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Hydroponic Optimization and Screening of Aluminum Tolerance on Finger Millet (*Eleusine coracana* (L.) Gaertn.) Accessions and Cultivars

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Abstract: Finger millet (*Eleusine coracana* (L.) Gaertn.) is an annual allotetraploid that belongs to the grass family Poaceae subfamily Chloridoideae. Using less productive cultivars, biotic and abiotic stresses affect the yield and productivity of finger millet in Ethiopia. This research was aimed at investigating the acidity/Al tolerance of 328 finger millet accessions and 15 cultivars from Ethiopia and Zimbabwe. Prior to screening the accessions, optimization was performed on 15 cultivars and 15 accessions under three Al concentrations (0, 75, and 100 µM), and, afterward, 100 µM of Al concentration was selected as the threshold level. Root length (RL) and shoot length (SL) were recorded after 10 days of treatment. Accessions 215836, 215845, and 229722 and cultivars Urji, Bareda, and Axum were found Al-tolerant, while cultivars Tadesse, Padet, and Kumsa and accessions 212462, 215804, and 238323 were found Al-susceptible. ANOVA on RL indicated that the variance due to environment (42.3) was higher than genotypic variance (0.37). Whereas, the ANOVA on SL indicated the variance due to environment was not significant, and genotypic variance (0.18) was higher than environmental (0.02). RL was highly affected due to Al stress, while no distinct and visible symptoms were observed on SL. Furthermore, the screening of 328 accessions under 100 µM and the control resulted in Al-tolerant (n = 20), intermediate (225), and Al-susceptible (83). The results of the present study reveal that the presence of acid-tolerant accessions can be used as inputs for breeders to improve the productivity of finger millet in acidic areas.

Keywords: Al tolerance; finger millet; hydroponic; optimization; root length

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1. Introduction

Finger millet (*Eleusine coracana* (L.) Gaertn.) is an annual, self-pollinating, allotetraploid (2n = 4x = 36; with AABB genome and 1593 Mb genome size), food and feed cereal crop belonging to the grass family *Poaceae* [1]. Studies have indicated that finger millet originated in tropical and subtropical parts of Africa, particularly in Ethiopia and Uganda, and it spread to India probably more than 3000 years ago [2–4]. In Ethiopia, finger millet is produced by small-scale farmers in Tigray, Wellega, Illuababora, Hararghe, Gonder, Gojjam, Gamo-Gofa, and Hossana [5,6].

In Ethiopia, finger millet is the sixth most important cultivated cereal crop after teff, wheat, maize, barley, and sorghum [7]. Grain of finger millet is rich in protein, minerals, dietary fiber, calcium, iron, and essential amino acids; it is also gluten-free, and has health-promoting benefits such as hypoglycemic, anti-hypocholesterolemia, and anti-ulcerative effects [8]. Finger millet is often mixed with other grain crops such as sorghum, maize, or teff to make composite flour for local food preparation such as cake, injera, porridge, and traditional local alcohols [9,10].

Even though it is a nutritionally important and environmentally resilient crop, its current productivity is low, i.e., 2.76 t ha^{-1}. This might be due to a shortage of improved cultivars, or to drought, blast, soil salinity, soil acidity, or moisture stress, as well as a poor attitude toward the crop [11,12]. Among the challenges, soil acidity is the most limiting factor to finger millet production in different parts of Ethiopia. This limitation can be reduced by developing finger millet cultivars, which are more tolerant or resistant to acidic soils.

Soil acidity is a plant growth limiting factor affecting the yield of many crops all over the world. It has been estimated that 50% of the world and over 43% of Ethiopia's potentially arable lands are acidic [13]. Among the 43% soil acidity, 27% of the arable lands are strongly acidic (pH < 5). The excessive presence of toxic compounds such as Al, Fe, and Mn and a deficiency in phosphorus are the challenges for acidic soils. Among these factors, Al toxicity is the main factor that affects yield and crop productivity, especially in developing countries relying on agriculture to feed their populations [14,15]. In the soil, at a low pH, Al changes into soluble form and affects plant growth [16]. Using inorganic fertilizers instead of using compost, the leaching of nitrogen below the plant root zone, and the accumulation of inorganic matter, together with natural processes such as flooding and acid rain, are factors that can increase soil acidity [17,18]. At neutral and basic soil pH conditions, a large amount of Al is incorporated into aluminosilicate soil minerals and becomes unavailable for plants, while at a low pH, Al becomes available for plants, and it inhibits root growth by inducing oxidative stress, affecting nutrient uptake, peroxidation of the cellular membrane, and reduces water and nutrient absorption [19].

To decrease soil acidity, the Ethiopian government has embarked on a massive soil reclamation program. Liming of the soil combined with the application of inorganic fertilizer has improved the quality of the topsoil to some extent, but this approach was found to be too expensive to be sustainable in the long term or even attainable in the short term for subsistence farmers [20]. Given the limited access of most farmers to phosphate fertilizers as well as liming services in Ethiopia, it is necessary to increase the production of crops such as finger millet in acidic soils in an environmentally friendly and sustainable manner. Arable lands in western and southern parts of Ethiopia such as Ghimbi, Nedjo, Hossana, Chencha, Sodo, Gozamin, Senan wereda, and Hagere-Mariam are predominantly covered by strong to weak acid soils [21].

Hydroponic-based screening of Al tolerance is preferred for stress-related research because it uses water and fertilizer efficiently. Hydroponic systems are suitable for early growth and seedling screening under submerged conditions. According to [22], relative root length (RRL) and relative shoot length (RSL) are better indicators of root growth under Al stress, as they can eliminate genotype-specific differences in root growth and normalize comparisons between genotypes. Since RRL and RSL are the relative growth of the genotype in Al solution compared with its potential growth without Al, this parameter is a real measure of Al tolerance [22]. Various findings have confirmed that hydroponic conditions are suitable for screening against Al stress because there are no soil-related challenges such as disease, salinity, and acidity in finger millet [23], wheat [24,25], rye [26], and chickpea [27]. The aim of this research was therefore to optimize the threshold level of Al tolerance in finger millet accessions and cultivars under different Al concentrations and to conduct the rapid screening of more accessions at the threshold level and control under hydroponic conditions.

