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*Agriculture
For Life And
Wealth Creation*



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Preface

This is the book of Extended Abstracts of the Agriculture Congress on 'Agriculture For Life and Wealth Creation' held at Putrajaya, Malaysia from December 12 to 15, 2006. This Congress, the second, was organized by the Faculty of Agriculture, Universiti Putra Malaysia in collaboration with Ministry of Agriculture and Agro-based Industry (MOA), Ministry of Plantation Industries and Commodities (MPIC), Malaysia Agriculture Research and Development Institute (MARDI) International Society for Southeast Asian agricultural Sciences (ISSAAS) and The Incorporated Society of Planter (ISP).

The increasing demand on the agriculture sector to feed the continuous rising of world population coupled with limited resources available for agriculture has made agriculture a critical global issue. There is also a need to develop agriculture as a profitable and lucrative business to draw more people and investors into this sector. Based on these scenarios, the Faculty of Agriculture, Universiti Putra Malaysia took the initiative to organize Agriculture Congress 2006 to address and deliberate topics pertaining to the above theme. The congress covered various disciplines in agriculture, which include aquaculture, agricultural and animal production technologies, extension services, food processing and industries, food safety, genetic resources and utilization, molecular techniques in agriculture, microbial technology, marketing and policies, new crops, nutraceutical, agriculture pest management, plant nutrition and fertilizer, plantation management technology and sustainable agriculture.

A total of 157 papers were presented in 3 keynote, 12 plenary, 74 oral and 68 poster. The presentations from 10 countries indicate the important of addressing these issues to ensure the agriculture sector is attractive and profitable.

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SCREENING AND SELECTION OF ACID SOIL TOLERANCE INBRED LINES IN MAIZE (ZEA MAIZE)

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INTRODUCTION

Malaysia has been very much dependent on the importation of grain maize from other countries in the past two decades, while local production is negligible (FAOSTAT, 2005). In order to achieve sufficiency, it is necessary to develop the local maize growing industry to reduce the dependence on imported maize in the long-term. However, most of the arable soils in the country are acidic. The highly weathered soils which are classified as Ultisols and Oxisols occupy 72 % of land area in Malaysia (IBSRAM, 1985). The soils have low cation exchange capacities (CEC), high soil solution aluminum concentration and are usually deficient in Ca and/or Mg. Some of these soils are being planted with corn during the early years of rubber and oil palm replanting phase, but the productivity is low on these soils due to Al toxicity and Ca and/or Mg deficiencies (Shamshuddin *et al.*, 1991). These soils can be made highly productive by application of lime (Shamshuddin *et al.*, 1989; Ismail *et al.*, 1993). However, the high application of lime in many tropical countries may not be economical and permanent solution.

Planting acid soil tolerant maize varieties offer the strategy for improving maize production under acidic soils. The identification of acid soil tolerant and high yielding inbred lines is potential to produce hybrids with complementary performance. Field trials have been proven to be effective in selecting aluminum tolerant plants, but are very expensive and time consuming. A rapid screening method is needed to identify and select a large number of genotypes in plant breeding. Screening using hematoxylin staining of seedling root has been widely used in screening relatively large populations based on the formation of an intense blue coloration in the root tips of sensitive genotypes (Polle *et al.*, 1978). This reaction occurs when hematoxylin forms a complex with Al so that the penetration and retention of Al in the roots can be assessed (Polle *et al.* 1978; Ownby, 1993).

Laboratory- and greenhouse-based techniques have been developed to identify Al-tolerant and Al-sensitive plants precisely, i.e. using nutrient solution culture and pot trials. Since the main effect of Al toxicity is the inhibition of root growth, the roots are not easily observed using soil culture. Screening in nutrient solution allows studying Al toxicity providing easy access to the root system and strict control over nutrient availability and pH (Blamey *et al.*, 1990). Some researchers use this technique to screen maize genotypes that were tolerant to Al (Kasim and Wasson, 1990; Cancado *et al.*, 1997; Giaveno and Miranda Filho, 2000). However, many nutrient solution culture studies have used the high ionic strength of nutrient solution with high Al concentrations. In low nutrient solution concentrations estimates of Al concentrations that result in decreased plant performance have been similar to those reported in soil (Blamey *et al.*, 1991).

