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Selection criteria for drought tolerance at the vegetative phase in early maturing maize

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Identifying drought tolerant maize (*Zea mays* L.) at the vegetative stage is a meaningful effort at reducing cost and time of screening large number of maize genotypes for drought tolerance. The primary objectives of this study were to assess the effectiveness of vegetative traits in discriminating between drought tolerant and drought sensitive hybrids and to determine the stage at which the stress should be imposed to achieve maximum difference between hybrids with contrasting responses to drought. A drought tolerant hybrid (TZEI 18 × TZEI 31) and a sensitive hybrid (TZEI 108 × TZEI 87) were evaluated in a pot experiment conducted in a screen house facility and in the field at the Teaching and Research Farm of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife in 2011. The experiment was laid out as a randomized complete block design in each of four groups of different water treatments, namely one week of watering for 1, 2, and 3 weeks after planting and withdrawing watering for the rest of the period of experimentation (43 days after planting), along with a treatment involving watering throughout the period of the experiment. Data were collected on root and shoot traits under the four levels of water treatment and the data were subjected to analysis of variance (ANOVA) and orthogonal contrasts. Results of the ANOVA showed significant mean squares for root length, root fresh weight, shoot length, number of root branches, shoot dry weight, root dry weight and number of shed leaves. Withdrawing water a week or two after planting induced large differences between the drought tolerant and drought sensitive genotypes for root length, root dry weight, number of root branches and number of shed leaves. In conclusion, root length, root fresh weight, shoot length, number of root branches, shoot dry weight, root dry weight and number of shed leaves were the most reliable traits for pre-anthesis drought tolerance. Watering for only one or two weeks after planting was the best treatment for identifying drought tolerant maize genotypes at the vegetative growth stage.

Key words: Drought, maize, pre-anthesis, seedling stage.

INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop cultivated for its high economic importance as a food crop

and an industrial raw material. It is grown extensively in Africa primarily for its carbohydrate-rich kernel. On the

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African continent alone, maize constitutes the staple diet for 300 million people (Shenaz, 2010). Maize productivity is greatly constrained by both biotic and abiotic factors, including drought which occurs at any stage of maize development. When drought stress occurs at the flowering stage, it causes an estimated annual yield loss of about 15% (Edmeades et al., 1995) but if it occurs at the seedling and early vegetative stage, reduced crop establishment, zero yield or complete crop failure, may result (Edmeades et al., 1994). Stress tolerant maize varieties offer a means of stabilizing yield at no additional cost to the farmer (Edmeades et al., 1997) and the development of drought tolerant maize genotypes by breeders have resulted in yield stability, thereby improving global maize production. Two mechanisms for adaptation to drought in maize have been reported; the first is the possession of drought-tolerance genes, which make the variety capable of continuing growth and grain formation under drought stress. The second is the drought-escaping mechanism which is the ability of the genotype to flower and produce grains before drought sets in (Badu-Apraku et al., 2011). Therefore, maize breeders use a two-pronged approach to combat the adverse effect of drought stress, including the development of early and extra-early maturing cultivars that are drought escaping and the introgression of drought tolerance genes into the early and extra-early cultivars to enable them withstand mid-season drought if and when it occurs during the flowering and grain-filling periods. Much of the research on drought tolerance in maize has been concentrated on the adult stage and traits such as anthesis-silking interval (ASI), stay green characteristic, plant and ear aspect and grain yield have been used singly or in a base index to select drought tolerant genotypes at the flowering and grain-filling periods (Banziger and Lafitte, 1997; Banziger et al., 1999; Badu-Apraku et al., 2011) without considering how the genotypes would respond if drought occurred at the vegetative stage. Information is limited on the improvement of maize for drought tolerance at pre-anthesis stage. Moser (2004) found that pre-anthesis drought significantly reduced the number of kernel rows, number of kernels per row, and 1000-kernel weight while it consistently increased harvest index. The study also showed significant interaction effects between moisture regime and cultivar on grain yield and other agronomic traits.

