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Genetic Variation for Seedling Traits in Hydroponics and Correlated Response with Mature Plant Traits on Acid Soil Field

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Authors' contributions

This work was carried out in collaboration between all authors. Author GOSO designed the study, wrote the protocol and wrote the first draft of the manuscript. Author GOSO managed the literature searches, analyses of the study, performed the hydroponics and field experiments as part of author GOSO PhD research under the supervision of authors LLB and SAA. All authors read and approved the final manuscript.

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ABSTRACT

Four levels of aluminium activity (0, 5, 50 and 300 μMAl^{3+}) were used to screen soybean seedlings in hydroponics for 3 days (3D) and 13 days (13D). The objective of the research was to determine genetic variation for aluminium stress tolerance in seedlings (primary root length, root and shoot dry weight) and to correlate the hydroponics result with that of mature plants on acid soil field. The four levels of aluminium activity constituted the main plots while 49 soybean genotypes constituted the subplots in a split-plot design, with three replications in both 3D and 13D. Genetic variance was higher than other variance components, leading to moderate to high heritability estimates (56.16 – 92.52%) for aluminium stress tolerance in both 3D and 13D hydroponics media. Genetic Coefficient of variation (GCV) ranged from a very low value of 1.88% for shoot dry weight in 13D hydroponics media to a very high value of 116.39% for primary root length in the 3D hydroponics media with

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higher phenotypic coefficient of variation (PCV) in 3D and 13D. The GA for primary root length was high in 13D (95.30%) and very high in 3D (187.37%) compared to very low values (2.71 – 9.01%) observed for the root and shoot dry weights in both 3D and 13D hydroponics media. Significant and highly significant positive correlations were observed between most of the seedling traits in 3D and 13D and between the hydroponics and acid soil field in general. The results of the current study indicate that aluminium stress tolerance in tropically adapted genotypes of soybean is heritable and that there is sufficient genetic variation for selection of seedling traits of the studied population in hydroponics. The results of correlation also implies that a two – stage selection process, beginning with selection of tender seedling root traits at 50/300 µMAl^{3+} in 3D, to be subsequently followed by selection for root dry weight at 0 µMAl^{3+} in 13D prior to acid soil field evaluation. Further studies on the activity of manganese and iron on the growth and development of soybean in the presence and absence of aluminium is required.

Keywords: Genotypic and phenotypic variances; PCV; GCV; heritability; GA.

1. INTRODUCTION

Soybean is a very important leguminous world crop with high value in human and livestock nutrition and in the industry. Soybean grains contain high percentage of protein (40%) and high quality oil and therefore processed into various human foods (soymilk, soyogi, vegetable oil, baby food, beverages, etc) and livestock feeds. The haulms are also utilized in feeding livestock. Soybean contributes to the fertility of the soil via its root nodules which are left in the soil after harvesting. Despite this importance of soybean, the continent of Africa, including Nigeria is yet to explore its full capacity utilization.

The International Institute for Tropical Agriculture (IITA) has the mandate for soybean improvement and has developed high yielding early, medium and late maturing varieties with resistance/ tolerance to abiotic and biotic stresses for the various Nigerian ecologies, particularly the savannas. However, not much attention has been accorded the breeding of soybean for acid stress tolerance, and aluminium stress tolerance in particular.

Nigeria is a large country with diverse soil types, ranging from acidic to saline soils and therefore require crop varieties that are best suited for each soil type. The rain forest ecological zone of South-East and South-South Nigeria is largely predominated by acid soils [1] and pockets of acid soils exist in various parts of Nigeria where soybean production is discouraged due to its sensitivity to acidity and aluminium (the major phytotoxic element in acid soils). There is therefore the need for concerted effort to breed for acid/aluminium stress tolerance in soybean with a view of expanding the area of land cultivated to the crop and increase its output in Nigeria. Limited effort at breeding for acid/ aluminium stress tolerance in hydroponics, sand culture and acid soil field had identified acid/ aluminium tolerant genotypes of soybean in the respective media [2,3,4]. However, whether there is enough variability to substantiate the basis for selection needs to be addressed. The presence and magnitude of genetic variability in a gene pool is the pre-requisite of a breeding programme [5]. Moreover, the degree of relationship in the relative performance of the genotypes in the various media (i.e. between hydroponics and acid soil field) needs to be addressed.

