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Reliability of seedling stage selection for aluminium stress tolerance in hot pepper (*Capsicum annuum* L)

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Abstract In Ultisol, the presence of aluminium (Al) in high concentration is the main constraint hampering growth and yield of many crops, including hot pepper. The use of varieties tolerant to Al stress is one most prospective manner, which is relatively low cost and environmentally friendly, in exploiting this acidic soil to increase the national hot pepper production. Appropriate screening method is required to make variety development more efficient. a concentration of 2 mM Al in nutrient solution gave enough selecting pressure to determine genotypes which tolerant to Al stress. Seedling stage selection was highly reliable to determine most tolerant genotypes against Al stress in hot pepper, with the key trait of plant fresh weight, plant dry weight, and stem diameter. The most tolerant genotype amongst 27 tested genotypes were 'HP', 'PBC621', 'PBC266', 'PBC 157', 'Mario', 'PBC155', 'PBC396', 'Sempurna' and the most sensitive ones were 'LPK' and 'Romario'. The result of field experiment confirmed the greenhouse finding. However, It is needed to be further evaluated more acidic ultisol to obtain more accurate aluminum tolerance property of selected genotypes.

Keywords: Acidity tolerance, Al, Chili, Screening

Introduction

Ultisol, an acidic and less fertile type of soil, occupied about 25% of the dry land of Indonesia accounted for about 48 million hectares (Prasetyo and Suriadikarta, 2006). It is a huge potential for increasing the national production of many crops, including hot pepper. However, this type of soil is characterized by high soil acidity, high Al^{+} and low P nutrient availability, low organic matter, and generally low fertility (Ifansyah, 2014) that might be unfavorable for growth and yield of hot pepper. Both soil correction and crop improvement are theoretically considered the best measureable approaches in exploiting this type of soil.

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Acidic properties of the soil can be corrected by the application of ameliorants, such as by lime, in the form of calcium carbonate or other alkaline materials (Drapanauskaite *et al.,* 2021), or any kind of manures, to increase the pH and to reduce exchangeable Al^{+} (Choudhary and Kumar, 2015) However, lime application to neutralize the soil pH is a machinery- and cost-intensive manner due to the need of re-application of lime after a period of time and sometime unfeasible, particularly where the source of lime is hardly accessible. Genetic manipulation to develop high yielding and more adaptive cultivars to acidic soil, consequently, is the other promising and dependable approach.

Effective breeding program to develop such cultivars requires, at least, genetic materials as sources of genes controlling tolerance to Al stress and potential high yielding, and effectual screening methods. Many previous works demonstrated that Al tolerant genotypes have been identified in many crop species, such as pigeon pea (Choudhary *et al.,* 2011), soybean (Akhter *et al.,* 2009), maize (Akhter *et al.,* 2009; Zhou *et al.,* 2014; Richard *et al.,* 2015a; Xu *et al.,* 2017a), common bean (Domingues *et al., 2013*), rice (Bidhan and Bhadra, 2014; Awasthi *et al.,* 2017), sorghum (Akhter *et al.,* 2009; Lestari *et al.,* 2017), wheat (Silva *et al.,* 2010), buckwheat (Wang *et al.,* 2015a), and hot pepper (He *et al.,* 2019) (Herison *et al.,* 2020). Tolerance to Al is the ability of plant withstand to Al stress conditions, survive, and reproduce successfully (Fritsche-Neto and Borém, 2012). In agronomical term, tolerance is defined as the less yield reduction in stress condition compared to that in the optimal cultivation (Miti *et al.,* 2010). Most of the previously mention studies, assessment of tolerance was merely on juvenile stage with the root length as the key trait. Little information was found to correlate the tolerance in the juvenile stage to the yield reduction in Al stress condition, more specifically in *Capsicum* sp.

Our present study aimed at determining the reliability of seedling stage screening method, correlated to the yield reduction in Al stress condition, and selecting the most tolerance genotype to Al stress. Establishment of a reliable, fast, and less-expensive screening method, and the most tolerant genotypes will be valuable assets in Al tolerant breeding programs in the future.

Materials and methods

Four consecutive experiments were performed from August 2016 to June 2018 to evaluate the reliability of seedling stage selection for Al tolerance and to determine hot pepper genotypes most tolerant to Al stress. They were experiment (1) determination of Al level for screening, (2) study on the reliability of early growth tolerance determination, (3) screening for Al tolerant genotypes at juvenile stage, and (4) selection for Al tolerant genotype in acidic soil.

All experiments were conducted in low altitude location, 15 m above sea level giving raise to the ambient temperature in the range of 28 - 33°C. Experiment 1, 2 and 4 were conducted in the greenhouse of Faculty of Agriculture, University of Bengkulu, with sand media assay of nutrient culture procedure following the method of (Choudhary *et al.*, 2011) modified with a wick irrigation system following (Kuntz, 2013). The sand was prepared by thoroughly washed to remove all dirt and other materials prior to use. Field experiment was performed in acidic ultisol land of Medan Baru Experimental Station of Faculty of Agriculture, University of Bengkulu, Indonesia.

