Genetic Analysis of Maize Response to Salt Stress in Hydroponic Medium

Langa Tembo [a*]

[a] *Department of Plant Science, School of Agricultural Sciences, University of Zambia, Box 32379, Zambia.*

Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

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 the use of phenotypic traits as a selection criterion and screening of maize genotypes for tolerance to salt stress was investigated. The objectives of this study were therefore to i) estimate heritability for genotypic response to root and shoot length ii) ascertain an appropriate genotypic maize screening criterion in saline medium and iii) identify tolerant maize genotypes in hydroponic medium. The experiment was set up as 5 x 11 factorial completely randomized design (CRD) with three replications at the University of Zambia, pathology laboratory. Thus 11 genotypes were evaluated in five doses of salinity concentrations giving 55 experimental units. 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 respectively. 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.

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*Corresponding author: Email: langatembo@yahoo.com, langa.tembo@unza.zm;

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" [1-3].

"Among the abiotic factors, soil salinity is an important factor causing significant 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 in plant cells permits the absorption of water and essential nutrients from a soil solution into the root cells. On the other hand, under salt (NaCl) stress conditions, the high concentration of salts in the soil solution prevents absorption of water and essential minerals at the expense of facilitating the entry of Na+ and Cl− ions into the cells.

"Thus absorbed sodium ion (Na+) and chlorine (Cl−) ions have an effect on maize productivity with germination 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 study I intended 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 nutrient under hydroponics has previously utilized number of root hairs, roots length and shoots [10-12]. 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' is key 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 approaches such as plot mean based heritability [14]. The objectives of this study were therefore i) estimate 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 to salinity in hydroponic medium.

2. MATERIALS AND METHODS

2.1 Location of Study and Germplasm Used

This research was carried out in Lusaka at the University of Zambia (Zambia), Department of Plant Sciences in the Plant Physiology laboratory (15^023 '41.3"S; 28^020' 11.8" E) in March 2022. The germplasms used in this study were maize hybrids from Seed-co company Zambia. Namely coded as: ZHYB-2, ZHYB-3, ZHYB-4, ZHYB-5, ZHYB-6, ZHYB-7, ZHYB-8, ZHYB-9, ZHYB-10, ZHYB-11 and ZHYB-12.

2.2 Design of the Experiment and Medium Preparation

The medium was prepared following a modified procedure by Kerridge 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

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	$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate
Mg	14.6	$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
Cu	0.06	$CuSO_4.5H_2O$	Copper sulphate pentahydrate
Fe	1.60	$FeSO_4.7H_2O$	Iron sulphate heptahydrate
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 hydroponic combinations with either 0, 0.075, 0.15, 0.225 and 0.3 NaCl M

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.

2.3 Placement of Maize Seedlings

Twenty five (25) seeds per genotype were germinated 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 uniform root length were selected and then transferred into 50 mL test tubes covered with black plastic having nutrient solution of varying salinity concentration and supported over the solution with the stopper. Covering 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 using aquarium air pump to provide oxygen and refilling of the solution was subsequently done.

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 root length 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 α = 0.05.

The entry mean heritability estimates for root length and shoot length were estimated from variance components as described by Nyquist and Baker [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 data was performed using GenStat statistical package (18th edition).

3. RESULTS

3.1 Evaluation of Genotypes in Various Levels of Salinity Concentration

Genotypes exhibited significant differences (P< 0.05) in response to both parameters (root length and shoot length) across salinity concentrations. Similarly the response of both parameters in different salinity concentration across genotypes was also significant (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 that genotype ZHYB-4 had a higher mean performance with respect to root length (8.74 cm) (Table 3). On the other hand ZHYB-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) between shoot and root length.

Table 3. Genotypic means of measured parameters across salinity concentration evaluated at UNZA plant Physiology laboratory

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 length than root length as evidenced by the root / shoot length ratio (Table 4, Plate 1).

3.2 Estimated Heritabilty on Entry Mean Basis

Heritability on entry mean basis with 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 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

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

Variance component	Trait	
	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 trait to utilize in selection of tolerant genotypes under salinity conditions is fundamental. In this study genotypic root 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 length as 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 trait responses may be due to increase in abscisic acid that rapidly reduces leaf growth rate due to a reduction in the number of elongating cells and/or the rate of cell elongation compared to roots [18,19]. This study revealed that the prevalent negative effect on mean shoot length
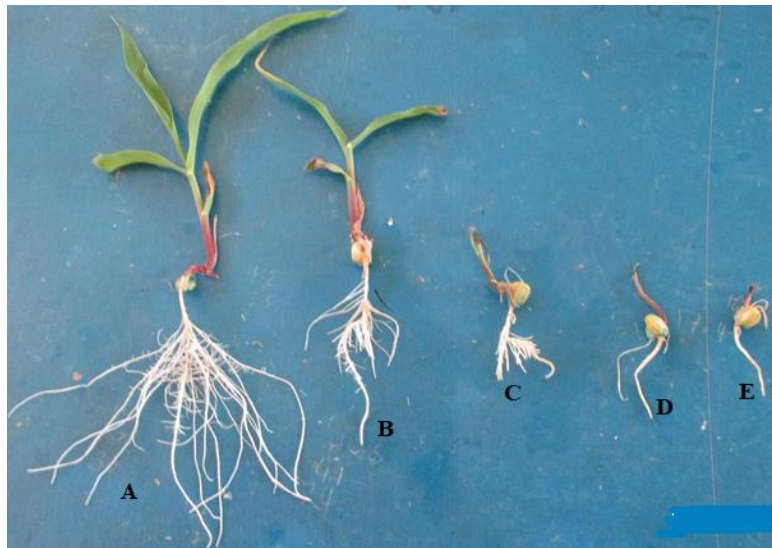
Plate 1. Comparison of representative maize seedlings evaluated in different sodium chloride concentration exposure. Concentration at A- 0 M; B- 0.075 M; C- 0.15 M; D- 0.23 M and E- 0.3 M

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 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 selection criterion for genotypic response in salinity medium, agreeing with this study [7,21].

To concretize, though shoot length response has been identified as an indirect selection criterion for genotypic response in salinity medium, it should only be used as a complement to direct selection owing to its low heritability value. It has been established that 'grain weight' and 'number' are associated to maize 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 demonstrated that 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 utilization of shoot length as a selection criterion for genotypic response to salt stress, ZHYB 2, ZHYB 7 and ZHYB 11 were identified as tolerant genotypes. Salt-tolerant genotypes were found to relatively accumulate lower sodium and 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 selection owing 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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COMPETING INTERESTS

Author has declared that no competing interests exist.

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