2. Materials and Methods

2.1. Plant Materials and Germination Conditions

A total of 328 accessions representing various agro-climatic zones of Ethiopia and Zimbabwe were obtained from the Ethiopian Institute of Biodiversity (EIB, Addis Ababa, Ethiopia), and 15 cultivars were obtained from the Bako Agricultural Research Center (BARC, Bako, Ethiopia). All accessions were selected randomly from the gene bank and used in this study (Supplementary Table S1). Optimization was performed on selected 15 cultivars and 15 accessions. There are only 16 cultivars of finger millet in Ethiopia. We collected and used all cultivars except Diga-02, which failed to germinate and was omitted from the study. The 15 accessions were selected randomly from the 328 accessions. Similar size seeds and similar seed color (n = 15) from each accession were selected and surface-sterilized by soaking in 3% sodium hypochlorite solution for 5 min and rinsing thoroughly with water. Sterilized seeds of each accession were wrapped and germinated in tissue paper, and then moistened with distilled water in separated Petri dishes for 36 h under dark conditions for later use as the germinated seedlings in hydroponic experiments. Then, the seedlings were transferred to hydroponic nutrient solution and treated for about 10 days within the greenhouse adjusted to a temperature of 18 °C and a humidity level of 65% at the Swedish University of Agricultural Sciences (SLU, Alnarp, Sweden).

2.2. Hydroponics Experimental Setup

The basic assumption for setting up the equipment for the hydroponic screening was that the system should enable growth and development of seedlings while ensuring the seeds and later seedlings had maximal exposure to Al stress. This requirement can be realized only under submerged conditions, which demands a mechanism of aerating the seedlings within the nutrient solution. For this purpose, dense narrow holes were introduced into small centrifuge tubes (5 mL) in such a way that the holes did not allow finger millet seeds to pass through but allowed air bubbles in for aerating the seedlings in the tube. Continuous aeration was supplied by an aquarium air pump with an air stone. A rack-like plate to hold the perforated tubes was prepared from a jar plastic plate having wide holes capable of holding and submerging tubes in the nutrient solution (Figure 1).

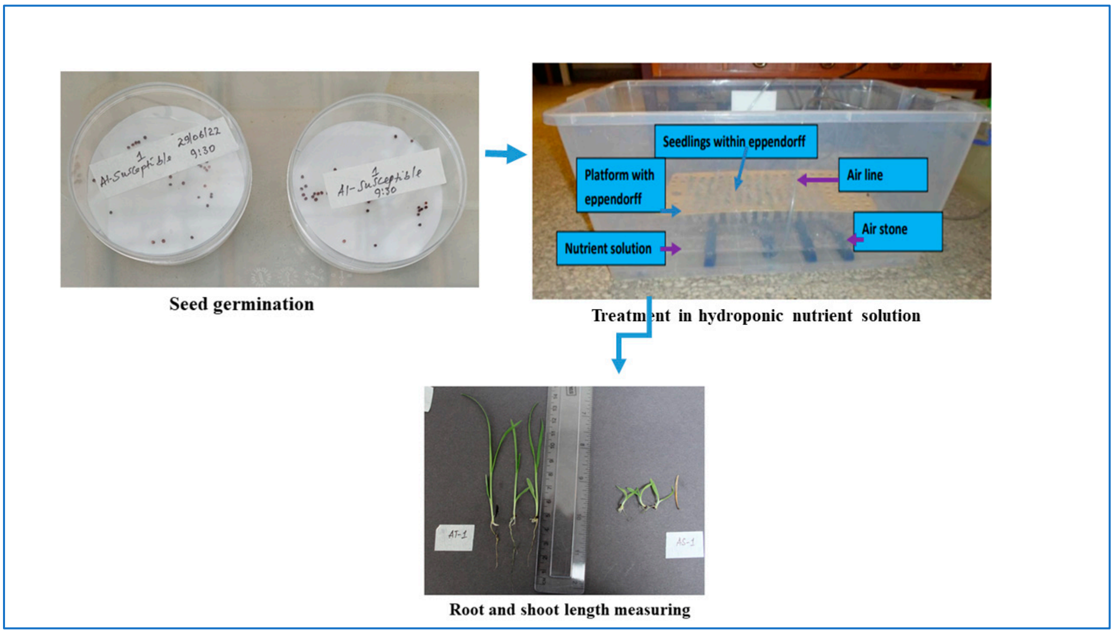

Figure 1. Overview representation of the hydroponic nutrient solution treatment including seed germination, nutrient solution preparation, treatment under hydroponics, and data recording.

2.3. Nutrient Solution Culture and Treatment

The nutrient solution culture was prepared according to [22] and composed of 500 µM KNO_3, 500 µM $CaCl_2$, 500 µM NH_4NO_3, 150 µM $MgSO_4.7H_2O$, 10 µM KH_2PO_4, 2 µM $FeCl_3$ (III), and different concentrations of Al_2 $(SO_4)_3$. In vitro-germinated seedlings (n = 10) of each accession with similar root lengths were transferred into the perforated tube, which was then arranged on plastic plate, and seedlings would be in full contact with the growth solution but would not be fully submerged. The control experiment was performed side by side with each treatment and composed of all the above nutrients except Al_2 $(SO_4)_3$. The pH of the nutrient was adjusted to 4.3 by using 1 M HCl or NaOH and the solution was renewed every day (24 h) in order to refresh the detoxified solution and ensure continuous exposure of the seedlings to Al ions. The seedlings were treated for consecutive 10 days under hydroponic nutrient solution. After 10 days, root length (RL) and shoot length (SL) were measured from five seedlings per accession.