Plant materials

Genetic materials in this study were commercial varieties commonly grown by local farmers with ultisol field, and genotypes rejuvenated from accessions of Asian Vegetable Research Development Center (AVRDC). In total, there were 25 genotypes, all of which were *Capsicum annuum* L. (Table 1).

Table 1. List of genetic materials used in this study

No	Code	Genotype name	Genotype information/origin
1	BGT	Bogota	Commercial non-hybrid variety
2	FRS	Ferosa	Commercial non-hybrid variety
3	KH	Keriting Hitam	Local landrace
4	LPK	Kopay	Local open pollinated variety
5	LRS	Laris	Commercial non-hybrid variety
6	MRO	Mario	Commercial non-hybrid variety
7	PBC067	PBC067	Accession of AVRDC
8	PBC137	PBC137	Accession of AVRDC from Brazil
9	PBC140	PBC140	Accession of AVRDC
10	PBC146	PBC146	Accession of AVRDC
11	PBC157	PBC157	Accession of AVRDC
12	PBC260	PBC260	Accession of AVRDC
13	PBC266	PBC266	Accession of AVRDC
14	PBC396	PBC396	Accession of AVRDC
15	PBC398	PBC398	Accession of AVRDC
16	PBC401	PBC401	Accession of AVRDC
17	PBC402	PBC402	Accession of AVRDC
18	PBC518	PBC518	Accession of AVRDC
19	PBC521	PBC521/Tiwari II	Accession of AVRDC from India
20	PBC592	PBC592	Accession of AVRDC
21	PBC621	PBC621/Kalmicho	Accession of AVRDC from Korea
22	PBC622	PBC622	Accession of AVRDC
23	RMR	Romario	Commercial non-hybrid variety
24	SMPR	Sampurna	Commercial non-hybrid variety
25	TNK	Tanaka Tsung	Commercial non-hybrid variety

Determination of Al level for selection

The experiment was carried out in a wick system hydroponic model with twin stacked plastic boxes with the dimension of 50 cm (length) x 40 cm (width) x 15 cm (height). The lower box was the nutrient container, and the upper one was the sand media container. The bottom part of the upper boxes was punched to create holes, 1 cm in diameter, twelve holes per box. Flannel-fabric wicks, 3 cm wide and 30 cm length, were set pass through the holes, halfway to the nutrient container and the rest to the sand media. The nutrient was a commercial AB-mix hydroponic nutrient solution ("Hydro J" produced by Jingga-Ag Inc.). The seeds of five randomly chosen hot pepper genotypes were germinated in a wet tissue for 4 days. Five germinating seeds of each genotype, with radicle length of 2-3 mm, were planted into the sand. The seedlings were maintained for 4 weeks before harvested.

The experiment was arranged in a randomized completely design with three replications, and the treatment was 0, 0.5, 1.0, 1.5 or 2.0 mM Al added into the nutrient solution. The Al stock solution of 100 mM was freshly prepared from un-hydrous $AlCl_3$ (Merck catalogue number 801081). All nutrient solutions with Al treatment were maintained at the pH of 4.0-4.2 by 0.1 N HCl or NaOH solution. The check nutrient solution was maintained at pH level of 6.5. The plants were grown in the greenhouse under the natural low-altitude tropical condition without any control of microclimate. The mean temperature in the greenhouse was 24°C (night) and 38°C (day). Nutrient solution was added weekly as needed. Plant height, root length and plant fresh-weight of all seedlings were measured at 4 week after seeding. The data analysis was performed by ANOVA followed by trend regression.

Study on the reliability of early growth screening

Seedlings of nine randomly picked genotypes were evaluated for Al tolerance in the nutrient culture supplemented with Al in comparison to the check. The level of Al was determined from the first experiment. The seedlings were grown in a hydroponic system similar to the first experiment in a randomized completely design with three replications. The experimental unit was ten seedlings. Measurement was performed on seedling height, number of leaves, stem diameter, root length, root volume, seedling fresh and dry weight, root-fresh and dry weight.

The same genotypes were grown in polybags containing 10 kg of sand media in a wick hydroponic system with and without the supplementation of Al to evaluate growth and yield reduction. Before being transplanted into the

polybag, the seedlings were grown in 72 cell seedling trays with a 1:1 mix media of manure and top soil (v/v) for 35 days. Each polybags were placed on a bucket containing nutrient solution supplemented with 0 or 2 mM Al. The pH of nutrient solution with Al was maintained at 4.0-4.2, and for the control was at 6.5 level. The experiment was arranged in a randomized completely design (RCD) with three replications, separately for the Al treatment and the check. The vegetative growth, i.e. plant height, first dichotomous height, stem diameter, number of branches, canopy diameter, shoot fresh and dry weight, root length, root was measured at the end of the experiment. Number of fruit set, fruit length, fruit diameter, and fruit weight were measured at harvesting time.

Analysis of variance was applied on all data collected in both juvenile assay and full grown evaluation, followed by the Duncan's Multiple Range Test (DMRT) at α=5% (Steel and Torrie, 1982). Evaluation on tolerance was performed by stress tolerance index (STI) following (Bahari *et al.*, 2013), with the modification for all observed variables.

$$STI = \frac{Ypi\ x\ Ysi}{\overline{Yp}^2}$$

Where STI, Ypi, Ysi and $\overline{Yp}$ were stress tolerance index, observed value of genotype i without stress, observed value of genotype i in a stress condition, and the average observed value of all genotype without stress, respectively. Pearson correlation on STIs of juvenile assay to that of full grown was performed to determine whether juvenile assay is reliable to evaluate tolerance to Al stress, and to identify the key traits most suitable for selection.

