL) for Al tolerance. Moderately Al-tolerant genotypes showed significantly higher relative *TaALMT1* expression than the Al-sensitive ones. A considerably high level of Al tolerance was found in Ljiljana genotype, which showed the highest Al-induced malate efflux along with the highest constitutive expression level of *TaALMT1* transcripts. The results also demonstrated that Al tolerance is based on a constitutive trait of high *TaALMT1* expression and malate efflux in wheat roots, resulting in a decrease in root length reduction. Likewise, Perekeira [21] also screened wheat genotypes by evaluating their relative root length. The alleles of two important genes (*TaALMT1* and *TaMATE1B*) were discriminated for Al tolerance in wheat. Both of these genes encode membrane transporters responsible for the efflux of organic acids by the root apices that were considered responsible in conferring tolerance by chelating Al.

Al tolerance in wheat may also be evaluated in hydroponic solution [22] whereby seedlings may be grown in both the presence and absence of Al [23-24]. Hematoxylin staining may be used as an indicator of Al tolerance. The objectives of this study were three folds; (1) identify genotypes of wheat tolerant to Aluminum toxicity, (2) ranking wheat genotypes according to their susceptibility and tolerance.

MATERIALS AND METHODS

Study area

The study was conducted in the laboratory of the Department of Crop Science at the National University of Lesotho, which is situated at Roma lying 34 kilometers from Maseru District in Lesotho.

Source of genotype material

The genotypes of wheat were collected from Department of Agricultural Research in the Ministry of Agriculture and Food Security, Lesotho. The material has been evaluated in four agro-ecological zones of Lesotho; namely; lowlands, foothills, mountains and Orange River valley. All of which are now planted country-wide by the farmers in their respective fields.

Laboratory procedure

Factorial design was applied with factor A being wheat genotypes and factor B being Alcl concentrations. Completely randomized design with four replications was applied to lay-out the treatments. Wheat genotypes were screened using three different Al concentrations, namely; 0.00, 0.014, and 0.027 gL^{-1} Al). The seeds were surface sterilized with 1% sodium hypochlorite. Ten wheat seeds were germinated on a wet filter paper placed on separate petri dishes. They were grown in an incubator that was set at 25 °C for 6 days. Al was added to the nutrient solution in the form of Aluminium Chloride ($AlCl_3$ + $3H_2O$= Al $(OH)_3$ + 3HCl) but there was no Al added to control treatment. Three healthy uniform seedlings of the same wheat genotypes were selected, measured, stained and planted on a filter paper in a plastic bowls covered with foil to prevent growth of algae.

For hematoxylin treatment, the stain was prepared a day before the experiment by adding 0.2 g of hematoxylin and 0.02 g of $KI0_3$ in 100 mL of water. The solution was left overnight to dissolve the hematoxylin [25]. The basic protocols were a bit modified to maintain consistency. The protocol which was used by Polle *et al*. [23] was adopted. The plant roots were shaken in 200 ml distilled water for 15 minutes. The water was then replaced by 200 ml of aqueous hematoxylin solution (0.2g hematoxylin) and left at the slow agitation for 10 min. Finally, the solution was replaced again by 200 ml water, thereby repeating the first step [26].

Data collection

The parameters measured were: germination percentage, initial root length, final root length and net root length. All these parameters were measured using a ruler with the units of cm. The plants were grown for 6 days in a climatically controlled growth chamber. The parameters were again re-measured and re-recorded after 10 days. The plant roots were photographed and placed under light microscopes to observe the hematoxylin-Al complexes in the internal tissues. To score the intensity of staining, the measurements were done on the arbitrary scale of 0-to-4, in which the total absence of color was assigned a zero (0) and maximum staining was (four) 4. The hematoxylin staining of root apices were performed after a 48-h exposure to Al.