The current research was therefore initiated with the objective of determining genetic variability for aluminium stress tolerance for seedling traits in hydroponics and correlated response with mature plant traits on acid soil field in tropically adapted genotypes of soybean.

2. MATERIALS AND METHODS

2.1 Hydroponics Experiment

The hydroponics experiment is part of a PhD thesis of Ojo [2] for which the experimental procedure had already been published [3,4]. A brief description of the procedure is therefore presented below.

Forty nine (49) tropically adapted soybean genotypes across the three maturity groups of early, medium and late, with diverse response to aluminium stress tolerance [2] were evaluated for aluminium stress tolerance at 4 levels of aluminium activity (0, 5, 50 and 300 µMAl^{3+}) for 3 days (3D) and 13 days (13D) in hydroponics.

Germinated four days old seedlings transferred to hydroponics solution, grew for three days (3D) and thirteen days (13D) in hydroponics and were harvested at the age of seven and seventeen days respectively. Nutrient composition of the hydroponics solution is presented in Table 1. The hydroponics nutrient media were changed after every three days of continuous bubbling in the 13D hydroponics to avoid the exhaustion of nutrient elements. This renewal of hydroponics nutrient solution sustained the growth of the seedlings throughout the thirteen days (13D) in hydroponics. The four levels of aluminium constituted the main plots in a split-plot design, while the forty-nine genotypes constituted the sub-plots in both 3D and 13D. Each experiment was replicated three times.

Table 1. Composition of nutrients in hydroponics

KH_2PO_4	0.50	mML^{-1}
KCl	0.50	$mM\ L^{-1}$
$NaH_2PO_4.2H_2O$	1.00	$mM\ L^{-1}$
NH_4NO_3	0.80	$mM\ L^{-1}$
$Ca(NO_3)_2.4H_2O$	1.50	$mM\ L^{-1}$
$MgSO_4$	1.00	$mM\ L^{-1}$
$Fe(NO_3)_2$	80.00	$\mu M\ L^{-1}$
H_3BO_3	20.00	$\mu M\ L^{-1}$
$ZnSO_4.7H_2O$	3.00	$\mu M\ L^{-1}$
$MnCl_2.4H_2O$	3.00	$\mu M\ L^{-1}$
$CuSO_4.5H_2O$	3.00	$\mu M\ L^{-1}$
$(NH_4)_6M_{O7}O_{24}.4H_2O$	0.80	$\mu M\ L^{-1}$

$mM\ L^{-1}$ = Millimole per litre; $\mu M\ L^{-1}$ = Micromole per litre; The various levels of Al^{3+} were supplied in the form of $Al_2\ (SO4)_3$; Adapted from Howell and Bernard (1961)

Data were taken on primary root length, root dry weight and shoot dry weight, as previously described [4].

2.2 Acid Soil Field Experiment

The acid soil field experiment is part of a PhD thesis of Ojo [2]. Below is a brief description of the experimental procedure:

The National Root Crops Research Institute (NRCRI), Umudike, Nigeria, was selected as the acid soil field site in the rain forest zone of South-East Nigeria. The physical and chemical properties of the acid soil field are presented in Table 2. Umudike is situated on Latitude 5º 29'N and Longitude 7º 32'E at an altitude of 122 m above sea level.

Forty nine genotypes of soybean were planted out in a randomized complete block design with three replications in July, 2003.

Table 2. Physical and chemical properties of the soil used for acid soil field experiment in Nigeria during the 2003 cropping season

Sand (%)	77.60
Silt (%)	13.60
Clay (%)	8.80
Textural class	Loamy sand
PH	4.50
Organic carbon (%)	2.16
Organic matter (%)	3.73
Total nitrogen (%)	0.20
Available P(mg kg^{-1})	8.90
Ca^{++} ($cmolkg^{-1}$)	1.60
Mg^{++} ($cmolkg^{-1}$)	0.80
K^{+} ($cmolkg^{-1}$)	0.04
Na^{++}($cmolkg^{-1}$)	0.15
H^{+}($cmolkg^{-1}$)	0.55
Al^{3+}($cmolkg^{-1}$)	1.65
Exchangeable acidity ($cmolkg^{-1}$)	2.20
ECEC	4. 79

Each plot consisted of 3 ridges of 5 m length, spaced 1.0m apart with plot size of 15 m^2. Seeds were drilled into the crest of ridges and later thinned down to 26 plants per meter after emergence. The field was sprayed with a pre – emergence herbicide immediately after planting and manually weeded at five (5) weeks after planting (WAP). Fertilizer was applied at the rate of 10 kg N/ha, 36 kg P_2O_5/ha and 20 kg K_2O/ha within the first two (2) weeks after planting (WAP). Harvesting was carried out in November of the same year and data were taken on days to flowering, plant height at maturity (cm), number of pods per plant and grain yield (tons/ha).