Juvenile stage screening for Al tolerant genotypes

The seeds of 25 genotypes (listed in Table 1) were germinated, seeded and maintained in nutrient culture with sand media similarly to the former experiment. The screening was established in two separate experiment, without Al stress or with 2 mM Al, each of which was arranged in a randomized completely design with three replications, and each experimental unit consisting of 10 seedlings. Evaluation on tolerance was performed during the juvenile stage at four week after seeding, based on the calculated STI on a couple of the key traits.

Field evaluation for Al tolerant genotypes in acidic soil

The experiment was conducted at the Medan Baru Experimental Station of Faculty of Agriculture, University of Bengkulu. The farm is situated at altitude of 20 m above sea level, 3º75’86”S latitude and102º28’811’’E

longitude. The soil is Ultisol and soil analysis by the Soil Laboratory (Faculty of Agriculture, University of Bengkulu) showed the pH was 4.3 and the nutrient content of N, P_2O_5, $K_{exchangable}$, and $Al_{exchangable}$ was 0.2%, 1.9 ppm, 0.77 me. $(100g)^{-1}$, 1.03 me.$(100g)^{-1}$, respectively, (Rustikawati *et al.,* 2020).

Land preparation was performed by plowing, loosening and bedding. The soil bed size was 1 m wide. Before layered with plastic mulch, the soil beds were broadcasted with cow manure at 10 ton. ha^{-1}, and N, P and K fertilizer at 60, 30, and 35 $kg.ha^{-1}$, respectively. Black-silver plastic film, 1.2 m wide, and 0.27 mm thick (Hidup Baru Plasindo Inc., Sukoharjo, Central Java, Indonesia) was laid manually, with the silver color was visible. The mulch was then punched by a sharpened 10 cm diameter can to make two row planting holes, 50 cm apart and 40 cm within the row.

Transplants of 25 hot pepper genotypes were produced in a greenhouse using 72-cell plastic trays containing a mix of soil and cow manure with a ratio of 1:2 (v/v). The plants were grown in the greenhouse and watered daily as needed. At three weeks after seeding, the seedlings were drenched with diluted multi-nutrient NPK (16% N – 16% P_2O_5 – 16% K_2O) at 2 $g.L^{-1}$. Five-week-old hot pepper transplants were planted singly in the planting holes previously scattered with a carbofuran containing insecticide at 20 $kg.ha^{-1}$, on September 23, 2018. Four weeks after transplanting, all plants were circle dressed using urea at 60 kg $N.ha^{-1}$.

Plant maintenance including of watering, staking, lateral shoot pruning and pest controlling were conducted as the standard of commercial hot pepper grower (Herison *et al.,* 2018). Watering up to a field capacity was carried every other morning, when there was no rain. The plants were supported by bamboo stake of 75 cm length and 4 cm width to prevent lodging. All lateral branches below the first dichotomous point were cut off manually. Pest and diseases control was done preventively every week with a mix of profenofos insecticide, pyridaben acaricide and mancozeb fungicide at 2 $ml.L^{-1}$, 2 $ml.L^{-1}$ and 2 $g.L^{-1}$, respectively. Mature fruits, indicated by at least 75% of the fruit have turned to red color, were harvested periodically, every 5 day.

The experiment was a randomized complete block design with three replications. Twenty five genotypes were considered as the treatment. The individual experimental units were 6 m long, consisting of 30 plants. Vegetative growth, i.e. plant height, first dichotomous height, stem diameter, number of branches, and canopy diameter, was measured at the end of the experiment. The yield and yield components, i.e. number of fruit set, fruit length, fruit diameter, were measured at harvesting time. Analysis of variance was applied on all data collected followed by a Duncan's Multiple Range Test (DMRT) at 5%, following the method of (Steel and Torrie, 1982).

Results

Determination of Al level for selection

Application of Al at low concentration (0.5 mM Al) tended to slightly increase plant height, root length and plant fresh-weight. Further increase of Al levels restricted the seedling growth. All variables responded in negative quadratic curve to the increase of Al concentrations up to 2.0 mM Al. Plant height response followed the equation of $y = -0.5848x^2 + 0.2935x + 4.713$ with coefficient of determination (R^2) was 0.7981. The response of root length followed the equation of $y= -1.6724x^2 + 1.8621x + 8.9151$ with R^2=0.7293. Whereas the trend of plant fresh-weight followed the equation of $y=--0.1375x^2 + 0.0657x + 0.7415$ with R^2=0.8027 (Fig. 1). The figure indicated that Al concentration of 2.0 mM was sufficiently restraint as high as 50 to 70% of growth of hot pepper seedlings. Those level, therefore, was strong enough to screen genotypes tolerance to Al stress.