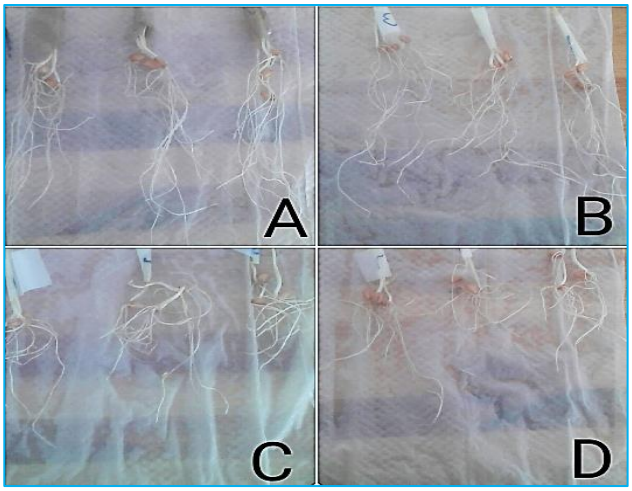

Fig 1 An example of different wheat genotypes (roots) exposed to different Aluminum concentrations illustrations from (PAN 3623). A=0g Alcl g L^{-1}, B= 0.007 Alcl gL^{-1}, C= 0.014 Alcl gL^{-1}, D=0.021 Alcl gL^{-1}

Data analysis

Data generated were analyzed using Genstat 16 version software to perform analysis of variance and mean separation (Duncan Multiple Range Test).

RESULTS AND DISCUSSION

Variability in wheat genotypes

The analysis of variance (Table 1) revealed highly significant differences ($P<0.05$) among wheat genotypes for germination percentage, initial root length, final root length and net root length. Genotypes that managed to score high germination percentage included SST 8154, SST 8154 and PAN 3541 with 95.83% and 92.50%, respectively. The lowest germination percentage was obtained from PAN 3195 with 5.00% and Gariep with 1.67%. PAN 3541 and SST 843 had the longest initial root lengths of 9.92 cm and 9.03 cm, respectively. The shortest length was obtained from PAN 3195 and Gariep by 0.63 cm and 0.41 cm, respectively. The final root length was longer with PAN 3541 being 7. 63 cm and SST 843 with 7.30 cm. The shortest length was measured from Gariep and PAN

3195 with 0.33 cm. SST 884 and PAN 3623 on the net root length (delta) had the greatest lengths of 4.52 cm and 3.48cm. PAN 3195 and Gariep had the shortest lengths of 0.30 cm and 0.08 cm, respectively (Table 2).

Table 1 Mean squares of wheat genotypes for various parameters

Source of variation	d.f.	Mean square			
		GP%	IRG	FRG	NRG
Genotypes	40	8169.30**	204.62**	74.72**	68.66**
Concentration	3	8448.20**	64.17**	37.24**	8.46**
Genotypes × Concentrations	120	468.80**	5.05**	2.43**	2.55**
Error	328	150.20	1.44	0.61	1.33
GM		65.55	7.21	5.13	2.12
DMRT (0.05)		19.69	1.93	1.25	1.85
CV%		18.70	16.70	15.2	54.3

Highly Significant at P> 0.05 probability level= **, GM= Grand mean, CV%= Coefficient of variance, GP= Germination percentage, IRL= initial root length, FRL=final root length, NRL= net root length

Table 2 Means of genotypes for various parameters

Genotypes	Means of genotypes for parameters			
	Germination percentage	Initial root length	Final root length	Net root length
Duzi	78.33	8.03	5.48	2.55
Eland	60.00	8.72	5.88	2.83
Gariep	1.67	0.41	0.33	0.08
Koonap	83.33	8.43	5.31	3.12
Kouglas	61.67	6.84	4.41	2.43
Krokodil	77.50	8.00	5.44	2.56
Kubetu	16.67	3.28	2.27	1.04
Matlabas	24.17	4.02	2.59	1.43
PAN 3111	77.50	8.52	5.08	3.43
PAN 3195	5.00	0.63	0.33	0.30
PAN 3368	90.00	8.80	6.97	1.83
PAN 3400	73.33	7.79	5.92	1.88
PAN 3471	59.17	7.86	5.48	2.38
PAN 3497	50.00	7.76	5.40	2.33
PAN 3515	76.67	7.88	5.58	2.30
PAN 3541	92.50	9.92	7.63	2.29
PAN 3623	48.33	8.36	4.88	3.48
PAN 3644	84.17	7.35	5.68	1.66
Sabie	74.17	8.10	5.49	2.74
Senqu	60.83	8.73	5.62	3.12
SST 3149	45.83	6.08	4.49	1.58
SST 316	77.50	7.36	4.91	2.45
SST 3161	55.00	6.32	4.08	2.24
SST 317	88.33	8.71	6.70	2.14
SST 347	12.50	2.19	1.30	0.81
SST 356	79.17	7.46	5.51	1.97
SST 374	86.67	7.59	5.82	1.78
SST 375	90.83	9.03	6.73	2.29
SST 387	60.00	6.19	4.95	1.23
SST 398	89.17	8.14	5.73	2.42
SST 806	68.33	7.73	5.98	1.75
SST 8135	76.67	8.90	6.31	2.59
SST 8154	95.83	8.42	6.07	2.35
SST 8156	88.33	8.76	7.43	1.35
SST 835	82.50	9.05	6.52	2.53
SST 843	90.00	9.58	7.30	2.28
SST 866	80.83	8.18	6.38	1.80
SST 884	86.67	8.97	5.98	4.52
SST 895	91.67	8.73	6.30	2.43
Tungela DN	16.67	3.23	2.23	1.08
Wedzi	30.00	5.39	3.69	1.70