In southwestern Nigeria, the rainfall pattern is bimodal. Rainfall stabilizes for early maize planting between April and May and this is the time early-season maize is usually planted. Developing specific maize genotypes, which tolerate drought at vegetative stage, will offer the farmers in this region the opportunity of planting maize earlier in the year (that is, late February to early April) immediately after the first few rains. Earlier studies conducted in this agro-climatic zone showed that maize planted that early significantly out-yielded those planted

later in the season primarily because grain-filling coincided with the period of relatively high incident solar radiation (Fakorede, 1984; Fakorede and Opeke, 1985). However, analysis of the long-term historical climatic data at Ile-Ife, a typical rainforest location showed that rainfall during the early part of the season is erratic and has been on a decreasing trend since 1975 (Fakorede and Akinyemiju, 2003), thus exposing maize planted at this time to the risk of unpredictable drought stress at the vegetative stage. One way to minimize the negative impact of drought at the vegetative stage of maize is to develop drought-tolerant genotypes for this growth stage. Unfortunately, suitable traits that could distinguish between drought-tolerant and drought-sensitive genotypes at this stage are not yet known. Therefore, assessment of the reliability of vegetative traits for selecting drought-tolerant genotypes which can identify maize with pre-anthesis drought tolerance will not only improve the indices used for selecting drought tolerant maize but will shorten the time for selection.

The objectives of this study were to (i) identify the vegetative traits that could be used in discriminating effectively between drought-tolerant and drought-sensitive maize genotypes and (ii) determine the period at the vegetative stage that water stress should be imposed to bring about the maximum differential response in the performance of resistant and susceptible maize genotypes.

MATERIALS AND METHODS

The present research was conducted in the screen house facility as well as the Teaching and Research Farm of the Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria in 2011. Two maize hybrids, namely TZEI 108 × TZEI 87 (drought sensitive) and TZEI 18 × TZEI 31 (drought-tolerant) were the test crops. The inbred parents from which the hybrids were developed were extracted from a genetically broad-based tropical maize population TZE W Pop DT STR, which combined drought tolerance with moderate resistance to *Striga*. The population was developed by maize scientists at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The hybrids had undergone multi-location field evaluation under induced drought stress and they were selected using IITA's drought tolerance base index that integrates increased grain yield under drought stress and well-watered environments with a short ASI, increased number of ears per plant, good stay green characteristic, and high scores for plant aspect and ear aspect under drought stress (Badu-Apraku et al., 2011).

Screen house experiment

The screen house experiment was conducted using 328 L plastic pots, each filled with 5 kg of well drained loamy soil. The pots were arranged in four groups of different water treatments. Within each group, pots were arranged using randomized complete block design (RCBD) with four replicates, and five seeds were sown per pot. Seedlings were later thinned to four per pot. Adequate watering of all pots was done immediately after planting to ensure good germination of the seeds. Subsequent watering was done at the rate of 0.6 L /pot/week. All the four blocks were watered for 7 days after planting (DAP); thereafter, the water treatments were imposed.

In the first block [water treatment (WT7)], watering was stopped 7 DAP while in the second block (WT14), the experiment was watered till 14 DAP. The third block was watered till 21 DAP (WT21) and the fourth block till 35 DAP (WT35). For each water treatment, data were collected on percentage seedling emergence, plant height (PH) in cm, total number of leaves per plant (LFNOS) and number of shed leaves (LFSHED). The experiment was terminated at 43 DAP, when the stover of the plants was harvested. To minimize breakage during data collection, soil in the pots were softened by watering and roots were carefully removed and washed gently under a flowing tap. Data were recorded on plant fresh weight (FPW), root fresh weight (FRW), and shoot fresh weight (FSW) using a weighing balance in the Seed Science Laboratory of the Department of Crop Production and Protection. Root length (RL= the length of the longest root branch), shoot length (SL), and number of root branches (RBG) were also recorded. The harvested samples were oven dried at 75°C to a constant weight after which data were taken on plant dry weight (DPW), root dry weight (DRW), and shoot dry weight (DSW). Moisture content (MC) was calculated using the formula:

$$MC (\%) = (\epsilon - \gamma) / \epsilon \times 100$$

Where, ϵ = plant fresh weight and γ = plant dry weight.