Screening techniques using soil in glasshouse are very representative of real conditions in the field (Howe *et al.*, 1990). Soil bioassays seem to reproduce more realistic field condition (Giaveno and Miranda Filho, 2002). Information on the use of hematoxylin staining assay and screening in nutrient solution using low ionic strength and low concentration of Al in maize is scarce. The objective of this study was to screen and select maize inbred lines tolerant to aluminum toxicity using hematoxylin staining assay, screening in nutrient solution and screening in pot trials.

METHODOLOGY

Genotype. Thirty-six genotypes consisted of 6 maize inbred lines from CYMMIT, 2 composites, 1 hybrid and 27 locally-developed inbred lines were used in hematoxylin staining assay and screening in nutrient solution. Ten of the five maize inbred lines short listed from these experiments were further evaluated in pot experiment.

INBRED LINES IN MAIZE (ZEA

S Napis³

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ze from other countries in the past two decades to achieve sufficiency, it is necessary to import maize in the long-term. However, soils which are classified as Ultisol and Oxisol have low cation exchange capacity in Ca and/or Mg. Some of these soils are in the planting phase, but the productivity is low (N et al., 1991). These soils can be managed (Mail et al., 1993). However, the nutrient solution.

maize production under acidic soils. The aim is to produce hybrids with complementary aluminum tolerant plants, but an aim is to identify and select a large number of seedling root has been widely used. The blue coloration in the root tips of sensitive forms a complex with Al so that the (Ownby, 1993).

identify Al-tolerant and Al-sensitive plants. The effect of Al toxicity is the inhibition of nutrient solution allows studying Al toxicity, availability and pH (Blarney et al., 1991). tolerant to Al (Kasim and Watson, 1998). nutrient solution culture studies have been similar to those reported in the field.

al conditions in the field (Howe et al., 1991) and Miranda Filho, 2002). Information on selection using low ionic strength nutrient solution and screening in pot trials.

YMMIT, 2 composites, 1 hybrid and 1 inbred and screening in nutrient solution. The results are presented in pot experiment.

Hematoxylin staining assay (HS). Maize seeds were surface sterilized with 1.5% and germinated inside the moisture paper until the roots were 2 cm or more in length. Three aluminum concentrations (5, 10 and 20 μM) were used to screen genotypes tolerant to Al toxicity in nutrient solution. The basic protocol was based on Polle et al. (1978). The roots of seedlings cultivated for 17 hours were gently shaken with distilled water for 30 minutes. The water was then replaced by 0.2% hematoxylin solution (2g hematoxylin + 0.2g KI) for 15 minutes and was replaced again by distilled water for 30 minutes. After staining, the photograph was taken and the pattern of HA was used to classify all genotypes into tolerant, moderately tolerant, moderately sensitive and sensitive based on no or less stain and a complete staining of the root tips.

Screening in nutrient solution (NS). Initially, twelve genotypes and 4 Al levels (0, 10, 20 and 30 μM) were used to determine Al concentration that could give different response among Al-tolerant and Al-sensitive based on the root growth and aluminum tolerant index variables under the absence and the presence of Al in aerated and low ionic strength NS (1332.74 μM). Al was added to the NS from 0.1M stock of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ and the pH was adjusted to 5.5 daily. The root growth variables were net seminal root length (NSRL), ratio seminal root length (RSRL), root dry weight (RDW) and shoot dry weight (SDW). Aluminum tolerant index was based on the ratio of the root growth in NS with and without Al.

Screening in pot trial (PT). Pot experiment was conducted using two aluminum levels, i.e. acid soils with and without lime. GML was applied at 2 t ha⁻¹, 30 days before planting. The plant height and plant top dry weight were measured 40 days after planting. The chemical properties of the soil are Table 1.