PEG-6000 concentrations

The analysis of variance showed highly significant difference (P<0.01) among PEG-6000 concentrations for germination percentage, root length, coleoptile length, plumule length, root fresh weight, coleoptile fresh weight and plumule fresh weight (Table 1). The grand mean of germination

percentage in all PEG-6000 concentrations and control were 90.56% and 92.40%, respectively. The highest germination percentage obtained with control was 94%, followed by 39g (-0.5 bars) concentration at 92.13%. The least germination percentage was obtained at 117g with 88.53%. The longest radical length measured in PEG-6000 was 39g (-0.5bars) with 20.54cm, followed by PEG 78g (-1.0 bars) with 19.30cm. The shortest length was recorded at 117g (-1.5 bars) with 17.63cm. The greater weight in radical fresh weight was found in control where it had 0.15g. It was then followed by PEG 39g (-0.5 bars) with 0.14g. The least weight was obtained at the concentration of 117g (-1.5 bars) with 0.11g. The longest plumule length was obtained at the concentration of 39g (-0.5 bars) with 9.77cm, followed by control which had 9.57cm. The shortest length of plumule measured was at 117g (-1.5 bars) which had 8.08cm. The plumule fresh weight seemed to be greater at the concentration of 39g (-0.5 bars) where it got 0.16g, and then followed by control with 0.14g. The least weight was obtained at 117g (-1.5 bars) with 0.10g. The coleoptiles length was high at the concentration of 78g (-1.0 bars) with 4.41cm which was followed by the concentration of 39g (-0.5 bars) with 4.17cm. The shortest length was recorded with control which had 3.88cm. The coleoptiles fresh weights were similar at the concentrations of 78g (-1.0 bars) and 39g (-0.5 bars) which had 0.14cm. The control together with 117g (-1.5 bars) had 0.12g (Table 3).

Table 3 Means for different concentrations of polyethylene glycol-6000

PEG (g)	Means of parameters on four concentrations						
	GP%	RL	CL	PL	RFW	CFW	PFW
0	92.40	18.42	3.880	9.573	0.1505	0.1232	0.1365
39	92.13	20.54	4.170	9.770	0.1433	0.1371	0.1596
78	91.02	19.30	4.413	9.106	0.1314	0.1429	0.1281
117	88.53	17.63	4.102	8.078	0.1083	0.1165	0.0986

Table 4 Means for wheat genotypes × Aluminum chloride interaction

AlCl (g)	GP%	IRL	FRL	NRL
0	61.38	8.87	5.74	3.14
0.007	77.72	7.35	5.85	1.50
0.014	62.44	6.85	4.63	2.22
0.021	60.65	5.76	4.30	1.62