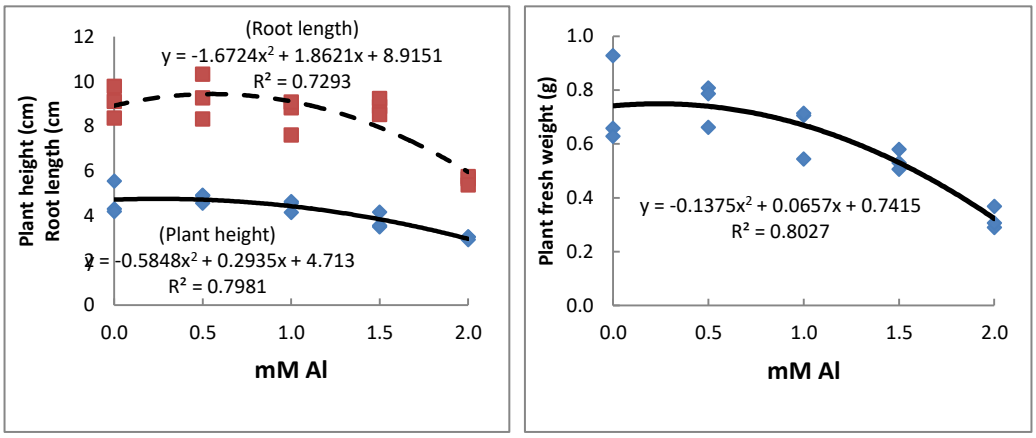

Figure 1. Trends of plant height, root length and plant fresh-weight to the increasing of Al concentration

Reliability of early growth tolerance screening

Calculated STI values of seedling growth were varied amongst genotypes and variable observed. It ranged from the lowest value of 0.03 and the highest value of 1.78 (Tabke 2). Among genotypes, considering of the average of all variables, PBC621 and KH showed the highest STI value, which mean they were the most tolerance to Al stress. Whereas Kopay and Bogota were the most sensitive ones. Among variables observed, root length produced the highest STI which means that the observed value on Al stress condition was

close to that of control. While the smallest average of STI value was on plant dry weight. This variable also exhibit the highest coefficient of variation (CV), followed by plant fresh weight.

Table 2. STIs calculated based on seedling growth traits

Geno-type	Calculated Stress Tolerance Index (STI)										
	Seed-ling height	Numb er of leaf	Leaf greeni sh	Stem dia-meter	Root length	Root volum e	Root fresh weight	Shoot fresh weight	Plant fresh weight	Plant dry weight	Leaf area
PBC396	0.37	0.66	0.80	0.49	0.90	0.68	0.39	0.17	0.18	0.04	0.19
PBC518	0.22	0.45	0.95	0.36	1.34	0.89	0.35	0.09	0.10	0.03	0.18
PBC521	0.23	0.64	0.83	0.32	1.01	0.43	0.36	0.07	0.08	0.03	0.17
PBC621	0.46	0.70	0.68	0.46	1.78	0.64	0.14	0.28	0.36	0.16	0.28
PBC622	0.28	0.83	0.95	0.39	1.00	0.59	0.31	0.18	0.19	0.05	0.20
Bogota	0.33	0.67	0.81	0.34	0.64	0.31	0.19	0.16	0.16	0.07	0.19
Ferosa	0.30	0.45	0.83	0.37	1.22	0.77	0.19	0.10	0.10	0.04	0.11
KH	0.64	0.78	1.04	0.33	1.26	0.62	0.41	0.15	0.17	0.05	0.16
Kopay	0.33	0.51	0.85	0.32	0.95	0.47	0.14	0.08	0.08	0.03	0.15
Min	0.22	0.45	0.68	0.32	0.64	0.31	0.14	0.07	0.08	0.03	0.11
Max	0.64	0.83	1.04	0.49	1.78	0.89	0.41	0.28	0.36	0.16	0.28
Mean	0.35	0.63	0.86	0.38	1.12	0.60	0.27	0.14	0.16	0.05	0.18
SD	0.13	0.14	0.10	0.06	0.32	0.18	0.11	0.07	0.09	0.04	0.05
CV (%)	37.55	21.43	12.08	16.54	28.97	29.78	39.65	47.24	54.88	79.84	25.80

The STI value on mature plant was even more varied among genotypes or variables, ranging from 0.01 to 2.74. With regard to all variables, PBC621 and PBC396 were consider the most tolerant genotypes, whereas genotype KH and Kopay were the most sensitive (Table 3). Comparing among variables, the highest mean STI value was on root length, followed by fruit weight, while the smallest value was on root dry weight. However, the STI calculated based on fruit weight per plant exhibit the highest variation, followed by that of fruit number.

The STI values base on growth traits of seedlings did not correlate to all STIs based on mature plant dry weight and root dry weight. STI based on seedling stem diameter significantly correlated to that of root fresh weight. STI calculated based on leaf greenish of seedlings showed highly negative correlation to that of root length, fruit number and yield per plant. On the contrary, STI based on seedling stem diameter significantly correlated to that of root length and number of fruits, and highly and significantly correlated to the STI based on the yield per plant. Similarly did the STI based on shoot fresh weight and leaf area of seedlings. The STIs based on seedling fresh and dry weight highly and significantly correlated to number of fruit and the yield (Table 4).

