Original Research Article

Genetic analysis of maize response to salt stress in hydroponic medium

ABSTRACT

Background: The productivity of maize (*Zea mays*L.) from the farmers' fields has been characterized as being low due to salinity among other contributing factors. In this study I intend to investigate the use of phenotypic traits as a selection criterion in a breeding program and to screen maize genotypes for tolerance to salt stress.

Aim: The objectives of this study were therefore i) estimate mean entry based heritability for genotypic response to root and shoot length ii) ascertain an appropriate genotypic maize screening criterion in salinity medium and iii) identify tolerant maize genotypes in hydroponic medium.

Methodology: The experiment was set up as 5 x 11 factorial completely randomized design (CRD) with three~~3~~ replications at the University of Zambia, pathology laboratory. Thus 11 genotypes were evaluated in five doses of salinity concentrations giving 55 experimental units.

Results: Genotypes exhibited significant differences ($P< 0.05$) in response to both parameters (root and shoot length) across salinity concentrations. Similarly the response of root and shoot length in different salinity concentration across genotypes was also significant ($P< 0.001$). The entry based heritability estimate was found to be 0.76 and 0.18 for responses to root and shoot ~~responses~~ respectively.

Conclusion: Shoot length was identified as a key trait to utilize in screening maize genotypes for tolerance to salt stress under hydroponic conditions despite possessing a low heritability estimate. In this study tolerant genotypes to salt stress were identified as ZHYB 2, ZHYB 7 and ZHYB 11.

Keywords: Salinity, *Zea mays*, Heritability, Shoot length, Indirect selection

1. INTRODUCTION

Maize (*Zea mays*) is an important cereal in Africa and the most popular products being maize flour and animal feed. In addition, it can be processed into cooking oil, maize snacks and the fresh grains can either be roasted or boiled on the cob. Over 30% of the caloric intake of people in sub-Saharan Africa comes from maize [1]. Maize occupies approximately 24% of farmland in Africa and the average yield stagnates at around 2 tons/hectare/year due to biotic and abiotic factors [1, 2, 3]

Among the abiotic factors, soil salinity is an important factor causingsignificant yield losses.Worldwide, it has been estimated that 20% of total cultivated and 33% of irrigated agricultural lands are afflicted by high salinity [4].Under normal soil situations, the higher osmotic pressure inplant cells permits the absorption of water and essential nutrients from a soil solutioninto the root cells. On the other hand, under salt (NaCl) stress conditions, the high concentrationof salts in the soil solution prevents absorption of water and essential mineralsat the expense of facilitating the entry of Na+ and Cl− ions into the cells.

Thus absorbedsodium ion (Na+) and chlorine (Cl−) ions have an effect on maize productivity withgermination and early seedling growth being more sensitive to salinity than later developmental stages[5].Furthermore,Na^+interfere with potassium uptake which results in disturbing stomatal undulations causing severe water loss and necrosis in maize [6, 7].In addition, physiological processes such as respiration, photosynthesis, nitrogen fixation and starch metabolism are also affected thereby causing losses in crop productivity primarily due to wilting [7].

The physiological responses of a plant to salt stress are often complicated, which makes experiments difficult to interpret. With advent changes in technology, physiological measurements have been revolutionized enabling the use of bioinformatics, molecular markers and genomic approaches to aid genotypic selection [8, 9].However, in this studyIintend to investigate the use of phenotypic (morphological) traits, as a selection criterion in a breeding program. Being that early seedling growth is more sensitive to salinity than later developmental, employment of phenotypic traits as a means of hydroponic screening of germplasm under salt stressed conditions is a viable option. Selection of maize responses to nutrientunder hydroponics has previously utilized number of root hairs, roots length and shoots [10, 11, 12]. However, the use of number of root hairs is a complicated and time consuming venture[13]. Making the use of root and shoot length as an easier and faster alternative.

In screening for a desirable trait, ‘response to selection’ iskey as it is associated with genetic gain in selected plants/ population and narrow sense heritability was computed to be a direct function for ‘response to selection’[14]. However, in an experimental trial, the use of entry mean based heritability estimate was identified as a reliable scheme compared to other approachessuch as plot mean based heritability[14]. The objectives of this study were therefore i) estimate mean entry based heritability for genotypic response to root and shoot lengthii) ascertain an appropriate genotypic maize screening criterion in salinity medium and iii)identifytolerantmaize genotypes to salinity in hydroponic medium.