2.4. Screening of Accessions under Hydroponic Assay

To find the threshold level of Al tolerance in finger millet, optimization on different Al^{3+} concentrations (0, 75, and 100 µM) was performed on 15 cultivars and 15 accessions in the hydroponic nutrient solution. The two Al concentrations (75 and 100 µM) were selected by considering the optimization protocol we developed previously [23]. After the threshold level of tolerance was decided (100 µM Al_2 $(SO_4)_3$), a large number of landraces (n = 328) were evaluated in the hydroponic system. Based on their RRL, the accessions were classified into three tolerance groups. Accessions grouped as Al-tolerant were those that had RRL $\geq$ 80%, whereas intermediates were between 80% and 20%, and susceptible were those below 20%.

2.5. Data Recording and Analysis

The root length (RL) of five seedlings per accession was measured from the base of the cotyledon to the tip of the roots, and shoot length (SL) was also measured from the base of the cotyledon to the tip of the shoot using a ruler. The normality of data collected from the hydroponic data was tested using R software. Analysis of variance (ANOVA) was performed using aov function in R software. Pairwise mean comparison was performed using Tukey test in R software. Root growth parameters such as relative root length (RRL) and relative shoot length (RSL) were estimated as described in [22]:

$$\text{RRL}(\%) = \frac{\text{Root length under treatment}}{\text{Root length under control}} * 100\% \quad (1)$$

$$\text{RSL}(\%) = \frac{\text{Shoot length under treatment}}{\text{Shoot length under control}} * 100\% \quad (2)$$

3. Results

3.1. Optimizing Threshold Level of Al-Toxicity on Finger Millet

We used morphological markers, RL and SL, to compare the Al tolerance of seedlings grown under control and Al-stress conditions. The dose–response experiment showed that finger millet accessions and cultivars grown under lower Al concentrations had higher RL than those treated with a relatively high level of Al concentration. In the control experiment, the highest RL was found in cultivar Tessema (2.26 cm) followed by Tadesse (1.98 cm), whereas the 203314 (0.58 cm), 215888 (0.66 cm), 203322 (0.68 cm), and Bareda (0.70 cm) had short root lengths. At 75 µM, Al-concentration 215897 (0.78 cm) and 215910 (0.80 cm) accessions had the longest RLs, whereas cultivars Padet, Kumsa, and Urji with 0.10 cm each had short RLs. At 100 µM Al-concentration, the top performing accessions were 228901 (0.56 cm), 215910 (0.40 cm), 243644 (0.38 cm), and 215897 (0.38 cm), while Tadesse (0.10 cm), Padet (0.10 cm), and Kumsa (0.10 cm) were the least performing cultivars

(Figure 2). Overall, RL-based evaluation showed that the landraces perform better than the cultivars in Al-stress conditions (Figure 2).

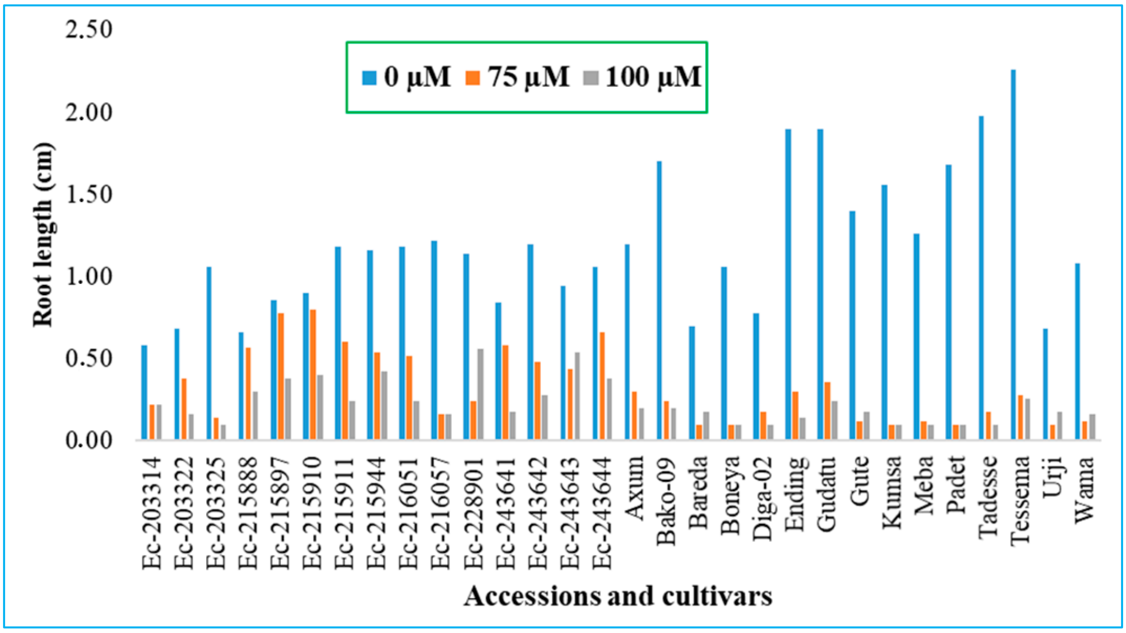

Figure 2. Histogram plot showing root length (cm) of finger millet accessions and cultivars grown at three (0 µM, 75 µM, and 100 µM) Al concentrations under hydroponic nutrient solution.

Shoot length (SL) of the accession and cultivars grown under control (0 µM Al-concentration) varied from 0.46 cm (Urji) to 1.18 cm (215888 and 215911). At 75 µM Al-concentration, SL ranged from 0.66 cm (215897) to 1.07 cm (243642), and at 100 µM Al-concentration SL ranged from 0.45 cm (Meba) to 1.64 cm (213314) (Figure 3). The effect of Al stress on shoots of finger millet was not observed at Al concentrations of 75 µM or 100 µM.