Table 3. STIs calculated based on growth and yield traits

Genotype	Calculated Stress Tolerance Index (STI)					
	Plant dry weight	**Root fresh weight**	**Root dry weight**	**Root length**	**Number of fruit**	**Fruit weight**
PBC396	0.50	1.09	0.62	0.86	0.85	1.31
PBC518	0.21	0.13	0.12	0.45	0.02	0.01
PBC521	0.29	0.47	0.27	0.57	0.16	0.05
PBC621	0.26	0.39	0.32	0.79	1.94	2.74
PBC622	0.31	0.55	0.30	0.32	0.21	0.09
Bogota	0.67	0.55	0.57	0.52	0.30	0.22
Ferosa	0.69	0.90	0.75	0.66	0.77	0.21
KH	0.19	0.08	0.07	0.24	0.03	0.03
Kopay	0.17	0.05	0.06	0.39	0.09	0.01
Min	0.17	0.05	0.06	0.24	0.02	0.01
Max	0.69	1.09	0.75	0.86	1.94	2.74
Mean	0.37	0.47	0.34	0.53	0.49	0.52
SD	0.20	0.36	0.25	0.21	0.62	0.93
CV (%)	55.39	76.97	74.09	39.16	128.64	178.44

Table 4. Correlation coefficient between STIs calculated based on seedling growth traits and growth- and yield traits under stress condition

STIs of seedling growth	Stress Tolerance Index (STI) of fully grown plant					
	Plant dry weight	Root fresh weight	Root dry weight	Root length	Fruit number	Fruit weight
Seedling height	-0.23 ^{ns}	-0.23 ^{ns}	-0.22 ^{ns}	-0.15 ^{ns}	0.24 ^{ns}	0.32 ^{ns}
Leaf number	-0.17 ^{ns}	0.03 ^{ns}	-0.12 ^{ns}	-0.22 ^{ns}	0.08 ^{ns}	0.20 ^{ns}
Leaf greenish	-0.34 ^{ns}	-0.43 ^{ns}	-0.48 ^{ns}	**-0.84** **	**-0.80** **	-0.75 *
Stem diameter	0.20 ^{ns}	0.60 *	0.45 ^{ns}	0.73 *	0.76 *	**0.81** **
Root length	-0.42 ^{ns}	-0.27 ^{ns}	-0.26 ^{ns}	0.19 ^{ns}	0.60 *	0.58 ^{ns}
Root volume	-0.12 ^{ns}	0.09 ^{ns}	0.01 ^{ns}	0.16 ^{ns}	0.18 ^{ns}	0.14 ^{ns}
Root fresh weight	-0.23 ^{ns}	0.07 ^{ns}	-0.19 ^{ns}	-0.20 ^{ns}	-0.44 ^{ns}	-0.30 ^{ns}
Shoot fresh weight	0.02 ^{ns}	0.17 ^{ns}	0.16 ^{ns}	0.33 ^{ns}	0.75 *	**0.81** **
Plant fresh weight	-0.06 ^{ns}	0.11 ^{ns}	0.10 ^{ns}	0.39 ^{ns}	**0.81** **	**0.88** **
Plant dry weight	-0.04 ^{ns}	-0.02 ^{ns}	0.07 ^{ns}	0.40 ^{ns}	**0.84** **	**0.86** **
Leaf area	-0.30 ^{ns}	-0.08 ^{ns}	-0.15 ^{ns}	0.35 ^{ns}	0.64 *	**0.80** **

Note: ns, * or ** means non significance (P>0.05), significance (P<0.05), or highly significance (P<0.01), respectively

Juvenile Stage Screening for Al Tolerant Genotypes

Under Al stress condition, there were significant (P<0.05) variety differences for all traits measured, except for plant fresh weight. For plant height, genotype KH was the tallest, and LRS and PBC067 were the shortest. Overall, genotype KH and SMPR were taller than LRS, PBC067 or PBC518. Regarding number of leaves, PBC622 was the highest and PBC067 or FRS was the least amount. Genotype PBC266 showed the biggest stem diameter, and LRS or PBC 402 was the smallest. PBC140 and PBC155 possessed the longest, whereas BGT the shortest root length. Regarding plant dry weight, PBC621 was the highest, and MRO, PBC137 were the lowest. Considering the performance in non-stress condition (data not shown) in the calculation of STI, PBC266, PBC621, PBC396, and MRO were the highest and categorized tolerant. Meanwhile, PBC521, PBC518, PBC592, FRS, RMR and TNK showed the lowest STI categorized sensitive (Table 5).