Table 1. Chemical properties of the soil

	pH(H ₂ O)	K	Na	Ca	Mg	CEC	Al	Al saturation	SOM
Soil acidity	1:1	cmol _c kg ⁻¹						%	%
Acidic Soil	4.6	0.06	0.21	1.41	0.1	6.67	1.82	51	1.49
Non Acidic	5.1	0.13	0.38	2.94	0.29	7.86	0.34	27	

Experimental Design. Screening in NS and PT was conducted in a split plot design with 3 replications. Main plots were Al concentrations or soil acidity and sub plots were genotypes. Analysis of variance was calculated for each variable from means of 6 seedlings per sub plot for NS. T-test was used to compare the plant growth under acidic and non acidic soil (application of lime) in pot trial.

RESULTS AND DISCUSSION

Hematoxylin staining assay (HS). There was no root stain in 5 μM Al for all genotypes, indicating there was no Al toxicity. The Al-tolerant genotypes showed only less stain in the root tip in 20 μM , while the Al-sensitive genotypes showed complete stain in 10 μM and the intensity of stain in the root increased in 20 μM Al. A visual identification of root stain revealed HS assay was sufficient to discriminate between Al-tolerant and Al-sensitive genotypes. The genotypes SM7-3, SM7-6, SM7-10, SM7-11, SM7-12, IPB-12, CML-1 and CML-6 were characterized as Al-tolerant, while SM5-9, IPB-8, CML-2, CML-3, IPB-18, SM5-4, and SM5-5 as Al-sensitive. Other genotypes were characterized as moderately tolerant and moderately sensitive to Al toxicity.

Screening in nutrient solution (NS). Table 2 shows highly significant effects of aluminum concentrations and genotypes on all root growth variables. The interaction of aluminum and genotype also shows highly significant for NSRL, RSRL and RDW.

Table 2. Mean squares for responses of NSRL, RSRL, RDW and R/S of 12 genotypes

Source of Variation	D.F.	Mean squares			
		NSRL	RSRL	RDW	R/S
Replicate	2	0.89	0.04	0.000002	0.0001
Aluminum	3	1110.30**	10.37**	0.000122**	0.0251**
Error (a)	6	0.21	0.01	0.000001	0.0005
Genotype	11	23.63**	0.18**	0.000055**	0.1977**
AlxGen	33	7.92**	0.08**	0.000004**	0.0015
Error (b)	88	1.50	0.03	0.000002	0.0009

**,* significant at $p \leq 0.01$ and $p \leq 0.05$, respectively

Analysis of variance based on the aluminum tolerant index variables was consistent with root growth variables. However, only Relative NSRL showed highly significant for the interaction of aluminum and genotype. The results are similar to Kasim and Wasson (1990) that used 18 maize genotypes at 83, 111 and 148 μM Al. Root growth decreased significantly as Al concentration in solution increased (Fig. 1). Al affected root growth since roots absorb Al from the solution. The most common symptom of Al toxicity is a stunted root system, thick, stubby and show little branching. The interaction of aluminum and genotypes was significant for NSRL, RDW, and Relative NSRL. Hence, the appropriate comparison of means would be between genotypes at same aluminum concentration (Table 3). All variables showed variation of tolerant to Al among the genotypes. However, there was no variation among 12 genotypes at 30 μM Al on Rel-NSRL. The highest mean ranking at 30 μM Al tended to be in the same position at 30 μM Al. The concentration of 20 μM Al was sufficient to give different responses among genotypes based on Al tolerant index.