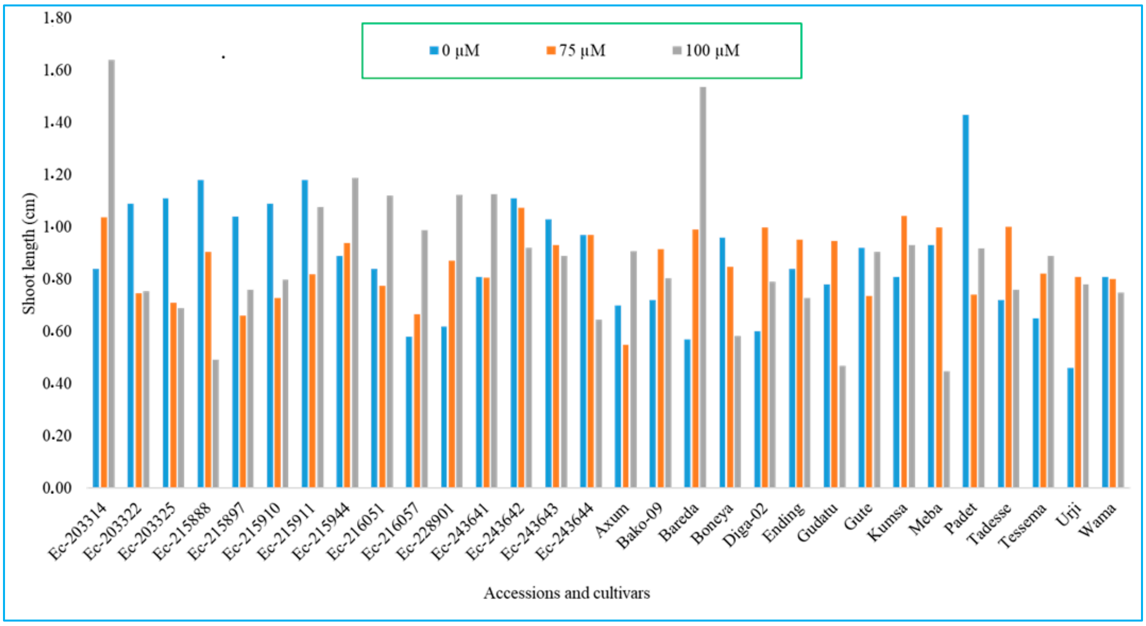

Figure 3. Histogram plot showing shoot length (cm) of finger millet accessions and cultivars grown at three (0 µM, 75 µM, and 100 µM) Al concentrations under hydroponic nutrient solution.

Analysis of variance (ANOVA) on RL indicated significant differences between finger millet accessions grown at 0 µM, 75 µM, and 100 µM Al-concentrations (Table 1; Figure 4). ANOVA on RL indicated that the variance due to environment (42.3) was higher than genotypic variance (0.37) and variance due to replications (0.15). Whereas the ANOVA on SL indicated that the variance due to environment and replication was not significant, and genotypic variance (0.18) is higher than environmental (0.02) and replication variance (0.04).

Table 1. Analysis of variance (ANOVA) of the accessions and cultivars grown at different Al concentrations (0 µM, 75 µM, and 100 µM).

Source	DFZ	MS	
		Root Length	Shoot Length
Concentration	2	8.5 ***	0.003 ns
Residuals	87	0.08	0.04
Environmental variance	2.00	42.03 **	0.02 ns
Replication variance	12.00	0.15 **	0.04 ns
Genotypic variance	29.00	0.37 **	0.18 **
Genotypic X Environment	58.00	0.46 **	0.26 **
Residuals	348.0	0.05	0.10

Key: DF^Z = degree of freedom, MS = mean of squares, ns = not significant; *** significant at $p < 0.001$, and ** significant at $p < 0.01$.

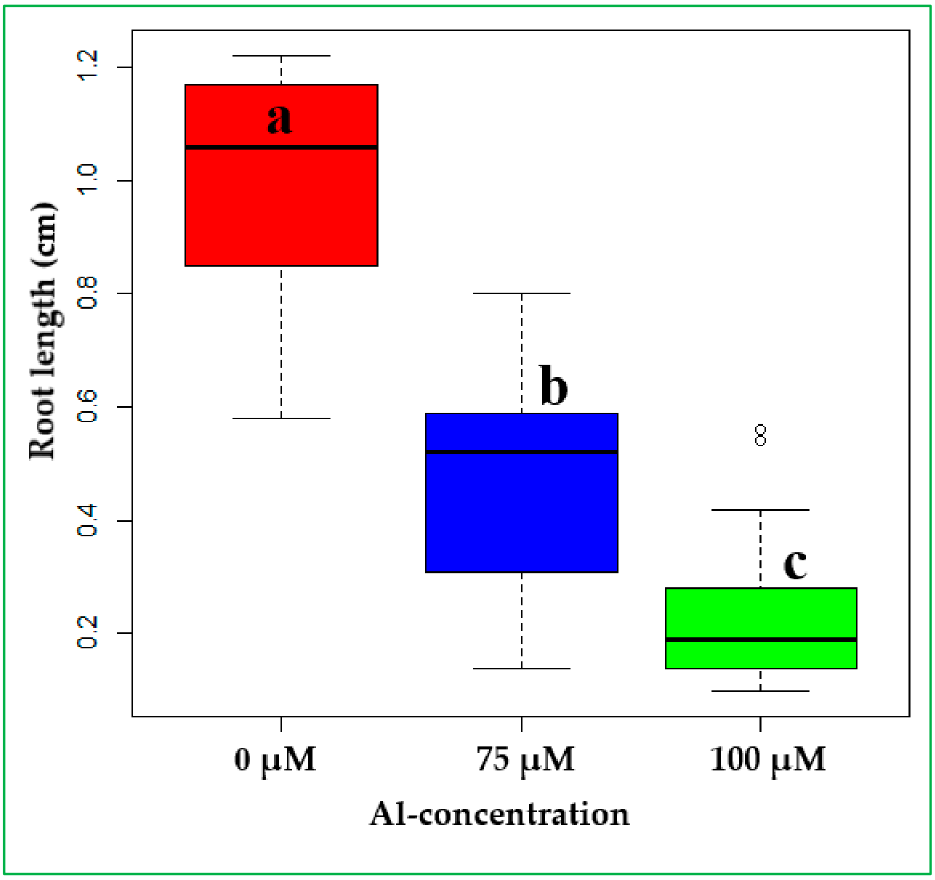

Figure 4. Boxplot showing effect of different Al concentrations (0 µM, 75 µM, and 100 µM) on plant root length. *X*-axis indicates different Al concentrations and *Y*-axis indicates upper and lower mean values of the 15 accessions and 15 cultivars at each Al concertation. Box plots with the same letter are not significantly different from each other.