Field experiment

A field experiment was carried out in the growing season of 2011 at the Teaching and Research Farm of the Obafemi Awolowo University. The land for the experiment was ploughed and harrowed to provide good tilth. Three seeds were sown per hill at a spacing of 75 cm x 50 cm to give a plant population of 53,000 plants per hectare. Each plot consisted of two rows of 5 m long and the experiment was laid out as a RCBD with three replicates. First fertilizer application (NPK 15:15:15) was carried out 3 weeks after planting (WAP) at the rate of 60 kg/ha. A post emergent non-selective herbicide (Gramoxone at 1.5 L/ha) was also applied 4 WAP to reduce weed competition with the maize seedlings. A special guide was attached to the nozzle to minimize drifting of chemical to non-targeted plants. The second fertilizer application was carried out 5 WAP using urea (46:0:0) at the rate of 60 kg/ha. Data were recorded on emergence count 7 and 9 days after planting (DAP). Plant aspects (PASP) and ear aspects (EASP) were recorded on a scale of 1 to 5. The PASP was rated based on plant type, architecture and physical appeal, where, 1 = excellent plant type and 5 poor, where, 1 = excellent plant type and 5 = poor. For ear aspect, 1 = clean, uniform, large, and well-filled ears and 5 = ears with undesirable features. In addition, number of ears harvested (EHARV), ear weight (EWT), ear diameter (EDIA), ear length (ELT), kernel row number (KROW), moisture content (MC), shelling percentage (SHELL), average leaf number (ALN), plant height (PLHT), leaf number above ear (LFNOS_AB), leaf number below ear (LFNOS_BL), and grain weight (GWT) were also recorded. Grain yield in kilogram per hectare adjusted to 15% moisture assuming 80% shelling percentage was computed using the following formula:

$$Y = \epsilon + \frac{(100 - n)}{85} \times \frac{(10000)}{\phi} \times 0.80$$

Where, Y = grain yield (kg/ha), ϵ = ear weight (kg/m²), n = grain moisture at harvest, ϕ = plot area m², 85 = percentage dry matter used to adjust for 15% moisture content, 10000 = total land area (in m²) of a hectare, and 0.80 = 80% shelling percentage.

Furthermore, data were taken on plant fresh weight (FPW), root

fresh weight (FRW), shoot fresh weight (FSW), root length (RL), shoot length (SL), and number of root branches (RBG) through destructive sampling of five plants per plot at 6 WAP. The stovers were then oven dried at a temperature of 75°C to a constant weight after which data on root dry weight (DRW), shoot dry weight (DSW), moisture content (MC), and plant dry weight (DWT) were recorded.

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) using PROC GLM of the Statistical Analysis System [(SAS), SAS Institute, 2002] to test for significant effects of the experimental treatments on all the hybrid traits and means were separated using the Least Significant Difference (LSD). Orthogonal contrasts were used to decompose the treatment sum of squares into single degrees of freedom to determine the critical moisture regime that showed differences between the two hybrids.

RESULTS

Results of the ANOVA show significant differences between the two hybrids for FRW, RL, SL, RBG, DSW, and DRW but not for other traits (Table 1). The water treatment effect was significant for all traits except FRW and DRW (Table 1). Variety x water treatment interaction mean squares were not significant for all traits except FRW and PH (Table 1). The results also show that for most traits, water treatment effects contributed more to the total sum of squares than hybrid effects, although for FSW and RL, the reverse was the case (Table 1). Results of the orthogonal contrasts showed in every case that the WT7 vs others accounted for the largest proportion of the variation due to the water treatment, ranging from 33% for number of leaves to 97% for number of root branches (Table 1). The WT14 vs WT21 + WTWT35 accounted for the next largest proportion of the water treatment effects although some of the values were not significant. The WT21 vs WT35 accounted for the least proportion of the water treatment effects. Across water treatment regimes, the drought-tolerant hybrid gave better response to induced drought stress than the drought sensitive hybrids, with differences ranging from about 11% for number of root branches to 35% for root length (Table 2). The V x WT effect was significant only for root fresh weight and plant height.