2.3 Data Analysis

Data generated from all the experiments were subjected to analysis of variance by the General Linear Model (GLM) and the Analysis of variance (ANOVA) procedures of SAS (1990) while SPSS statistics 17 was used to correlate genotypic means of phenotypic traits.

Components of variance were estimated according to Bliss et al. [6]. Variance components were obtained by equating the mean square for a source of variation to its expectation and solving for the unknown (Eq. 1, 2, 3).

$$\delta^2_e = M_3 \quad (1)$$

$$\delta^2_{ge} = M_2 - M_3/r \quad (2)$$

$$\delta^2_g = M_1 - M_2/rt \quad (3)$$

Where: $\boldsymbol{\delta^2_e}$, $\boldsymbol{\delta^2_{ge}}$ and $\boldsymbol{\delta^2_g}$ are components of variance for error, genotype by aluminium interaction and genotype, respectively. M_1, M_2, and M_3 are the observed values of the mean squares for the genotype, interaction and error, respectively [7].

Broad sense heritability ($\mathbf{H_{BS}}$) was calculated as the ratio of the genotypic variance to phenotypic variance using the formula of Allard [8]:

$$\delta^2_g / \delta^2_{ph} \times 100 \quad (4)$$

Where $\mathbf{H_{BS}}$ = broad sense heritability (%), $\boldsymbol{\delta^2_g}$ = genotypic variance, $\boldsymbol{\delta^2_{ph}}$ = phenotypic variance

$$\boldsymbol{\delta^2_{ph}} = \boldsymbol{\delta^2_e} + \boldsymbol{\delta^2_{ge}} + \boldsymbol{\delta^2_g} \text{ as defined by Fehr [7].} \quad (5)$$

PCV (phenotypic coefficient of variation) and GCV (genotypic coefficient of variation) were calculated from the formula:

PCV = phenotypic standard deviation/mean;
GCV = genotypic standard deviation/mean

Genetic advance (GA) as percentage of the mean was calculated at 10% selection intensity (I=1.76). The GCV, PCV, $\mathbf{H_{BS}}$ and GA were all expressed in %.

3. RESULTS

Means, range, variance components, coefficients of variation, heritability and genetic advance of seedling traits of tropically adapted soybean genotypes evaluated in acid short term hydroponics for 3D and 13D are presented in Table 3.

Higher mean values were observed for the primary root length (PRL), root dry weight (RDW) and shoot dry weight (SDW) in 13D compared to the 3D hydroponics media with very wide range for all the three seedling traits in both media. Genetic variance was generally higher than genotype x aluminium variance and error variance, leading to moderate to high heritability estimates (56.16 – 92.52%) for all the three seedling traits in both 3D and 13D hydroponics media. Genetic Coefficient of variation (GCV) ranged from a very low value of 1.88% for shoot dry weight in 13D hydroponics media to a very high value of 116.39% for primary root length in the 3D hydroponics media. Higher values were observed for the phenotypic coefficient of variation (PCV) compared to the GCV in both 3D and 13D. The trends in both GCV and PCV were however the same, and similar to that for the genetic advance as percentage of the mean (GA). The GA for primary root length was high in 13D (95.30%) and very high in 3D (187.37%) compared to very low values (2.71 – 9.01%) observed for the root and shoot dry weights in both 3D and 13D hydroponics media.