Finger millet accessions and cultivars grown under the hydroponics displayed three distinct Al-tolerance phases in different Al concentrations. A high phase tolerance was observed between 0 and 75 µM, slight tolerance at 100 µM, and an intolerance phase above 100 µM. This indicates that low Al concentrations were not strong enough to create stress conditions on finger millet root, and high Al concentrations above 100 µM inhibit growth in all finger millet varieties without discrimination. Therefore, the 100 µM Al-concentration was selected as the threshold concentration for extensive screening activities due to its multiple advantages. Firstly, it allows for the distinguishing of the various tolerance classes (tolerant, intermediate, and susceptible) at the highest accuracy level (that is, $p < 0.01$, unlike the lower concentration levels). At Al concentrations above 100 µM, the growth of roots of all the varieties was greatly hampered to the extent that there were nearly no differences among them. Therefore, 100 µM was selected as the optimum Al concentration for the screening of 328 finger millet accessions.

3.2. Screening Finger Millet Accessions

Finger millet accessions (n = 328) were rapidly screened after initially deciding the optimum Al concentration (i.e., 100 µM). There were observable differences among individuals within an accession such as variation in grain color and grain size, and to take this heterogeneity into account, each accession was evaluated systematically by recording data from similarly performing individuals. There were significant variations among accessions grown at 100 µM Al-concentration. The RRL of tolerant accessions ranged from 79.4% (245084) to 127.9% (215836), while the most susceptible accessions had an RRL of less than 10% (215804, 212462, and 238323) (Tables 2–6). There was a significant difference between the most extremely tolerant accession 215836 with 127.9% RRL and the most susceptible accession 215804 with 7.1% RRL (Figure 5). Among the total accessions screened, 20 of them were better performing and grouped as Al-tolerant (Table 2), while 225 of them were grouped as intermediate (Tables 2–5), and 63 of them were highly susceptible to Al-stress and least perform and grouped as Al-susceptible (Tables 5 and 6).

Figure 5. Examples of seedlings grown for 10 days under 100 µM Al^{3+}-containing nutrient solution. Accession 215836 was scored as tolerant, and accession 215804 was scored as sensitive.

Table 2. Relative root length (RRL), relative shoot length (RSL), phenotypic class (PC), tolerant (T), and intermediate (I) performance of 20 tolerant and 55 intermediate finger millet accessions (Acc.) grown under control and 100 µM Al^{3+}-concentration.

No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC
1	215836	127.9	98.2	T	26	215847	71.9	126.7	I	51	216031	57.6	105.4	I
2	215845	120.9	110.0	T	27	215896	71.4	132.5	I	52	242118	57.1	59.2	I
3	229722	113.3	106.7	T	28	238306	70.0	81.8	I	53	215883	56.6	103.6	I
4	215919	107.0	41.9	T	29	215840	69.8	95.1	I	54	242610	55.9	44.6	I
5	216023	101.8	58.6	T	30	203336	69.8	140.0	I	55	238299	55.6	135.4	I
6	207962	97.8	66.7	T	31	237443	68.9	91.5	I	56	215936	54.9	50.0	I
7	215905	93.9	47.2	T	32	215996	68.3	55.9	I	57	238342	54.8	94.5	I
8	203356	92.5	48.3	T	33	203364	67.7	91.7	I	58	240506	54.7	64.4	I
9	216055	91.9	25.0	T	34	212134	67.3	51.7	I	59	203314	54.2	68.7	I
10	215875	90.9	47.9	T	35	203343	66.7	47.0	I	60	234147	53.6	61.5	I
11	215994	90.3	43.8	T	36	225893	65.9	70.8	I	61	215888	53.3	136.6	I
12	215841	90.0	103.8	T	37	242621	64.7	70.0	I	62	215914	52.6	59.3	I
13	216034	86.1	68.1	T	38	235156	64.4	23.6	I	63	237971	52.6	133.3	I
14	216027	84.5	83.3	T	39	215930	64.3	56.1	I	64	215860	52.0	85.9	I
15	100093	82.9	96.5	T	40	215945	63.5	53.7	I	65	238317	51.1	56.6	I
16	215906	81.4	89.6	T	41	245086	62.9	70.9	I	66	229724	51.0	84.0	I
17	215852	80.9	47.5	T	42	215831	62.1	39.6	I	67	208725	50.8	55.7	I
18	215868	80.6	115.1	T	43	208730	61.8	46.9	I	68	215871	50.0	90.3	I
19	215827	80.4	47.5	T	44	243643	60.5	91.9	I	69	215944	50.0	69.0	I
20	245084	79.4	50.7	T	45	242116	60.3	29.8	I	70	203368	50.0	83.6	I
21	215910	78.9	64.7	I	46	237970	60.0	62.6	I	71	215902	50.0	85.1	I
22	219827	75.5	37.0	I	47	215957	59.6	75.5	I	72	215880	50.0	58.9	I
23	242114	75.0	51.7	I	48	215877	59.0	34.2	I	73	203311	49.2	41.7	I
24	215942	74.1	31.1	I	49	208445	58.6	63.4	I	74	215933	49.1	103.8	I
25	216030	73.8	59.7	I	50	215829	58.0	100.0	I	75	216025	49.0	53.3	I

Table 3. Relative root length (RRL), relative shoot length (RSL), phenotypic class (PC), and intermediate (I) performance of 75 finger millet accessions (Acc.) grown under control and 100 μM Al^{3+}-concentration.