The patterns of response of the two genotypes to increasing water stress for traits with significant G and G x E interaction are shown in Figures 1A to 1D. For root length, the two genotypes were significantly different ($p < 0.05$) from each other with the tolerant genotype having the higher values when the water stress was imposed from 7 DAP till the termination of the experiment (Figure 1A). Similarly, differences between the two hybrids occurred for induced stress from 7 DAP for the other traits, with the widest difference occurring at 7 to 14 days of stress for root fresh weight and number of root branches, and 21 DAP for number of shed leaves. For each of the four traits, there was no significant difference ($p > 0.05$) between the two hybrids when the plants

Table 1. Sum of squares derived from analysis of variance for some vegetative traits of two early-maturing (one drought-tolerant and one drought-sensitive) maize hybrids under four water treatments in the screen house of Obafemi Awolowo University, Ile-Ife, in 2011.

Source of variation	DF	FPW (g)	FRW (g)	FSW (g)	RL (cm)	SL (cm)	RBG	DRW (g)	DSW (g)	MC (%)	DPW (g)	PLHT (cm)	ALN	LFSHE D
Replication	3	25.3ns	1.5ns	17.9ns	223.2ns	3.0ns	4.5ns	0.03ns	0.16ns	30.4ns	29.9ns	6.6ns	1.02ns	0.2ns
Hybrid (V)	1	57.5ns	3.2*	33.4ns	927.5**	4.6ns	3.5**	0.06**	0.80**	0.3ns	0.4ns	5.1ns	0.04ns	6.3**
Water treatment (WT)	3	1242.2**	0.6ns	1206.8**	682.5**	511.4**	10.6**	0.06ns	6.47**	240.7**	240.5**	499.1**	27.2**	35.9**
WT7 vs others	1	702.4**	0.3ns	676.0**	609.8**	308.4**	10.3**	0.04*	4.04**	213.3**	211.5**	323.4**	10.7**	24.9**
WT14 vs (WT21,WT35)	1	443.9**	0.2ns	426.7**	72.6ns	163.1**	0.3ns	0.01ns	2.18**	19.7ns	21.3ns	151.6**	9.3**	8.3ns
WT21 vs WT35	1	95.8*	0.2ns	104.0*	0.01ns	40.0**	0.01ns	0.01ns	0.25ns	7.71ns	7.7ns	24.2*	7.2**	2.8ns
V × WT	3	26.5	5.7*	24.45	238.2ns	8.16	2.43	0.03	0.21	12.36	1.83	26.2*	1.48ns	1.8ns
Error	24	402.7	14.1	362.2	840.3	115.81	12.3	0.21	2.76	305.09	308.9	97.1	3.5	11.9
Corrected Total	31	1727.8	19.4	1620.4	2673.4	634.88	30.9	0.36	10.19	576.49	579.7	607.9	31.8	56.1
%Contribution of genotype SS		3.33	16.69	2.06	34.69	0.72	11.29	16.34	7.88	0.05	0.07	0.83	0.11	11.2
%Contribution of treatment SS		71.90	3.04	74.48	25.53	80.56	34.29	17.09	63.46	41.76	41.48	82.10	85.74	64.0

*, **, Significant F-test at 0.05 and 0.01 level of probability, respectively; ns = not significant at $P \leq 0.05$. FPW = plant fresh weight; DSW = shoot dry weight; FRW = root fresh weight; MC = moisture content; FSW = shoot fresh weight; DPW = plant dry weight; RL = root length; PLHT = plant height; SL = shoot length; ALN = average leaf number; RBG = root branches; LFSHE = number of leaf shed; DRW = root dry weight.

Table 2. Mean values of root fresh weight, root length, number of root branches, root dry weight, shoot dry weight, and number of shed leaves of drought-tolerant and sensitive maize hybrids evaluated under different moisture regimes under screen house conditions.