Interaction of PEG concentrations and wheat genotypes

Analysis of variance (Table 1) showed the interaction between PEG-6000 concentrations and the genotypes to be significantly different ($P<0.05$) for germination percentage and plumule fresh weight, and highly significant difference ($P<0.01$) for coleoptiles fresh weight, coleoptiles length, plumule length, radical fresh weight and radical length. At the highest PEG-6000 concentration of 117g (-1.5 bars), a drastic reduction of 88.5% in germination percentage was realized from the results (Table 4). The germination percentage in distilled water varied from 80% to 100% in all genotypes except Gariep which did not perform well in all the treatments, thus; 43% at 0g, 36.7% at 39g (-0.5 bars), 16.7% at 78g (-1.0 bars) and 6.7% at 117g (-1.5 bars). The evidence from varying response of wheat genotypes against various concentrations of PEG-6000 was also observed. Furthermore, it was realized that an increase in concentration led to a decrease in germination percentage. For instance, SST 317 at 39g (-0.5 bars) had 96.7% germination. At 78g (-1.0 bars), germination percentage was 93.3% while at 117g (-1.5 bars) 90% germination was obtained. The responses of wheat genotypes against varying PEG-6000 levels were different depending on each concentration. From (Table 4) of the results, the longest radical length of 22.03cm was obtained from PAN 3471 while the shortest length was obtained from Gariep with 12.50cm. At control, Senqu had the longest radical length of 22.10cm. Where PEG-6000 39g (-0.5 bars), 78g (-1.0 bars) and 117g (-1.5 bars) were applied, these genotypes had the longest radical length; SST 317 with 25.20cm, PAN 3497 with 23.2cm and SST 806 with 21.1cm, respectively. Radical fresh weight on (Table 4) indicated the highly significant difference among the wheat genotypes. Duzi obtained more weight than other genotypes with 0.25g while the lowest was Gariep with 0.07g from (Table 3). From (Table 1) of the results, high significant difference on the coleoptiles length was noticed. The genotypes that managed to obtain the longest length include PAN 3368 and SST 8154 with 5.38cm and 3.20cm, respectively (Table 4). Coleoptiles fresh weight was high with SST 347 having 0.20g and SST 845 having 0.08g. PAN 3368 measured the longest length of 11.95cm while the shortest length was obtained of 7.01 cm on Gariep. Plumule fresh weight was high on SST 347 with 0.16g and the least was SST 8154 with 0.08g.

Ranking

The genotypes were ranked according to their tolerance to Al stress (Table 5). The genotypes were designated 1 to 41 according to the manner in which they followed each other based on their tolerance and susceptibility. Tungela DN was designated 1, followed by PAN 3515, PAN 3541, and SST 317. The moderately susceptible genotypes include: Elands, SST 356, SST 8156 and Koonap. The genotypes that were susceptible appeared down on the rank and they include: Kougas, Duzi, Matlabas and SST 387.

Variability in wheat genotypes

The 41 wheat genotypes responded differently to four levels of stress induced by Al concentrations as measured by four parameters, being; germination percentage, initial root length, final root length and net root length. In wheat, although some studies have used soil experiments in glasshouse or growth chambers [27], most of the reports assessing Al stress response have used hydroponic culture. The advantages in using hydroponics are the isolation of the Al stress, greater control of environmental conditions and easy observation of the roots. Like in this study, the roots were easily assessed. On (Table 3) of the results, Aluminium toxicity seemed to have high impact on the lines tested because the longest mean root (NRG) was observed on SST 884 with 4.52 cm and PAN 3623 with 3.48 cm while the shortest length was obtained from Gariep with 0.08 cm and PAN 3195 with 0.30 cm. At soil pH values of 5 or below, toxic forms of Al are solubilized and excess levels of toxic Al inhibit root growth and function [28]. The primary and earliest symptom of Al toxicity is a rapid inhibition of root elongation [29], and thus crops suffering from Al toxicity are assumed to be at greater risk of drought stress due to limited root development [30]. So, the tolerant genotypes may be an option under the aluminium toxicity stress. Al tolerance is an Al resistance mechanism in which Al ions are sequestered and detoxified in sub-cellular compartments and translocated away from the root tip [31].

Table 5 Ranking of genotypes with different concentrations

Number	Genotype	0.007g	0.014g	0.021g1	Ranking
1	SST 387	3	3	3	41
2	Matlabas	3	3	3	40
3	Duzi	2	3	3	36
4	Kouglas	1	3	3	25
5	SST 347	3	-	3	39
6	PAN 3471	1	2	3	24
7	PAN 3400	1	2	3	23
8	SST 866	1	2	3	22
9	Gariep	3	-	-	38
10	PAN 3195	3	-	-	37
11	SST 3149	2	2	2	35
12	PAN 3644	2	2	2	34
13	Kubetu	2	2	2	33
14	SST 8135	2	2	2	32
15	SST 8154	2	2	2	31
16	Elands	2	2	2	30
17	SST 356	2	2	2	29
18	SST 895	2	2	2	28
19	SST 8156	2	2	2	27
20	Koonap	2	2	2	26
21	Senqu	1	2	2	21
22	PAN 3515	1	2	2	20
23	Krokodil	1	2	2	19
24	SST 843	1	2	2	18
25	SST 875	0	2	2	5
26	SST 374	0	2	2	4
27	PAN 3368	1	1	2	16
28	PAN 3623	1	1	2	15
29	SST 316	1	2	1	17
30	SST 398	1	1	2	14
31	Sabie	1	1	2	13
32	PAN 3111	1	1	1	12
33	SST 806	1	1	1	11
34	SST 884	1	1	1	10
35	PAN 3161	1	1	1	9
36	Wedzi	1	1	1	8
37	SST 835	1	1	1	7
38	SST 317	1	1	1	6
39	PAN 3497	0	1	1	3
40	PAN 3541	0	1	1	2
41	Tangela DN	0	-	0	1