Significant (*P*<0,05) and highly significant (*P*<0,01) positive correlations were observed between the seedling traits (primary root length, root and shoot dry weight) at all levels of aluminium activity in 3D except the control (0 μMAl^{3+}) and root traits at almost all the levels of aluminium activity in 13D hydroponics (Table 4). No significant correlation was observed between any of the root traits (primary root length and root dry weight) in 3D and shoot traits in 13D except between root dry weight at 5 μMAl^{3+} (RDW5) in 3D and shoot dry weight at 50/300 μMAl^{3+} (SDW50/SDW300) in 13D. However, highly significant positive correlation was observed between shoot dry weight at almost all the levels of aluminium activity in 3D and shoot dry weight at 50/300 μMAl^{3+} activity in 13D hydroponics.

Generally, higher correlations were observed between root dry weight at 50/300 μMAl^{3+} and field traits in 3D, while higher correlations were observed between root dry weight at 0/5 μMAl^{3+} and field traits in 13D. Seedling traits at the highest levels of aluminium activity (50 and 300 μMAl^{3+}) in 3D hydroponics were significantly (*P*<0.05) and highly significantly (*P*<0.01) correlated with all the plant traits (days to flowering (DF), plant height (PHT) and number of pods/plant (PPP)) except grain yield (Table 5). However root dry weight at 50 and 300 μMAl^{3+} (RDW50 and RDW300) were highly significantly correlated with grain yield. Better correlative response was observed between seedling traits in 13D hydroponics and field traits. Seedling root traits at all levels of aluminium activity in 13D hydroponics were significantly and highly significantly correlated with all the field traits except grain yield. Grain yield was however highly significantly correlated with seedling root traits at all levels of aluminium activity except PRL0 and PRL5. Except for the control (SDW0), shoot dry weight at all levels of aluminium activity was highly significantly correlated with days to flowering and plant height in both 3D and 13D hydroponics.

Table 3. Means, range, variance components, coefficients of variation, heritability and genetic advance of seedling traits of tropically adapted soybean genotypes evaluated in acid short term hydroponics for 3D and 13D

Traits	Mean	Range	σ_g^2	σ_{ge}^2	σ_e^2	σ_{ph}^2	GCV (%)	PCV (%)	H_{BS} (%)	GA (%)
				3D						
PRL	3.85	2.12 – 14.50	39.21	7.34	0.31	46.86	116.39	127.24	83.67	187.37
RDW	13.25	7.20 – 29.80	0.82	0.59	0.05	1.46	6.83	9.12	56.16	9.01
SDW	79.38	35.40 – 161.70	5.31	2.02	0.72	8.05	2.90	3.57	65.96	4.15
				13D						
PRL	10.89	3.11 – 24.00	46.42	15.31	0.25	61.98	62.56	72.29	74.90	95.30
RDW	39.90	11.00 – 87.30	0.99	0.04	0.04	1.07	2.49	2.59	92.52	4.22
SDW	192.80	120.00 – 251.50	13.20	3.20	3.20	19.73	1.88	2.30	66.90	2.71

PRL: *Primary root length;* ***RDW:*** *Root dry weight;* ***SDW:*** *Shoot dry weight*

Table 4. Phenotypic correlation of genotypic means between seedling traits in 3D and 13D of tropically adapted soybean seedlings evaluated in acid short term hydroponics

Traits	13D Hydroponics											
3D hydroponics	**PRL0**	**PRL5**	**PRL50**	**PRL300**	**RDW0**	**RDW5**	**RDW50**	**RDW300**	**SDW0**	**SDW5**	**SDW50**	**SDW300**
PRL0	0.47**	0.43**	0.34*	0.34*	0.11	0.12	0.10	0.06	-0.13	-0.06	-0.04	-0.03
PRL5	0.53**	0.51**	0.63**	0.63**	0.28*	0.29*	0.07	0.31*	-0.03	-0.17	-0.07	0.01
PRL50	0.67**	0.65**	0.83**	0.83**	0.56**	0.56**	0.29*	0.53**	-0.07	0.02	0.02	0.11
PRL300	0.69**	0.67**	0.81**	0.81**	0.50**	0.50**	0.27	0.47**	-0.05	-0.01	-0.04	0.07
RDW0	0.23	0.21	-0.02	-0.22	-0.01	0.05	-0.01	-0.31	-0.02	0.22	0.10	0.08
RDW5	0.49**	0.45**	0.59**	0.58**	0.53**	0.53**	0.26	0.53**	-0.01	0.06	0.32*	0.38**
RDW50	0.58**	0.57**	0.77**	0.76**	0.78**	0.76**	0.36*	0.75**	0.01	0.03	0.17	0.26
RDW300	0.47**	0.46**	0.70**	0.70**	0.82**	0.78**	0.42**	0.82**	-0.04	-0.06	0.20	0.27
SDW0	-0.16	-0.18	-0.31*	-0.30*	-0.37**	-0.36*	-0.19	-0.42**	0.26	0.65**	0.26	0.21
SDW5	0.31*	0.35*	0.46**	0.47**	0.47**	0.52**	0.15	0.43**	-0.13	0.26	0.44**	0.46**
SDW50	0.38**	0.41**	0.54**	0.55**	0.49**	0.54**	0.17	0.45**	-0.13	0.23	0.43**	0.45**
SDW300	0.36*	0.39**	0.51**	0.53**	0.44**	0.49**	0.14	0.41**	-0.15	0.19	0.40**	0.42**