Source	Tolerant hybrid	Sensitive hybrid	LSD _(0.05)	Difference (%)
Root fresh weight (g)	2.22	1.58	0.56	28.82
Roots length (cm)	30.68	19.91	4.32	35.10
Number of roots branches	6.09	5.43	0.52	10.84
Root dry weight (g)	0.29	0.20	0.07	31.03
Shoot dry weight (g)	1.59	1.27	0.25	20.13
Number of shed leaves	0.53	0.69	0.03	-30.12

were well watered throughout the period of the experiment. Results of the ANOVA for the field experiment showed that the two hybrids were not significantly different for all traits measured except ear length (Table not presented). The tolerant hybrid had longer ears than the susceptible hybrid.

DISCUSSION

The primary objective of this study was to identify vegetative traits that can be used for selecting drought tolerant maize genotypes. The significant mean squares of the ANOVA for root length, shoot fresh weight, number of root branches, and

shoot dry weight and number of shed leaves showed that these vegetative traits could efficiently discriminate between tolerant and susceptible maize genotypes at pre-anthesis stage. It is striking to note that the two hybrids showed no significant difference for all traits when watered throughout the period of experimentation.

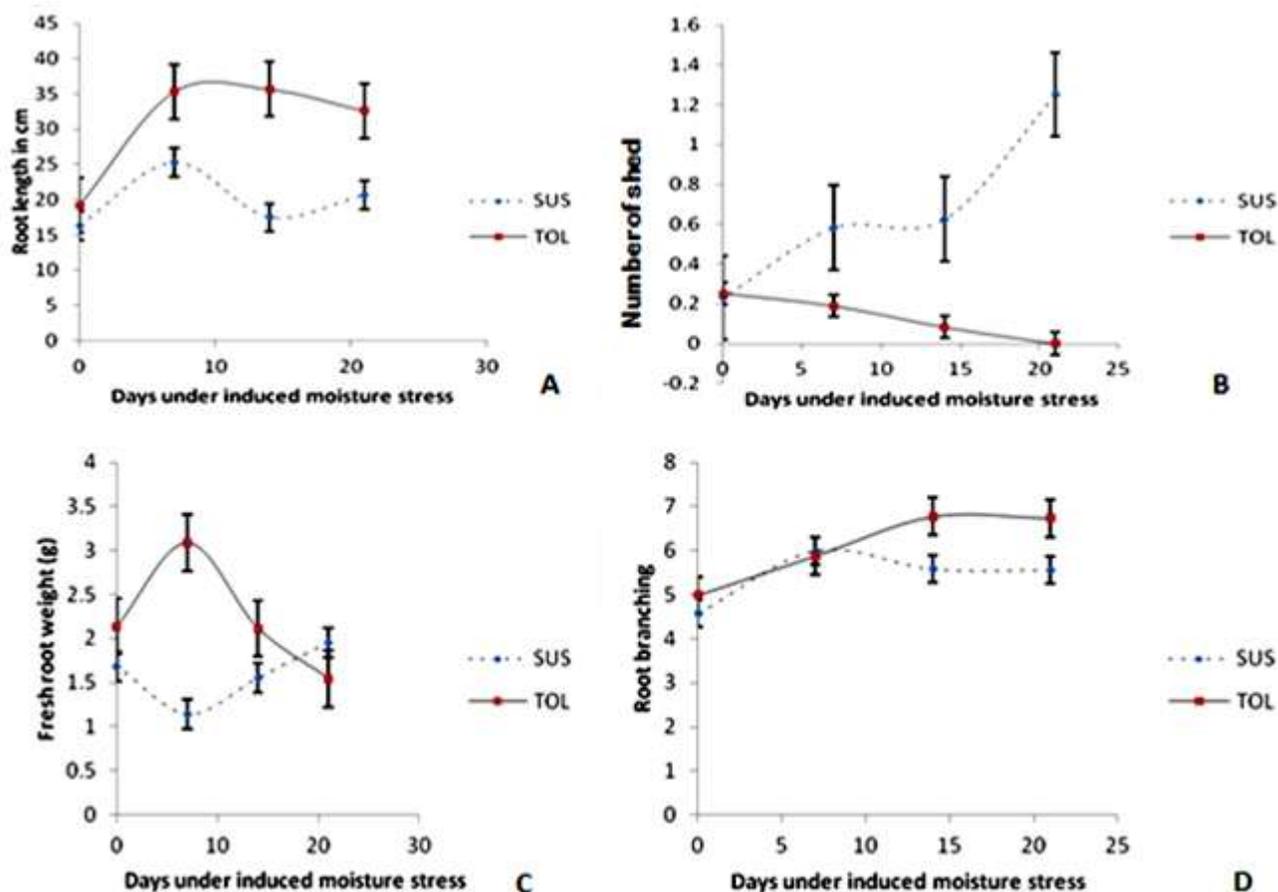


Figure 1. Pattern of response of drought-tolerant (TOL) and drought-sensitive (SUS) maize hybrids under different levels of induced drought stress. (A) root length (B) number of shed leaves (C) fresh root weight (D) number of root branches.

This indicated that the two hybrids expressed different genetic responses to water stress only. Evidently, water stress induced growth of longer roots and more root branches in the tolerant hybrid to increase its ability to search for water both vertically and laterally in the soil profile so as to buffer the effects of water loss due to evapotranspiration. Aggarwal and Sinha (1983), Nour and Weibal (1978) and Thakur and Rai (1984) attributed increase in root weight and length under drought stress to the accumulation of different solutes. Similar results have been reported for maize (Hund et al., 2006) and several other crops such as sunflower (*Helianthus annuus* L) (Tahir et al., 2002); rice (*Oryza sativa* L.) (Ekanayake et al., 1985); and alfalfa (*Medicago sativa* L.) (Zeid and Shedeed, 2006). Indeed, the importance of increased volume of the root systems of crops in acquiring water during severe moisture stress has long been recognized. A prolific root system can confer the advantage to support accelerated plant growth during the early crop growth stage and extract water from shallow soil layers that is otherwise easily lost by evaporation. This is particularly needed by maize planted early (late Feb-