2. MATERIALS AND METHOD

2.1 Location of Study and Germplasm Used

This research was carried out in Lusaka at the University of Zambia (Africa), Department of Plant Sciences in the Plant Physiology laboratory (15^{0}23 ’41.3”S; 28^{0}20’ 11.8” E)in March 2022. The germplasmsused in this study were promising candidate (yet to be released) maize hybrids from Seed-co company Zambia. Namely coded as:ZHYB-4, ZHYB-8, ZHYB-9, ZHYB-7, ZHYB-12, ZHYB-3, ZHYB-11, ZHYB-10, ZHYB-5, ZHYB-2 and ZHYB-6.

Comment [AM1]: Why did you not put this with ordered number? I mean ZHYB-2, then 3, 4, 5, 6, 7, 8, 9, 10,11,12

2.2 Design of the Experiment and Medium Preparation

The medium was prepared following a modified procedure by K~~k~~erridge and Kronstard [15] (Table 1).The experiment was set up as 5 x 11 factorial completely randomized design (CRD) replicated three (3) times. Thus five (5) doses of sodium chloride in molar concentration of 0 M, 0.075M, 0.15M, 0.225M and 0.3 M in nutrient media and eleven (11) genotypes were used in the experiment.Giving a total of 165 experimental units randomly assigned to each plot (test tube). Each test tube had a diameter and height of 2.3 and 14.5 cm respectively. The pH was adjusted to pH 6.5 using HCl and NaOH buffer solutions.

Table 1. Nutrient solution used in hydroponics study at University of Zambia, School of Agricultural Science

Nutrient	Con (mg/ L)	Chemical formula	Compound name
N	42.61	NH_4NO_3	Ammonium Nitrate

K	23.5	$K_2HPO_4.3H_2O$	Potassium hydrogen phosphate trihydrate
Zn	0.16	$ZnSO_4.7H_2O$	Zincsulphateheptahydrate
Mg	14.6	$MgSO_4.7H_2O$	Magnesium sulphateheptahydrate
Cu	0.06	$CuSO_4.5H_2O$	Copper sulphate pentahydrate
Fe	1.60	$FeSO_4.7H_2O$	Iron sulphateheptahydrate
Ca	48.10	$CaCl_2.2H_2O$	Calcium chloride dehydrate
Mo	0.03	$NaMoO_4.2H_2O$	Sodium molybdate dehydrate
Mn	0.03	$MnSO_4.H_2O$	Manganese sulphate monohydrate
B	0.32	H_3BO_3	Boric acid
Na, Cl	Varied*	NaCl	Sodium Chloride

N- Nitrogen, K-Potassium, Zn- Zinc, Mg- Magnesium, Cu- Copper, Fe- Iron, Ca- Calcium, Con- concentration
Mo- Molybdenum, Mn- Manganese, B- Boron, *- Factorial scheme under
hydroponiccombinations with either 0, 0.075, 0.15, 0.225 and 0.3 NaClM

2.3 Placement of Maize Seedlings

Twenty five (25) seeds per genotype weregerminated on separate petri dishes lined with filter paper soaked in distilled water and placed in the germination chamber at 25^0C for 5 days. After 5 days, 15 seedlings of each genotypes having uniformroot length were selected and then transferred into 50 mL~~l~~ test tubes covered in black plastichaving nutrient solution of varying salinity concentration and supported over the solutionwith the stopper. Covering ~~of~~ test tubes with black plastic was done so as to prevent any possible algae growth in solution. Aeration of the nutrient solution was done twice daily, morning and evening usingaquarium air pump to provide oxygen and refilling of the solution was subsequently done.

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2.4 Data Collection and Analysis

On the 11th day after placement the seedlings were harvested. Data was collected on all experimental units with respect to rootlength and shoot length. Measurements on root and shoot length on all experimental units were done as by Ndeke and Tembo[10]. Analysis of variance on root length and shoot length were performed assuming a fixed model. The means for the main effects were separated using fisher protected least significant difference (LSD), at significant level of $\alpha = 0.05$.