Table 5. Seedling performance of 25 hot pepper genotypes under Al-stress condition, their STI values and tolerance categories

No	Geno-type	Plant height (cm)	Number of leaves	Stem diameter (mm)	Root length (cm)	Plant fresh weight (g)	Plant dry weight (g)	STI	*
1	BGT	4.37 bcd	2.9 abc	0.65 abc	4.92 d	0.26 a	0.04 abcd	0.11	MS
2	FRS	4.57 bcd	2.4 c	0.79 abc	7.25 abcd	0.26 a	0.02 cd	0.07	S
3	KH	7.17 a	3.3 abc	0.70 abc	8.60 abcd	0.33 a	0.03 bcd	0.12	MS
4	LPK	5.80 abcd	3.2 abc	0.63 bc	6.72 abcd	0.34 a	0.04 abcd	0.06	S
5	LRS	3.48 d	2.7 bc	0.55 c	5.40 bcd	0.36 a	0.03 bcd	0.08	S
6	MRO	6.28 abc	3.7 ab	0.80 abc	7.71 abcd	0.40 a	0.01 d	0.21	T
7	PBC067	3.39 d	2.3 c	0.73 abc	8.03 abcd	0.17 a	0.04 abcd	0.10	S
8	PBC137	5.03 abcd	3.2 abc	0.67 abc	8.19 abcd	0.17 a	0.01 d	0.12	MS
9	PBC140	6.38 abc	2.7 bc	0.79 abc	10.44 a	0.18 a	0.06 ab	0.14	MS
10	PBC146	5.97 abc	3.2 abc	0.77 abc	7.18 abcd	0.19 a	0.03 abcd	0.11	MS
11	PBC155	5.08 abcd	3.4 abc	0.76 abc	10.36 a	0.20 a	0.04 abcd	0.16	MT
12	PBC157	5.27 abcd	2.6 bc	0.78 abc	9.96 ab	0.20 a	0.04 abcd	0.13	MS
13	PBC266	5.77 abcd	3.5 abc	0.91 a	9.22 abcd	0.21 a	0.04 abcd	0.29	T
14	PBC396	5.62 abcd	3.3 abc	0.89 ab	8.08 abcd	0.23 a	0.03 abcd	0.22	T
15	PBC398	5.62 abcd	3.2 abc	0.64 bc	6.09 abcd	0.23 a	0.02 bcd	0.11	MS
16	PBC401	4.10 bcd	3.5 abc	0.63 bc	7.48 abcd	0.23 a	0.02 cd	0.11	MS
17	PBC402	6.10 abc	2.8 abc	0.60 c	7.28 abcd	0.24 a	0.03 bcd	0.16	MT
18	PBC518	3.99 cd	2.7 bc	0.78 abc	8.30 abcd	0.24 a	0.03 bcd	0.07	S
19	PBC521	4.18 bcd	3.2 abc	0.69 abc	7.64 abcd	0.25 a	0.02 bcd	0.06	S
20	PBC592	5.17 abcd	2.7 bc	0.74 abc	5.21 cd	0.25 a	0.05 abc	0.07	S
21	PBC621	5.47 abcd	2.7 bc	0.76 abc	8.67 abcd	0.25 a	0.07 a	0.25	T
22	PBC622	4.31 bcd	3.9 a	0.81 abc	8.62 abcd	0.26 a	0.03 bcd	0.14	MS
23	RMR	4.98 abcd	2.6 bc	0.62 bc	5.89 abcd	0.41 a	0.02 bcd	0.09	S
24	SMPR	6.47 ab	3.0 abc	0.72 abc	9.57 abc	0.45 a	0.04 abcd	0.12	MS
25	TNK	5.15 abcd	3.4 abc	0.80 abc	8.47 abcd	0.52 a	0.04 abcd	0.10	S

Note: means in the same column followed by different letter indicate significant according to Duncan's Multiple Range Test (DMRT) (P<0.05). * T, MT, MS, and S stand for tolerant, medium tolerant, medium sensitive, and sensitive, respectively.