ruary to early April) in the first season of the rainforest ecology of southwestern Nigeria when rainfall is erratic, unsteady and therefore, unpredictable (Fakorede and Akinyemiju, 2003). An increased root growth due to water stress has also been reported in sunflower (Tahir et al., 2002).

The initial sharp increase in the root weight of the drought-tolerant hybrid with increasing water stress could be attributed to the accumulation of different solutes as the roots searched for water, as previously reported for maize, wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* L.) by Thakur and Rai (1984), Aggarwal and Sinha (1983) and Nour and Weibal (1978), respectively. Mehdi et al. (2001) also reported significant differences in root weight among S_1 families, treatments and interaction between families and treatments in a similar study in maize. Moreover, the sensitive hybrid showed significantly higher number of shed leaves under drought conditions than the tolerant genotype and this could be attributed to its inability to obtain and retain water under moisture stress with the accompanying increased evapotranspiration due to high temperatures.

Jason et al. (2004) and Moussa (2006) attributed high number of shed leaves in plants during drought to high oxidative stress resulting from increased rate of transpiration from plant relative to water absorption rate by the plant. Water stress causes stomata closure, which reduces the carbon dioxide to oxygen (CO_2/O_2) ratio in leaves and inhibits photosynthesis. These conditions increase the occurrence of reactive oxygen species (ROS) such as superoxide radical ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^\cdot) particularly in chloroplast and mitochondria, via enhanced leakage of electrons to oxygen (Mittler, 2002; Neill et al., 2002). The superoxide radicals and their product, hydrogen peroxide, can directly attack membrane lipid and inactivate enzyme activity (Sairam et al., 2000). The hydroxyl radical, one of the most reactive oxygen species, is responsible for oxygen toxicity *in-vivo*, causing damage to DNA, protein, lipids, chlorophyll and almost every other organic constituent of the living cell (Becana et al., 1998). The lower number of shed leaves in the tolerant hybrid is evidence of its inherent ability to effectively protect its cellular and sub-cellular systems from the cytotoxic effects of active oxygen radicals with anti-oxidative enzymes such as superoxide dismutase (SOD), peroxidase (POX), catalase (CAT), as well as metabolites like glutathione, ascorbic acid, tocopherol and carotenoids (Alscher et al., 2002).