PRL0: *Primary root length at 0 μM*Al^{3+}*;* ***PRL5:*** *Primary root length at 5 μM*Al^{3+}*;* ***PRL50:*** *Primary root length at 50 μM*Al^{3+}*;* ***PRL300:*** *Primary root length at 300 μM*Al^{3+}*;* ***RDW0:*** *Root dry weight at 0μM*Al^{3+}*;* ***RDW5:*** *Root dry weight at 5 μM*Al^{3+}*;* ***RDW50:*** *Root dry weight at 50 μM*Al^{3+}*;* ***RDW300:*** *Root dry weight at 300 μM*Al^{3+}*;* ***SDW0:*** *Shoot dry weight at 0 μM*Al^{3+}*;* ***SDW5:*** *Shoot dry weight at 5 μM*Al^{3+}*;* ***SDW50:*** *Shoot dry weight at 50 μM*Al^{3+}*;* ***SDW300:*** *Shoot dry weight at 300 μM*Al^{3+}

Table 5. Phenotypic correlation of genotypic means between seedling traits in hydroponics (3D and 13D) and mature plant traits of tropically adapted soybean

Traits						**3D hydroponics**						
FIELD	**PRL0**	**PRL5**	**PRL50**	**PRL300**	**RDW0**	**RDW5**	**RDW50**	**RDW300**	**SDW0**	**SDW5**	**SDW50**	**SDW300**
DF	.27	.17	.42**	.42**	.18	.43**	.52**	.49**	-.22	.36*	.37**	.30*
PHT	.19	.16	.39**	.40**	.19	.51**	.50**	.49**	-.15	.46**	.44**	.38**
PPP	.15	.23	.45**	.33*	-.21	.20	.47**	.46**	-.15	.19	.23	.25
GYLD	-.01	.09	.31*	.23	-.19	.09	.42**	.41**	-.13	.13	.15	.16
Traits						**13D hydroponics**						
FIELD	**PRL0**	**PRL5**	**PRL50**	**PRL300**	**RDW0**	**RDW5**	**RDW50**	**RDW300**	**SDW0**	**SDW5**	**SDW50**	**SDW300**
DF	.50**	.49**	.43**	.42**	.59**	.60**	.38**	.50**	.00	.38**	.43**	.43**
PHT	.45**	.45**	.37**	.37**	.55**	.55**	.32*	.49**	.11	.52**	.61**	.61**
PPP	.29*	.32*	.54**	.55**	.64**	.61**	.37**	.65**	-.10	-.02	.06	.10
GYLD	.19	.22	.43**	.45**	.60**	.57**	.39**	.61**	-.06	-.07	.05	.14

PRL0: *Primary root length at 0 μMAl^{3+};* ***PRL5:*** *Primary root length at 5 μMAl^{3+};* ***PRL50:*** *Primary root length at 50μMAl^{3+};* ***PRL300:*** *Primary root length at 300 μMAl^{3+};* ***RDW0:*** *Root dry weight at 0μMAl^{3+};* ***RDW5:*** *Root dry weight at 5μMAl^{3+};* ***RDW50:*** *Root dry weight at 50μMAl^{3+};* ***RDW300:*** *Root dry weight at 300μMAl^{3+};* ***SDW0:*** *Shoot dry weight at 0 μMAl^{3+};* ***SDW5:*** *Shoot dry weight at 5 μMAl^{3+};* ***SDW50:*** *Shoot dry weight at 50 μMAl^{3+};* ***SDW300:*** *Shoot dry weight at 300 μMAl^{3+}* ***DF:*** *Days to flowering;* ***PHT:*** *Plant height at maturity;* ***PPP:*** *number of pods/plant;* ***GYLD:*** *Grain yield*