The entry mean data broad heritability estimates for root length and shoot length were estimated from variance components as described by Nyquist and B~~b~~aker [16]

$$H = \sigma^2_G / \sigma^2_G + \frac{\sigma^2_{GM}}{m} + \frac{\sigma^2_e}{mr}$$

Where H- heritability on the entry mean basis; σ^2_{G-} Variance component due to genotypic effect, σ^2_{GM} – Variance component due to interaction of genotype and salinity molarity effect. m- number of molarity (concentration) levels; r- replications

Correlation (r) was computed using regression analysis with shoot length and root length being the response and explanatory variable respectively. All the datawas performed using GenStat statistical package (18th edition).

3.RESULTS

3.1 Evaluation of Genotypes in Various Levels of Salinity Concentration

Genotypes exhibitedsignificantdifferences ($P< 0.05$)inresponsetobothparameters(rootlength and shootlength)acrosssalinityconcentrations.Similarly theresponseofbothparametersindifferentsalinityconcentrationacrossgenotypeswasalsosignificant($P<0.001$) (Table 2).

Table 2. Analysis of variance for mean squares for measured parameters evaluated in hydroponics at the University of Zambia, School of Agricultural Science

SOV	Root length	Shoot length
Genotype	56.13***	11.69*

Molarity	269.76***	759.95***
Genotype x Molarity	5.53**	5.9
Error	2.9	5.56

SOV- Source of variation;***, ** and *- Significant at P= 0.001, 0.01 and 0.05 respectively DF: Degrees of freedom

Further analysis, revealed thatgenotype ZHYB-4 had a higher mean performance with respect to root length (8.74 cm) (Table 3). On the other handZHYB-11 (10.7 cm) was the best performer with respect to shoot length(Table 3). Further analysis showed that there was no significant (P> 0.05) genetic correlation (r= 0.18)associated between shoot and root length

Table 3. GenotypicmeansofmeasuredparametersacrosssalinityconcentrationevaluatedatUNZAplantPhysiologylaboratory

Genotype	RL (cm)	SL (cm)
ZHYB-4	8.7	7.1
ZHYB-8	8.3	7.1
ZHYB-9	8.0	6.7
ZHYB-7	7.5	9.9
ZHYB-12	7.2	6.1
ZHYB-3	7.0	5.7
ZHYB-11	6.8	10.7
ZHYB-10	6.7	4.2
ZHYB-5	6.4	5.0
ZHYB-2	6.2	8.0
ZHYB-6	6.0	6.8
LSD (α= 0.05)	1.7	1.2

LSD- Least significant difference, RL- root length; SL- shoot length

Further more the results exhibited, a decrease in root and shoot length across genotypes as the concentration increased (Table 4). However, the rate of decrease was higher with shoot lengththan root length as evidenced by the root / shoot length ratio. (Table 4, plate 1)

Table 4.Means of measured parameters in varying salinity concentration across genotypes

NaCl (M)	Root length (cm)	SL (cm)	Ratio- RL/SH
0	11.44	15.18	0.75

0.75	8.27	8.11	1.02
0.15	5.8	4.56	1.27
0.23	5.01	3.99	1.26
0.3	4.55	3.98	1.14
LSD (α= 0.05)	**0.84**	**1.15**	

NaCl- Sodium Chloride, Least significant difference (LSD). SL- shoot length, RL- root length. M- morality

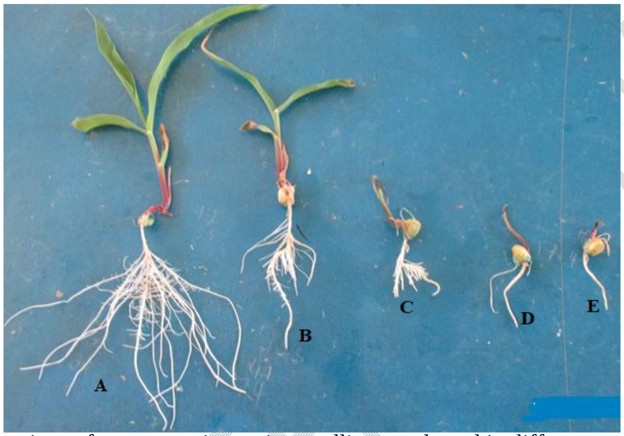

Plate 1: Comparison of representative maize seedlings evaluated in different sodium chloride concentration exposure. Concentration at **A**- 0 M; **B**- 0.075 M; **C**- 0.15 M; **D**- 0.23 M and **E**- 0.3 M

3.2 Estimated Heritabilty on Entry Mean Basis

Heritability on entry mean basiswith regards to root length response to salinity was high (H= 0.76). On the contrary it was low for shoot response trait (H= 0.18) (Table 5).