Although reduced plant height is a characteristic of drought stress, it was not significantly different between the two hybrids under stress conditions and, therefore, was not a reliable trait for selecting for tolerance to drought at the vegetative stage. This finding corroborates the results of Badu-Apraku et al. (2005; 2011) who reported that plant height was not a reliable secondary trait for selecting for improved grain yield under drought stress at flowering and grain filling stages. Another objective of this study was to determine the best period to impose moisture stress in order to achieve maximum difference between drought-tolerant and drought-sensitive maize genotypes. This was achieved by partitioning the total sum of squares due to water treatment into single degree of freedom to detect the source of variation accounting for the largest proportion of the water treatment effect. Graphical representation was also employed to investigate the trends of response of the two contrasting hybrids to the different water treatments. Pieces of evidence from the two approaches showed that withdrawing water after 7 DAP (WT7) accounted for the largest proportion of the total variation due to watering for root length, plant dry weight, moisture content of the plant, root dry weight, number of root branches and number of shed leaves. Graphical analysis of the response pattern of the two hybrids over the different treatment levels further showed that the drought tolerant hybrid had longer roots and lower number of shed leaves than the sensitive hybrid under the first three levels of drought stress (i.e., WT7, WT14, and WT21) but

not under the WT35 treatment. For number of root branches, the significant difference was only for WT7 and WT14. Therefore, when screening maize for drought tolerance at the vegetative phase, watering should be done for the first 1 or 2 weeks of planting and withdrawn for the next 2 or 3 weeks before assaying for genotypic responses of any of these traits. The results of the field evaluation agreed with that of the greenhouse when the experiment was watered throughout the period in which the two hybrids were not significantly different for most traits. This could imply that the results under induced drought in the greenhouse will be similar if the experiment is conducted under field conditions. However, the experiment will need to be conducted under induced drought in the field for comparison with the results of the greenhouse under moisture stress. Based on the results of this experiment, a large number of varieties of maize are being screened under greenhouse conditions using the reliable traits identified in the present study for selecting drought tolerant genotypes at the vegetative stage. Promising varieties selected will be field-tested to confirm their drought tolerance.

A major limitation of this study is that only two hybrids with contrasting responses to drought were assayed. Perhaps, more and/or different information would have been obtained if more drought-tolerant and drought-sensitive genotypes had been used. This is because the pattern of response and the level/mechanism of tolerance of a drought-tolerant or drought-sensitive genotype under the different levels of drought stress may differ from one another; that is, genotype x water treatment interaction would have been detected with each type of hybrid. Nevertheless, vital information about the reliability of the measured traits in determining drought tolerance at the vegetative stage was provided through the present study. The results obtained in the study should, therefore, be useful in screening a larger number of genotypes for drought tolerance at the vegetative growth stage of maize.

Conclusion

Two maize hybrids, one drought-tolerant and the other drought-sensitive were subjected to varying levels of induced moisture stress in a greenhouse study conducted for 43 days. Six vegetative traits, including root length, root fresh weight, number of root branches, shoot dry weight, root dry weight and number of shed leaves showed highly significant differences between the two hybrids and could therefore be considered of primary importance in determining drought tolerance at the vegetative growth stage of maize growth and development. Some or all of the traits could be used with appropriate weights to construct a base index for screening and selecting for drought tolerance at the vegetative stage. The results of this study also revealed that withdrawing water from the experiment a week or

two after planting is sufficient to induce the required water stress level in order to differentiate drought-tolerant from drought-sensitive maize genotypes at the vegetative phase.

Conflict of interests

The authors have not declared any conflict of interest.

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Abbreviation

ALN, Average leaf number; **ANOVA**, analysis of variance; **ASI**, anthesis-silking interval; **DAP**, days after planting; **DPW**, plant dry weight; **DRW**, root dry weight; **DSW**, shoot dry weight; **FPW**, plant fresh weight; **FRW**, root fresh weight; **FSW**, shoot fresh weight; **IITA**, international institute of tropical agriculture; **LFSHED**, number of leaf shed; **MC**, moisture content; **PLHT**, plant height; **RBG**, root branches; **RL**, root length; **SL**, shoot length; **WAP**, weeks after planting.

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