No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC
76	211553	48.3	88.0	I	101	215870	41.9	90.2	I	126	215927	37.2	85.4	I
77	211506	48.3	49.0	I	102	203363	41.2	38.3	I	127	215890	36.7	116.4	I
78	215886	47.5	92.0	I	103	208444	41.2	110.5	I	128	215961	36.2	56.9	I
79	243635	47.5	56.7	I	104	242135	41.0	85.4	I	129	215943	36.0	100.0	I
80	227973	47.4	52.2	I	105	225896	40.9	50.0	I	130	215865	35.9	83.3	I
81	208448	47.3	67.2	I	106	215952	40.7	40.0	I	131	237972	35.8	144.0	I
82	203312	47.1	93.0	I	107	216041	40.5	66.7	I	132	238321	35.7	41.0	I
83	203355	46.8	89.3	I	108	237456	40.3	128.3	I	133	208427	35.3	84.6	I
84	203353	46.7	92.9	I	109	238308	40.2	82.4	I	134	242121	35.3	36.0	I
85	238313	46.4	68.8	I	110	225894	39.8	48.4	I	135	215954	35.3	122.4	I
86	244798	45.8	103.6	I	111	215805	39.4	175.0	I	136	215913	35.0	112.2	I
87	215995	45.5	83.3	I	112	215837	39.3	50.0	I	137	228901	34.5	51.2	I
88	216050	45.5	68.4	I	113	203272	39.1	43.1	I	138	216049	34.5	54.8	I
89	215937	45.1	83.3	I	114	215893	38.9	79.6	I	139	216032	34.4	66.2	I
90	242110	45.1	141.9	I	115	237969	38.9	116.7	I	140	203345	33.3	88.1	I
91	203354	44.7	51.4	I	116	211504	38.7	107.0	I	141	220337	33.3	94.1	I
92	216026	43.3	70.4	I	117	203342	38.7	56.8	I	142	242613	33.3	52.9	I
93	215833	43.2	78.0	I	118	215932	38.6	75.8	I	143	230562	33.3	97.2	I
94	203322	42.9	106.0	I	119	238310	38.5	45.1	I	144	212694	33.3	95.8	I
95	216051	42.9	47.4	I	120	215869	38.3	54.2	I	145	215978	33.3	109.4	I
96	216042	42.6	126.1	I	121	216057	38.3	53.3	I	146	215986	32.6	37.9	I
97	208443	42.4	102.5	I	122	237973	38.2	54.0	I	147	216024	32.5	37.3	I
98	215842	42.4	55.7	I	123	203358	38.2	81.8	I	148	242637	32.3	69.8	I
99	216054	42.3	35.8	I	124	207460	38.1	51.9	I	149	219828	32.3	70.8	I
100	237458	41.9	110.5	I	125	242612	37.5	90.3	I	150	215928	32.0	120.8	I

Table 4. Relative root length (RRL), relative shoot length (RSL), phenotypic class (PC), and intermediate (I), performance of 75 finger millet accessions (Acc.) grown under control and 100 µM Al^{3+}-concentration.

No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC
151	245091	31.8	69.0	I	176	215803	29.0	62.0	I	201	242689	25.9	72.9	I
152	242117	31.7	93.0	I	177	215948	28.8	96.6	I	202	216028	25.9	53.1	I
153	203325	31.3	87.0	I	178	241769	28.6	43.4	I	203	203344	25.8	73.5	I
154	203374	31.0	59.3	I	179	215926	28.6	65.4	I	204	215918	25.8	72.0	I
155	208728	30.8	45.3	I	180	245087	28.6	104.8	I	205	234148	25.6	79.8	I
156	208441	30.8	78.2	I	181	203357	28.2	82.1	I	206	242108	25.5	38.8	I
157	215949	30.3	75.0	I	182	215889	28.2	98.1	I	207	242115	25.0	80.0	I
158	238312	30.2	110.0	I	183	337584	28.0	87.1	I	208	203377	25.0	71.4	I
159	203365	30.1	109.8	I	184	229721	27.9	56.3	I	209	215915	25.0	60.6	I
160	237583	30.0	22.4	I	185	215862	27.8	84.6	I	210	203352	25.0	59.2	I
161	215857	30.0	65.4	I	186	238316	27.8	69.9	I	211	237457	24.6	76.0	I
162	228202	29.7	75.7	I	187	207459	27.6	58.5	I	212	215959	24.6	71.7	I
163	215979	29.6	66.1	I	188	203388	27.6	77.3	I	213	242109	24.5	70.4	I
164	242624	29.6	78.9	I	189	242623	27.5	51.2	I	214	215863	24.3	37.7	I
165	242638	29.5	97.1	I	190	215854	27.3	68.1	I	215	219829	24.1	72.5	I
166	215941	29.4	72.6	I	191	216052	27.2	68.9	I	216	242622	24.1	45.1	I
167	215848	29.4	57.1	I	192	238319	27.1	86.8	I	217	216036	24.1	53.8	I
168	207963	29.4	54.4	I	193	203360	26.6	36.5	I	218	242119	24.0	67.6	I
169	228902	29.4	36.6	I	194	237447	26.5	24.0	I	219	238346	24.0	118.8	I
170	203386	29.4	62.2	I	195	215916	26.5	93.3	I	220	215861	23.8	100.0	I
171	215980	29.4	105.6	I	196	215938	26.3	44.7	I	221	215920	23.7	32.8	I
172	203340	29.4	75.0	I	197	242111	26.2	87.5	I	222	208726	23.3	88.7	I
173	215901	29.4	116.5	I	198	215838	26.1	98.1	I	223	230561	23.3	60.8	I
174	215903	29.3	68.0	I	199	216029	26.1	75.9	I	224	243641	23.1	88.0	I
175	203315	29.2	45.3	I	200	203335	26.0	49.1	I	225	215849	23.1	83.6	I

Table 5. Relative root length (RRL), relative shoot length (RSL), phenotypic class (PC), intermediate (I), and susceptible (S) performance of 20 intermediate and 55 susceptible finger millet accessions (Acc.) grown under control and 100 µM Al^{3+}-concentration.