PEG-6000 concentrations

Drought is the major threat in crop production. The water deficit results in the reduction of seedling growth, which is a critical factor for crop productivity [32]. The exposure of crops to drought stress affected most of the processes. The stomatal closure is considered as an early plant response to drought that allows relative water maintenance [33] and consequently reduces photosynthesis [34]. The scientists have devised drought simulation methods for screening genotypes that may be able to withstand drought conditions. A PEG-6000 method is the most popularly used to induce stress and help to screen the genotypes [35]. PEG-6000 is a widely used chemical compound and maintains lower osmotic potential at a comparatively lower temperature under hydroponic culture [36].

The results of this study indicated a decrease in germination percentage with increasing in PEG-6000 concentration. At control, the germination percentage was high. It was followed by PEG 39g and 78g. The least germination percentage was found in PEG 117g. The results were consistent with Datta *et al.* [37] who assessed the tolerance of wheat genotypes under laboratory conditions and observed that there was a declining pattern in water uptake by seeds. High concentration of PEG might hamper the process of water uptake by seeds and thereby inhibiting the process of seed germination because the enzymes and hormones excretion may consequently be disordered [38]. Seed germination is a mechanism, in which morphological and physiological alterations result in activation of the embryo [39]. Before germination, seed imbibes water which will make it swell and activate enzymes. The enzymes will in turn act upon stored compounds to release energy required to trigger embryonic growth, consisting of radicle and plumule. This is a sensitive stage for water deficit, hence seed and seedling parameters are used as indicators for water deficit tolerance and susceptibility. When the radical has grown out of the covering seed layers, the process of seed germination is completed [40].

Radical length and plumule length were long at the concentration of 39g (-0.5 bars) and decreased with an increase in PEG concentrations. The shortest lengths were found at the concentration of 117g (-1.5 bars). Similar results were found by Kaya *et al*. [41] when examining the different NaCl concentrations on germination, radical length and plumule length of clover genotypes where the relevant traits decreased with the increase in salt concentrations. The results are also supported by the findings of Oz *et al.* [42]. However, this may be an adaptive strategy of seed to prevent germination under stressful environment for ensuring proper establishment of seeds. Drought stress decreases with an increase in root growth rate in comparison with the growth of the aerial parts of the plant [43]. The reduction in longitudinal growth of shoot and root length growth are primary mechanisms in the face of stress. It is evident that, high root development under drought stress enables plant to reach deeper available water in the soil, hence survive to maturity [44]. Plumule fresh weight was also high at the concentration of 39g (-0.5 bars).

Coleoptile is defined as the protective sheath covering the emerging shoot. They do not divide but increase in size as they accumulate more water. Studies show that having long coleoptiles improves seedling establishment under drought stress and is considered as a major factor in plant production [45]. The results of the present study showed the better performance of the genotypes in coleoptiles length and coleoptiles fresh weight where the genotypes were treated with the PEG concentration of 78g (-1.0 bars). The similar coinciding results were also been reported by Alaei *et al.* [46] who was evaluating the germination characteristics of different durum wheat genotypes under osmotic stress. They discovered that the genotypes showing osmotic resistance may also be drought tolerance and may therefore survive drought stress with substantial production in terms of quality and yield. Movement of water and nutrients will not be hindered.