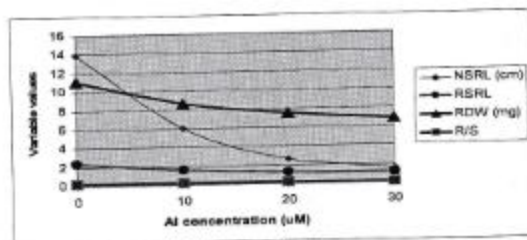


Fig. 1. Effect of 4 Al concentrations on root growth for 12 genotypes

Table 3. Mean squares for responses of Relative NSRL, NSRL, RSRL and RDW at 10, 20 and 30 μM Al

Al (μM)	Relative NSRL	NSRL	RSRL	RDW
10	0.11**	21.15**	0.16**	0.00002**
20	0.14**	22.48**	0.06**	0.00001**
30	0.096	3.39**	0.02*	0.00001**

**,* significant at $p \leq 0.01$ and $p \leq 0.05$, respectively

Using 36 genotypes and 2 Al concentrations (0 and 20 μM Al), aluminum and genotype showed highly significant effects on all root growth variables. However, the interaction of aluminum and genotype was highly significant for NSRL and R/S (Table 4).

R/S of 12 genotypes

Mean squares	
RDW	R/S
0.000002	0.0001
0.000122**	0.0251**
0.000001	0.0005
0.000055**	0.1977**
0.000004**	0.0015
0.000002	0.0009

ables was consistent with root growth variables. The interaction of aluminum and genotype for the genotypes at 83, 111 and 148 μM Al affected root growth. The increase of Al toxicity is a stunted root system. The interaction of aluminum and genotype was significant for the comparison of mean would be between genotype tolerant to Al among the genotypes on Rel-NSRL. The highest mean resistance of 20 μM Al was sufficient to give

NSRL (cm)
RSRL
RDW (mg)

L and RDW at 10, 20 and 30 μM Al

RSRL	RDW
0.16**	0.00002**
0.06**	0.00001**
0.02*	0.00001**

aluminum and genotype showed high resistance. The interaction of aluminum and genotype was highly significant

Mean squares for responses of NSRL, RSRL, RDW and R/S of 36 genotypes

Source of Variation	D.F	Mean squares			
		NSRL	RSRL	RDW	R/S
Replicate	2	1.4	0.8	0.00001	0.02
Aluminum	1	7731.9**	138.7**	0.0031**	2.16**
Genotype (a)	2	1.1*	1.4	0.00001**	0.02
Genotype	35	22.3**	1.9**	0.0002**	0.77**
Aluminum	35	14.3**	1.5	0.00017	0.56**
Genotype (b)	146	2.5	0.3	0.00003	0.01

* significant at $p \leq 0.01$ and $p \leq 0.05$, respectively

the ranking of means in each root growth and aluminum tolerant index variables, SMRG, IPB-14, SM7-6, IPB-1 and IPB-12 were classified as Al-tolerant, CML-5, IPB-8, IPB-11, IPB-15, IPB-17, IPB-18, IPB-19, SM5-5, SM7-12, Sel-A, Sel-G and Suwan as Al-sensitive and another genotypes as moderately tolerant and moderately sensitive to aluminum toxicity.

Screening in pot trial (PT). Analysis of variance for plant height and plant top dry weight showed significant for soil acidity and highly significant for genotype. However, there was no interaction between soil acidity and genotypes. The result from 2 series of pot experiments, IPB-8, CML-4, CML-5, IPB-11, IPB-13, IPB-15, IPB-20, SW-2, SM7-12, Putra J-58 and Sel-G were classified as Al-sensitive genotypes. IPB-8 as Al-sensitive inbred line was consistent result on 2 series pot experiments, while SMRG, SM7-11, IPB-12, IPB-14, CML-1, SM7-6, CML-6, SM 5-5, SM 5-4, SM 5-9, IPB-19, MT-13, Suwan, Sel-A, CML-3 and IPB-18 were classified as Al-tolerant genotypes due to no significant differences for plant height and plant top dry weight under acidic and non acidic soil.

CONCLUSION

Screening in benzoxazin staining assay, screening in nutrient solution and screening in pot trial, 36 maize genotypes were classified into four groups i.e. tolerant, moderately tolerant, moderately sensitive and sensitive to Al. The Al-tolerant lines are IPB-12, SM 7-6 and SM 7-11 and the Al-sensitive inbred lines are IPB-8 and IPB-11.

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