No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC
226	215895	23.1	67.4	I	251	203369	20.588	67.606	S	276	203372	17.5	59.5238	S
227	208447	22.8	50.0	I	252	238307	20.588	82.278	S	277	215962	17.2	132.258	S
228	203328	22.7	101.6	I	253	215947	20.588	89.474	S	278	215985	17.1	73.7705	S
229	215856	22.7	64.3	I	254	215846	20	151.72	S	279	215940	17.1	61.0169	S
230	335141	22.6	128.0	I	255	215929	20	68.889	S	280	215908	17.1	78.5714	S
231	216020	22.2	69.3	I	256	223146	19.355	78.571	S	281	242112	17.1	76.0563	S
232	216046	22.2	100.0	I	257	238322	19.231	56.522	S	282	216021	16.7	64.7059	S
233	215859	22.2	135.4	I	258	215934	19.231	75	S	283	215993	16.7	76.5625	S
234	208729	21.7	54.5	I	259	203346	19.231	68.919	S	284	238460	16.7	86.1111	S
235	215843	21.7	55.6	I	260	203317	19.231	65	S	285	215894	16.3	84.1463	S
236	238300	21.7	84.4	I	261	219825	18.75	32.075	S	286	208442	16.3	82.5	S
237	235142	21.7	53.3	I	262	219832	18.667	47.826	S	287	203347	16.2	46.0526	S
238	242133	21.7	87.0	I	263	245092	18.548	61.905	S	288	243642	16.2	112.903	S
239	203371	21.6	54.4	I	264	216038	18.519	68.919	S	289	216039	16.1	123.077	S
240	211505	21.3	50.0	I	265	216035	18.519	35.294	S	290	203339	16.1	112.5	S
241	203327	21.3	37.7	I	266	242107	18.519	45.455	S	291	207964	15.6	108.333	S
242	215873	21.3	60.7	I	267	243640	18.519	88.406	S	292	238345	15.6	58	S
243	245088	21.3	101.9	I	268	215904	18.421	137.74	S	293	208440	15.56	70	S
244	215802	21.2	70.7	I	269	215834	18	103.92	S	294	242120	15.15	59.155	S
245	238343	21.1	151.7	I	270	216033	17.857	94.444	S	295	215911	15.15	136.36	S
246	243644	20.9	68.2	S	271	238320	17.857	82.474	S	296	215887	15.09	98.148	S
247	242132	20.8	43.1	S	272	203359	17.8	58.9744	S	297	245090	15	67.647	S
248	215832	20.8	59.3	S	273	215946	17.8	28.8462	S	298	215799	14.89	78.481	S
249	215867	20.7	35.8	S	274	235699	17.6	62.766	S	299	203318	14.81	78.667	S
250	242106	20.6	69.8	S	275	243623	17.5	70.2703	S	300	215872	14.46	36.585	S

Table 6. Relative root length (RRL), relative shoot length (RSL), phenotypic class (PC), and susceptible (S) performance of 33 susceptible finger millet accessions (Acc.) grown under control and 100 µM Al^{3+}-concentration.

No.	Acc.	RRL (%)	RSL (%)	PC	No.	Acc.	RRL (%)	RSL (%)	PC
301	238311	14.29	116.67	S	325	215826	10.64	95.161	S
302	215931	14.04	52.083	S	326	215967	10.53	69.048	S
303	203326	13.89	53.488	S	327	238309	10.42	51.456	S
304	243639	13.56	62.069	S	328	215899	9.804	90.741	S
305	215966	13.33	25.263	S	329	203370	9.615	50.943	S
306	215956	13.33	55.172	S	330	215892	9.434	82.54	S
307	242614	13.33	45.882	S	331	215804	7.143	62.712	S
308	215897	13.16	69.643	S	332	212462	6.849	154.55	S
309	215992	13.16	65.909	S	333	238323	4.372	77.358	S
310	215955	13.11	45.455	S					
311	215898	12.94	66.197	S					
312	203362	12.5	71.642	S					
313	215858	12.07	65.591	S					
314	215851	12	69.091	S					
315	219826	11.9	33.898	S					
316	245085	11.9	52.727	S					
317	216048	11.76	75.862	S					
318	215876	11.63	202.13	S					
319	203331	11.43	62.037	S					
320	242105	11.36	81.481	S					
321	203338	11.32	72	S					
322	242625	10.71	52.381	S					
323	241768	10.64	85.714	S					
324	215951	10.64	79.348	S					

4. Discussion

Among the abiotic factors, soil acidity is a major constraint for plant development and growth as well as the yield and productivity of crops. It has been estimated that over 50% of the world's potentially arable lands are acidic [13]. In this study, a hydroponic system was used to study the Al tolerance of finger millet accessions and cultivars under different Al concentrations. Hydroponic systems are suitable for early growth and seedling screening under submerged conditions. Previously published research on wheat, rice, and chickpea has used hydroponics to screen against Al stress by measuring root and shoot length [23,28]. Therefore, the present study also confirmed the suitability of using hydroponics while exercising an Al-tolerance study on finger millet. The morphological markers, RL and SL, were important traits to study Al tolerance as the primary response to Al stress occurs in the plant roots, with the Al-susceptible genotypes showing retarded root growth.