4. DISCUSSION

The higher values observed for genetic variance compared to other variance components observed for all the seedling traits in both 3D and 13D hydroponics was due to the preponderance of additive genes, indicating that aluminium stress tolerance is heritable. This finding is consistent with the conclusion of Bianchi – Hall et al. [9] that aluminium stress tolerance is a heritable trait in their studies of F_4 derived soybean population in short term hydroponics. The moderate to high heritability observed for the seedling traits in hydroponics indicates their suitability for selection for aluminium stress tolerance. The highest heritability observed for primary root length (83.67%) and root dry weight (92.52%) in 3D and 13D respectively, is an indication that these traits should be concentrated on, in any selection work for the respective media.

The inconsistent pattern of correlation observed between seedling traits in hydroponics and mature plant traits on the field could be due to inconsistent ratings arising from the inability of hydroponics to discriminate all genetic sources of aluminium tolerance as previously observed [10]. It could also be attributed to the relative response of the studied genotypes to aluminium stress at different ages. The highly significant positive correlation (0.70 - 0.83) observed between tender seedling root traits at 50/300 µMAl^{3+} in 3D and older seedling root traits in 13D is an indication that tender seedlings could be indirectly used to select for aluminium stress tolerance in older seedlings. The higher number of correlations observed between tender seedling traits at 50/300 µMAl^{3+} and field traits compared to fewer number of correlations between tender seedling traits at 0/5 µMAl^{3+} and the field traits is an indication that selection for aluminium stress tolerance in 3D is more effective at high aluminium activity in 3D. The correlation of 0.55 – 0.64 observed between field traits and seedling root dry weight at 0/5 µMAl^{3+} compares favourably with that observed between field and seedling root dry weight at 300 µMAl^{3+} (0.50 – 0.65), and higher than the correlation range of 0.32 – 0.39 between field traits and root dry weight at 50 µMAl^{3+} in 13D. This is an indication that little or no aluminium is required in selecting aluminium tolerant older seedlings in hydroponics. The tender seedlings in 3D are more sensitive to changes in aluminium levels as reflected in their changes in genotypic ranking than older seedlings. This observation is consistent with the findings of Villagarcia et al. [10], Emorlayev et al. [11] and Ojo [2], indicating that the phytotoxic effect of aluminium on the growth and development of crop plants is age dependent. The phytotoxic effect on older seedlings at 0 µMAl^{3+} in the absence aluminium in 13D could be attributed to the effect of iron (Fe) and manganese (Mn). The growth of leguminous crops on acid soils and acidic medium in general, is affected by toxicities of Al, Mn and Fe [12,13,14,15]. Hence the need for an in depth studies on iron and manganese activity on the growth and development of tropically adapted soybean genotypes in an acid medium.

The results of the current study indicate that aluminium stress tolerance in tropically adapted genotypes of soybean is heritable and that there is sufficient genetic variation for selection of seedling traits of the studied population in hydroponics. The results of correlation also implies that a two – stage selection process should be adopted, beginning with selection of tender seedling root traits at 50/300 µMAl^{3+} in 3D, to be subsequently followed by selection for root dry weight at 0 µMAl^{3+} in 13D prior to acid soil field evaluation. Such double stage approach will enhance efficiency in selection and minimize the cost of evaluation, owing to reduction in population size from stage to stage.

Further studies on the activity of manganese and iron on the growth and development of soybean in the presence and absence of aluminium is required to deepen our knowledge on elemental phytotoxicity in crop plants.

5. CONCLUSION

Results indicate that there is sufficient genetic variability for aluminium stress tolerance in acid short term hydroponics in the studied soybean population. The highly significant correlation observed between the hydroponics and the field indicate that soybean genotypes could be selected in acid short term hydroponics for acid soil field stress tolerance evaluation. The highly significant correlation observed between the field and hydroponics when aluminium was excluded from hydroponics, suggest the significant effect of iron and manganese activities on the growth of soybean in an acidic medium that need to be addressed in subsequent studies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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