Table 5.Genotypic variance component and heritability entry mean basis estimate

	Trait	
Variance Component	**Root**	**Shoot**
Genotypic	3.55	0.41
G X C	0.88	0.11
Error	2.90	5.56
H (entry)	0.76	0.18

H(entry)- Heritability on entry mean basis; G x C- Genotypic x concentration

4. DISCUSSION

Salinity affects maize growth leading to reduction in productivity. Identifying appropriate maize traitto utilize in selection of tolerant genotypes under salinity conditions is fundamental.In this studygenotypicroot and shoot response were evaluated with the view of ascertaining the appropriate trait to utilize in screening maize germplasm in salt stressed hydroponic medium. Overall the results obtained showed that they were genetic differences among genotypes across salinity concentrations for both root and shoot length response. However, the rate of decrease in shoot length with increased salinity concentration was greater than root lengthas evidenced by the root/ shoot length ratio(Table 4). Earlier researchers also deduced that disastrous effect of salt stress on shoots is more than roots [7, 17].The differences in traitresponses may be due to increase in abscisic acid that rapidly reduces leaf growth rate due to a reductionin the number of elongating cells and/or the rate of cellelongation compared to roots[18, 19]. This study revealed that the prevalent negativeeffect on meanshootlength across genotypes was observed at a concentration rate of 0.23 M and no significant effect was observed with the further increase in concentration across genotypes (Table 4).An earlier study deduced that 0.25 M NaCl damages maize plants and may stunt growth and cause severe wilting [20]. Similar to what was observed in this study (Table 4; Plate 1).

Heritability estimates showed that genotypic gain to selection was more likely to be achieved with utilization of root length (H= 0.78) as a selection criterion. However shoot length (H= 0.18) was identified as a preferable trait to utilize in screening germplasm as it was associated with a greater severe effect (Table 4; Plate 1). This may give a more reliable attribute of selected genotypes if shoot at the expense of root is used as a selection criterion in salt stressed medium conditions. This is further backed by the results entailing ~~that~~ that there was no significant correlation (r= 0.18) associated between shoot and roots responses among genotypes across concentration. Previous studies found that shoot length should be used as a selectioncriterion for genotypic response in salinity medium, agreeing with this study [7, 21].

To concretize, though shoot length responsehas been identified as an indirect selection criterion for genotypic response in salinity medium, it should only be used as a complementto directselection owing to its low heritability value. It has been established that ‘grain weight’ and ‘number’ are associatedtomaize productivity under varying salt stress [7]. This implies that ‘grain weight’ and ‘number’can be used as a direct selection criteria for screening maize genotypes under salt stressed field conditions. Complementation can be achieved through employment of initial indirect selection using shoot length under hydroponic experiment, to reduce evaluated germplasm to affordable numbers, for direct evaluation in the field. Previous studies have also demonstratedthat traits with low phenotypic variation explained or heritability can be used as a supplement to direct selection and not a substitute of it[12, 22].

Arising from utilizationof shoot length as a selection criterion for genotypic response to salt stress, ZHYB 2, ZHYB 7 and ZHYB 11were identified as tolerant genotypes. Salt-tolerant genotypes were found to relatively accumulate lower sodiumand more potassium to sodium and calcium to sodium ratio than sensitive genotypes [21]. This entails that screening for accumulation of sodium ions can be used to identify tolerant genotypes but the process is destructive and time consuming [23].

5. CONCLUSION

The entry based heritability estimate was found to be 0.76 and 0.18 for responses to root and shoot responses respectively. However the disastrous effect was higher for mean genotypic response to shoot than roots with increase in salt concentration. Implying that shoot responses to salinity concentration can be used to compliment direct genetic selectionowing to low estimated heritability. In this study tolerant genotypes were identified as ZHYB 2, ZHYB 7 and ZHYB 11, employing shoot length.

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