Table 6. Field evaluation on 25 hot pepper genotypes in acidic Ultisol

No	Genotype	Plant Height (cm)	Dichotomous height (cm)	Number of dichotomous point	Yield (g/plant)	Percent survival (%)
1	BGT	69.0 $^{a-g}$	23.0 $^{b-f}$	188 $^{c-g}$	95.08 ^{fg}	40.0 ^{f}
2	FRS	63.0 $^{d-h}$	19.7 $^{c-g}$	162 ^{efg}	171.09 ^{c}	90.0 ^{b}
3	KH	73.0 $^{a-f}$	29.7 ^{ab}	241 $^{b-e}$	153.92 ^{d}	100.0 ^{a}
4	LPK	45.7 ^{h}	14.0 ^{fgh}	107 ^{g}	25.50 ^{n}	30.0 ^{g}
5	LRS	70.0 $^{a-g}$	23.3 $^{b-e}$	197 $^{c-g}$	88.63 $^{f-i}$	40.0 ^{f}
6	MRO	67.3 $^{b-g}$	23.7 $^{b-e}$	186 $^{c-g}$	83.45 $^{g-j}$	40.0 ^{f}
7	PBC067	70.7 $^{a-g}$	18.0 $^{d-h}$	253 ^{bcd}	73.67 $^{h-k}$	100.0 ^{a}
8	PBC137	68.0 $^{a-g}$	27.0 ^{abc}	224 $^{c-f}$	105.12 ^{f}	76.7 ^{c}
9	PBC140	55.3 $^{e-h}$	17.0 $^{d-h}$	137 ^{fg}	128.29 ^{e}	100.0 ^{a}
10	PBC146	84.0 ^{ab}	24.7 $^{a-d}$	235 $^{b-e}$	80.82 $^{g-j}$	100.0 ^{a}
11	PBC157	79.3 $^{a-d}$	16.3 $^{d-h}$	376 ^{a}	192.42 ^{b}	96.7 ^{a}
12	PBC260	52.7 ^{gh}	16.0 $^{d-h}$	159 ^{efg}	67.18 ^{jkl}	33.3 ^{g}
13	PBC266	45.7 ^{h}	16.0 $^{d-h}$	173 $^{d-g}$	90.65 ^{fgh}	96.7 ^{a}
14	PBC396	66.7 $^{b-g}$	14.7 $^{e-h}$	170 $^{d-g}$	216.68 ^{a}	100.0 ^{a}
15	PBC398	54.7 $^{e-h}$	17.0 $^{d-h}$	174 $^{d-g}$	62.83 ^{kl}	40.0 ^{f}
16	PBC401	57.7 $^{e-h}$	11.0 ^{gh}	155 ^{efg}	80.53 $^{g-j}$	60.0 ^{d}
17	PBC402	64.3 $^{c-h}$	32.7 ^{a}	158 ^{efg}	25.03 ^{n}	80.0 ^{c}
18	PBC518	61.7 $^{d-h}$	9.3 ^{h}	165 $^{d-g}$	102.24 ^{f}	80.0 ^{c}
19	PBC521	58.7 $^{e-h}$	19.3 $^{c-g}$	174 $^{d-g}$	85.51 ^{ghi}	100.0 ^{a}
20	PBC592	82.0 ^{abc}	30.7 ^{ab}	370 ^{a}	45.20 ^{m}	20.0 ^{h}
21	PBC621	72.0 $^{a-f}$	23.7 $^{b-e}$	264 ^{bc}	202.60 ^{ab}	100.0 ^{a}
22	PBC622	54.0 ^{fgh}	27.0 ^{abc}	187 $^{c-g}$	79.52 $^{g-k}$	100.0 ^{a}
23	RMR	86.3 ^{a}	24.7 $^{a-d}$	233 $^{b-e}$	57.08 ^{lm}	50.0 ^{e}
24	SMPR	73.7 $^{a-d}$	15.3 $^{e-h}$	216 $^{c-f}$	74.88 $^{h-k}$	40.0 ^{f}
25	TNK	68.3 $^{a-g}$	11.3 ^{gh}	277 ^{b}	72.85 $^{i-l}$	40.0 ^{f}

Note: means in the same column followed by different letter indicate significant according to Duncan's Multiple Range Test (DMRT) ($P<0.05$).

Field evaluation for Al tolerant genotypes in acidic soil

Plant stand in the field was very good in the first three weeks. All genotypes grew very well. However, starting at from week four to the end of the experiment some of the plants were not survive. There were significant ($P<0.01$) variety differences on percent survival, ranging from 20% to 100%. Genotype KH, PBC067, PBC140, PBC146, PBC396, PBC521, PBC621, PBC622, PBC157 and FRS had the highest percent survival, more than 90%. In contrast, PBC592, LPK, PBC260, PBC398, LRS, MRO, SMPR, TNK, and RMR were less than 50%.

Observation on the survived individuals, there were significant ($P<0.05$) variety differences on plant height, dichotomous height, number of dichotomous point and yield per plant. With regard to plant height, RMR was the tallest genotype, whereas LPK and PBC 266 were the shortest. Generally, genotype RMR, PBC146, and PBC592 were significantly taller than PBC266, LPK, FRS, PBC140, PBC260, PBC398, PBC401, PBC518, PBC521, and

PBC622. For the first dichotomous height, genotype PBC402 was the highest and PBC518 was the lowest. Overall, PBC402, PBC592, and KH had higher the first dichotomous position than PBC401, LPK, FRS, PBC067, PBC140, PBC157, PBC260, PBC266, PBC396, and PBC398. With respect to the number of dichotomous point, PBC157 had the highest number, and, on the contrary, LPK was the lowest one. Genotype PBC157 and PBC592 had significantly higher number of dichotomous point than the other genotypes. Regarding to the yield per plant, PBC396 had the highest yield (216 g/plant), and PBC402 was the lowest one (25 g/plant). The yield of PBC396 or PBC621 was significantly higher than that of the other genotypes (Table 6).

Discussion

Aluminium (Al) toxicity is the main limiting factor for plant growth in acidic soils. The problem becomes more severe when the pH level is lower than 5.0. Al bioavailability, and in consequence, toxicity, is responsible for the inhibition of root and plant growth. When pH drops below 5.0, aluminosilicate clays and aluminum hydroxide minerals dissolve, releasing aluminium-hydroxy cations ($Al(H_2O)_6^{3+}$) and Al^{3+}, which exchange to other cations (Kochian *et al.,* 2004); (Zhang *et al.,* 2007). In acidic conditions, Al^{3+} also forms the mononuclear species $AlOH^{2+}$, $Al(OH)_2^{+}$, $Al(OH)_3$, and $Al(OH)_4$ (Panda and Matsumoto, 2007). The mononuclear Al^{3+} species is considered as the most toxic forms (Giannakoula *et al.,* 2008; Silva *et al.,* 2010).