Interaction of Aluminium concentrations with wheat genotypes

The factors that were under study were genotypes and A1 concentrations. There was a highly significant interaction of genotypes with A1 concentrations ($P < 0.05$). Different genotypes showed diverse response for seed germination. Many genotypes started germinating the third day after sowing. Except from control, the seeds were exposed to mild stress (0.007g) but germination percentage increased. It showed that,

seeds still germinate well in the mild stress. The stress was increased to 0.014g where a slide decrease in germination was realized. The seeds were further exposed to increased levels of Al (0.027g) therefore, highly significant germination percentage was recorded and this indicated that, Al stress may still be a threshold value for good germination (Table 4). Some genotypes including: Gariep and SST 3149 decreased significantly when the concentration was increased.

The analysis of variance over initial root growth, final root growth and net root growth revealed the presence of highly significant ($P>0.05$) differences. The expressions of mean performance of these seedling traits were higher in control than in stress exposed ones. Root length decreased with increasing stress (concentrations) and these was in accordance with Szabo-nazy *et al*. [47], who followed the in vivo effect of soluble Al^{3+} form on winter wheat for two weeks**.** The sharp decrease was observed at 0.014g of Al. It was of great interest to compare the results of Iqbal [48] who studied on the effects of elevated Al and pH on the growth and root morphology of Al tolerant and Al sensitive wheat seedlings in an acid soil. The results indicated that, the number of root tips reduced as Al application increased. Based on the results obtained, the concentration of 0.014g may be used to discriminate the varieties.

Ranking

The general ranking of wheat genotypes for Al tolerance was previously found by [49-50] when the genotypes were screened with different methods being: solution culture and hematoxylin. The seedlings differing in Al tolerance were differentiated by staining with hematoxylin after exposures to Al stress. Al sensitive seedlings exposed to Al solution and stained with hematoxylin showed the strains at the root apices and within the root tissues, whereas Al tolerant seedlings treated in the same way showed little staining. Al tolerant and Al sensitive genotypes were identified by hematoxylin staining before differences in root elongation were taken. Because hematoxylin is nondestructive to root apices, the stained seedlings continued to grow after transfer to solutions without Al, indicating that the seedlings were not adversely affected by the hematoxylin staining. The seedlings were then separated according to their hematoxylin staining pattern.

The results of the test genotypes gave varied results. It is widely accepted that Al tolerance in wheat is species and genotype dependent as reported by Jones and Ryan [51]. Some genotypes were highly susceptible while others were highly tolerant to induced Al stress. Besides, there were some that were moderate. For instance, the genotypes that showed tolerance to Al toxicity stress were Tungela DN, PAN 3515, PAN 3541, and SST 317. The moderately susceptible genotypes were: Elands, SST 356, SST 8156 and Koonap (Table 5). The genotypes that were susceptible were: Kougas, Duzi, Matlabas and SST 387. The most tolerant genotypes could be used when drought prevails so that a substantial yield can be obtained. The ranking of genotypes to Al tolerance can be correlated with severity of drought that brings about stresses.

CONCLUSION

The present investigation attempted to measure Al stress on different wheat genotypes. This investigation compared roots cultured in the presence or absence of toxic levels of Al. The Al cultured roots were severely stunted and gross anatomical lesions was apparent after using haematoxylin. The hematoxylin staining, helped to analyze the total Al in the root apices, and microscope analysis all provided evidence that the methods that we used for detecting Al in root apices were complementary and present a picture of Al uptake and distribution in root apices. Some of the difference observed in Al uptake could be explained by differences in root growth, since Al tolerant roots continued to grow in the presence of Al and would have effectively diluted the Al in apices. Assessing the results, PAN 3541 and PAN 3111 were among the best performing variety but were susceptible to Al toxicity stress. Tungela DN was a poorly performing variety but it was able to tolerate Al stress. SST 317, SST 843, PAN 3623 was also considered as the tolerant varieties. Based on the findings, the concentration of 0.014g worked best for most of the varieties and is considered as the standard concentrations.

Recommendations

The varieties that managed to outperform others in face of Al toxicity stress may be recommended for further developments if yield wise were not performing well. Those that performed well under Al stress are recommended as the best varieties that may be grown under the changing environmental conditions and they include SST 843 and SST 317.

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Conflict of interest

In this research, there was no conflict of interest by authors and funders alike. All had expressed interest of seeing the University conducting scientific research and publishing the results of their findings, thus increasing popularity of an institution. Besides, the University wants to see academicians presenting findings where there is a fraternity of scientists such as symposium and conferences.

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