It is advisable to use seedlings with similar vigor and this is achieved by selecting seedlings with similar-sized endosperm, similar initial root length, and similar seed age to consider better performing individuals [25,29]. These accessions were sometimes comprised of two or more genotypes since there was a large variation in performance between individual plants of the accession. Furthermore, there were visually observable differences within an accession such as variations in grain color. To take this heterogeneity into account, an accession was scored based on its best-performing seedling. The use of the average performance of plants in representing an accession would have resulted in the rejection of many accessions because of poor average performance such that a single plant within the accession with an acceptable level of Al^{3+} tolerance would be lost [25].

According to [22], RRL and RSL are morphological markers to study Al stress as they can eliminate genotype-specific differences in root growth and normalize comparisons between genotypes. Since RRL and RSL are the relative growth of the genotype in Al solution compared with its potential growth without Al, this parameter is a real measure of Al tolerance [22]. Short root length is considered to be the primary consequence of aluminum toxicity, resulting in a smaller volume of soil explored by the plant. Consequently, reducing its mineral nutrition and water absorption. Furthermore, it reduces cell membrane permeability and binds to the phosphate groups of the deoxyribonucleic acid, decreasing replication and transcription [15].

In this study, a hydroponic nutrient solution was employed to identify the threshold level of Al concentration in finger millet landraces and cultivars. Finger millet accessions and cultivars were evaluated at three Al concentrations including the control (0, 75, and 100 μM). At low Al concentrations, it is difficult to properly discriminate finger millet accessions and cultivars in relation to their Al tolerance. The reason could be that low Al concentrations (less than 75 μM) were not strong enough to create Al-stress conditions at finger millet roots. Similarly, at high Al concentrations above 100 μM, the Al stress inhibited growth in all finger millet accessions and cultivars, making it difficult to differentiate between the tolerant and susceptible groups. However, better discrimination among the genetic materials was observed at 100 μM, and it was selected and used as an optimum concentration level for the wider screening of 328 landraces.

Comparatively, the threshold level of Al tolerance in finger millet accessions was found higher than the Al tolerance of barley accessions, which had 30 μM [30], and maize accessions, which had a 20 μM threshold level of Al tolerance. Whereas, in line with the tolerance level of finger millet at 112.5 μM [23], chickpea accessions had Al-concentration thresholds of 110 and 120 μM [27,31]. The higher Al-tolerance level noted in finger millet might be because finger millet is a climate-resilient crop that is able to grow in marginal lands, which helps the crop to perform better than other crops in biotic and abiotic-stress-prone environments [31]. Moreover, most of the accessions used in this study were collected from western and northern parts of Ethiopia, where soil acidity is predominant, and they developed a mechanism to tolerate this type of stress. Genotypes collected from acidic environments may accumulate mutations that adapt to acidic environments and develop rapid Al-tolerance mechanisms by activating genes responsible for the secretion of mucilage and organic acid anions when they are exposed to phototoxic forms of Al within minutes of exposure. Thus, due to natural selection, only the tolerant genotypes survive.

At the 100 μM Al-concentration screening, cultivars Tadesse, Padet, and Kumsa, as well as accessions 212462, 215804, and 238323, were the least performing (Al-susceptible). On the other hand, Urji, Bareda, and Axum cultivars, as well as 215836, 215845, and 229722 accessions, were relatively tolerant against Al stress. Accessions were found to be more tolerant against Al stress than cultivars. This indicates that landraces have a better Al tolerance compared to cultivars, implying that breeding activities have a significant effect on the stress tolerance, including on the Al tolerance of the crop.

In the present study, we did not observe any distinct and visible symptoms of Al toxicity in the SL of finger millet, which is in agreement with previous studies on pigeon pea using a 20 μM Al-concentration [32]. No significant effect of Al stress on SL was detected in our study due to the short exposure time in the hydroponic system.

The RRL considers control and treatment conditions. It allows for a comparison of accessions with a constant ranking according to their performance. The dose–response experiment on the wider number of accessions demonstrated that 20 (6.9%) of them were Al-tolerant, whereas 268 (93.05%) of them were ranked from low to medium tolerance. The majority of the accessions collected from Wellega and Gojam were found Al-tolerant, while those collected from the northern part of Ethiopia were found Al-susceptible. According to [21], acidic soil is prevalent in western Ethiopia. Accessions collected from soil-acid-prone areas were found Al-tolerant. Thus, their enhanced tolerance against Al concentrations was likely developed due to long-term exposure to soil acidity. Accessions identified as Al-tolerant in the hydroponic experiment often showed improved agronomic performance compared to Al-susceptible accessions [25–27,29]. Potential finger millet accessions identified here can be used as inputs for breeders to improve the Al tolerance of finger millet.

5. Conclusions

The results of the present study suggest that there are individual accessions that can better tolerate acidic soils and some of them are highly susceptible. Lower Al concentrations had no significant effect on the RL of most finger millet cultivars and accessions, while their growth starts to decline with an increasing Al concentration. At 100 µM Al-concentration, cultivars Tadesse, Padet, and Kumsa, as well as accessions 212462, 215804, and 238323, were Al-susceptible. Thus, these cultivars should not be recommended in areas where soil acidity is predominant. On the other hand, Urji, Bareda, and Axum cultivars, as well as 215836, 215845, and 229722 accessions, were relatively tolerant against Al and can be promoted in areas where soil acidity is highly prevalent. To confirm their performance, the accessions should be tested on multi-site fields by considering controlled and treated environments. Furthermore, association studies should also be considered to correlate field performance with genomic background. Transcriptomic analysis on the most tolerant and least susceptible should be tested by taking samples from different plant tissues (root, leaf, and stem) at different time intervals (0, 12, 24, 48, and 72 h). Finally, the Al-tolerant lines identified in this study should be used as inputs to finger millet breeding programs in relation to Al tolerance in Ethiopia, Zimbabwe, and elsewhere. If anyone is interested in studying Al tolerance on finger millet, we suggest that they include wild types for comparative analysis.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13061596/s1, Table S1: List of finger millet accessions used in present study with their passport data.

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