Since amelioration to improve soil is not always technologically or economically feasible, especially in strongly acid soils, breeding program to develop Al tolerant and high yielding varieties is considered a useful alternative approach. Some crops are considered tolerant to high levels of exchangeable Al^{+} which for others is a serious constraint. Species and genotypes within species greatly differ in their tolerance to Al (Fritsche-Neto and DoVale, 2012). Therefore, it is imperative to explore which genotypes, within a species, are more suitable to grow in acidic soils in order to increase production.

Artificial stress conditions for selection in nutrient culture most of the time is required to improve the effectiveness of screening to abiotic stress tolerant. Stressing agents, either chemicals or environmental condition, are added to mimic natural suboptimal condition at a designate level (Brhane *et al.,* 2018). Our study found that 2 mM Al was strong enough to provide stress that significantly inhibited growth of pepper plant. The strength of Al stress required for selection may differ depended on the plant species. For finger millet, as high as 0.125 mM is enough to screen for Al tolerant genotypes (Brhane *et al.,* 2018). Screening for cowpea tolerant to Al required up to 3 mM

Al (Kushwaha *et al.,* 2017). Therefore, it is imperative to determine the level of Al before screening for Al tolerant genotypes.

Recognizing the plant response to Al stress and the mechanisms of the plant tolerate to Al are beneficial in developing Al tolerant varieties (Zheng *et al.,* 2014). Crop improvement for acid soil adaptiveness via molecular assisted breeding and biotechnology approaches has exploited the understanding on Al tolerance mechanism in molecular and genetics basis (Kochian *et al.,* 2015). Aluminum (Al) in its ionic form rapidly inhibits root growth and uptake of water and nutrients. Toxic effect mechanism, manifested by root growth inhibition, may be directly/indirectly responsible for the loss of plant production by (Silva *et al.,* 2012; 2011). Other works also demonstrated that Al toxicity induced thickness of leave structure (Konarska, 2010) and root structure (Alvarez *et al.,* 2012a). All of these physiological and morphological changes in response to Al stress may be useful to identify sensitive of tolerant genotypes.

Effective screening methods is prerequisite in crop improvement to develop new varieties. Selection on early stage of plant development become more interesting as it reduces screening duration. In selection for aluminum tolerant genotypes, early growth stage screening have been practiced for many plant species, such as in finger millet (Brhane *et al.,* 2018), maize (Richard *et al.,* 2015b) (Xu *et al.,* 2017b), alfalfa (Khu *et al.,* 2012). Agronomically, tolerant genotype is expressed as the reduction of the yield under stress condition compared to under optimum condition (Fritsche-Neto and DoVale, 2012). However, none of the previously mentioned studies correlated the early growth observation to the reduction of yield of the genotypes under stress condition. Our present study justified the reliability of seedling stage selection for Al tolerant genotype. The STIs calculate from seedling stage highly and significantly correlated to the STI calculated from the yield per plant with the key traits were seedling fresh weight, seedling dry weight, and stem diameter.

Many studies reported that root length was the prime indicator for tolerance to Al stress (Alvarez *et al.,* 2012b), (Richard *et al.,* 2015b). In our study, however, the STI based on the root length trait did not correlate to the STI based on the yield. This may be because morphological changes of the root indicate the sensitivity this plant part to Al toxicity which may or may not influence the yield.

Evaluation on 25 hot pepper genotypes at seedling stage indicated that PBC266, PBC621, PBC396, and MRO were considered the most tolerant genotype. Those genotypes were able to accommodate high level of Al in the plant tissue. The distribution of Al in the plant body is controlled mainly by transpiration and when it is accumulated in the vacuole of a leaves, it becomes

immobile (Shen and Ma, 2001). Agronomically, all of those most tolerant genotypes were not high yielding ones and owned preferable size of fruits. However, they were of valuable genotypes as the source of gene(s) controlling tolerance to aluminum which acted through several ways.

Mechanism for Al tolerance includes external detoxification of Al by secreting oxalate from the roots and internal detoxification by formation of non-phytotoxic Al complexes with organic acids (Wang *et al.*, 2015b). Internal detoxification of Al may be achieved by forming non-phytotoxic complexes with oxalate and by sequestrating Al into the vacuoles (Ma, 2007). Al accumulation in some plants appears to be facultative and varies across different organs and tissues. The higher Al concentration was found in leaves and bark tissue than other organs (Schmitt *et al.*, 2016). Al accumulated rapidly in the developing leaves (Osawa *et al.*, 2013). The capability to exclude or accumulate Al differs between plant species or within a species giving rise to variety of Al tolerance level (Pattanayak and Pfukrei, 2013).

Field evaluation of 25 genotype in acidic soil showed that genotype PBC396 and PBC621 had the highest yield although their growth were not superior. Some other genotypes, which were consider sensitive to Al in nutrient culture, grew well vegetatively. This indicated that the acidic soil used in the field evaluation did not give enough stress to the population. Many other factors also influenced growth and yield, instead of aluminum, interfered the result.

Our present study concluded that seedling stage screening was a reliable method to identify the most tolerant genotypes to Al stress. Out of 25 genotypes under study, PBC266, PBC621, PBC396, and MRO were considered the most tolerant to Al stress. PBC396 and PBC621 also highly adaptive in acidic field.

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