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Morphological and tolerance assessments of vegetative growth under waterlogged conditions in Edo State maize landraces

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ABSTRACT

Waterlogging is a major constraint to maize production in tropical regions. This study assessed growth and root adaptive responses in six maize accessions from Aduwawa, Agbede, Auchi, Enwan, Ighara and Uselu, all in Edo State, Nigeria. This study involved a two-year screening was carried out under screen house conditions to evaluate waterlogging tolerance of six landraces collected from various part of Edo State. For 30 days (four weeks), plants were subjected to waterlogging stress at two key seedling stages, three leaf (V3) and five leaf (V5). Tolerance was assessed through growth traits (plant height, leaf length, leaf width, leaf area, number of leaves and chlorotic leaves) and incidence of adventitious root formation were recorded. Waterlogging significantly reduced most growth traits ($p < 0.05$) relative to the control. Plant height under no waterlogging was 92.37 cm but fell to 38.49 cm at V5 and 35.36 cm at V waterlogging. Leaf area dropped from 172.26 cm² (control) to 57.29 cm² (V5) and 42.15 cm² (V3). Enwan displayed considerable adventitious root formation at V3, with moderate responses observed in Igarra and Agbede while most accessions lacked adventitious roots under V5 or no waterlogging. The interaction of accession × waterlogging was not significant for growth traits, suggesting generally uniform sensitivity across genotypes. The capacity for adventitious root formation under waterlogging may serve as an adaptive trait to ameliorate stress. Thus, providing valuable resources for future breeding efforts aimed at creating resilient maize varieties.

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INTRODUCTION

Maize (*Zea mays* L.) is one of the world's most important staple crops, yet its productivity is severely threatened by abiotic stresses such as waterlogging (Liang *et al*., 2020). Waterlogging occurs when soil pores become saturated with water, thereby reducing oxygen availability to roots, impairing root respiration, nutrient uptake, and leading to reduced growth and yield (Salah *et al*., 2019). Root morphological adaptations such as adventitious root formation, changes in root-to-shoot ratio, and enhanced root porosity are among the key traits enabling some maize genotypes to tolerate waterlogging (Zhang *et al*., 2025).

Studies on natural genetic variation in maize have identified quantitative trait loci (QTL) associated with root traits under waterlogging, indicating that root dry weight, root length, and total root biomass under flooded versus non-flooded conditions are heritable and can be exploited for breeding (Qiu *et al*., 2007). Also, in landraces of maize from other geographies, high plasticity in root traits under waterlogging, such as development of adventitious roots has been shown to be important for tolerance (Ye *et al*., 2018).

In Edo State, Nigeria, diverse indigenous maize landraces are underutilised in terms of documented waterlogging tolerance, especially root morphological adaptations. Given increasing incidences of flooding and poorly drained soils in many parts of the tropical region, assessing these landraces for root adaptation traits under waterlogging could inform selection and improvement of more resilient varieties. Thus, this study aims to assess morphological traits of root growth and tolerance under controlled waterlogged and non-waterlogged conditions in maize landraces from Edo State, with a view to identifying phenotypic markers useful in breeding for waterlogging tolerance.

MATERIALS AND METHODS

Experimental site and plant materials

The experiment was conducted during 2023 and 2024 in the screen house of the Department of Crop Science, Faculty of Agriculture, University of Benin, Benin City, Edo State, Nigeria. The site is located at approximately 6°23' and 6°24' North latitude and between 5°36' and 5°38' longitude.

The experimental soil type was topsoil consisting of sandy loam, collected from farmlands in Ovia North East Local Government of Edo State. 15 dm^3 buckets were used for the waterlogging experiment obtained from the Ring Road, Benin City. The Genotypes used were Maize Landrace accessions obtained from the Edo State, Nigeria, comprising Aduwawa, Agbede, Auchi, Enwan, Ighara and Uselu locations.

Experimental design and treatments

Healthy, spotless, and clean seeds were selected from the Landrace accessions to ensure high germination. The selected seeds were sterilized by soaking in NaOCl, 1 % for 30 min, then washed with sterile water, then kept in a growth chamber at room temperature, under dark conditions for 72 hours. After two days, five properly germinated seeds were selected and sowed in plastic planting buckets (width 32 cm, height 30 cm, and depth 34 cm). The buckets were filled with 15 kg of sieved dry topsoil amended with NPK fertilizer, i.e., 0.14 g urea, 0.14 g diammonium phosphate, and 0.18 g potassium chloride per kg of soil. Maize seedlings were thinned to two per bucket at the first fully expanded leaf stage. Standard screen house disease/pest control protocols were strictly followed throughout the experiment. Waterlogging treatments were imposed at three-leaf growth stages (V_3) and five-leaf (V_5) growth stages, and for 4 weeks (Severe waterlogging). The buckets were filled with water 1.5 cm above the soil surface to cover the seedling root completely. One control treatment was used for each experiment, i.e., buckets that did not have waterlogging treatment (CK). In the CK treatment, soil moisture was kept at optimum throughout the experiment. The experiments were laid out as a Split-Plot in completely randomized design (CRD), with Maize Waterlogging treatments as Main-Plots and Accessions treatments in Sub-Plots. Three replications per treatment were maintained throughout the trial. The distance between each experimental unit was 30 cm, with 50cm between Main-Plots and 30 cm between Sub-Plots. Each replicate had 18 pots, and a total of 54 pots for each experiment, including control and treatment groups.

Collection of data for plant morphology evaluation

Randomly selected plants from each bucket in each treatment were used to evaluate the effect of waterlogging treatments on plant characters at V_3 and V_5 growth stages. Data were collected on some vegetative growth characters such as plant height, leaf length, leaf width, leaf area, number of chlorotic leaves and adventitious development. Data was collected at 6 and 8 weeks after sowing for V_3 and V_5 stages, respectively.

Statistical Analysis

Data collected were subjected to Analysis of variance (ANOVA) using GENSTAT 12th Edition statistical software and significant mean differences were separated using student New-mann Kuels Test t at a 0.05 probability.

RESULTS

Effects of Waterlogging on Growth Traits

The growth characters of maize accessions from Edo State, Nigeria after a waterlogging treatment for four weeks at the three (**V_3**) and five-leaf (**V_5**) stages are presented in Table 1. The values for different maize accessions, averaged across all waterlogging treatments, showed there were no significant differences among the accessions for all growth characters considered. Effects of no waterlogging treatment (NWL) during maize vegetative growth had the tallest plant (92.37 cm), longest leaves (56.76), widest leaves (3.53 cm), largest leaf area (172.26 cm²) and number of leaves per plant (8.17). It also had the highest number of chlorotic leaves (1.24) but at par with plants waterlogged at V5 which significantly ($p < 0.05$) higher those of plant waterlogged at V3. Number of leaves were higher in V5 (1.21) than in V3 (0.94) plants. The interaction effect between the accession and waterlogging treatment (A x WL), for all the measured growth characters, were not significant.

Adventitious Root Formation

The ratings of adventitious root formation in Edo State maize accessions under water logging stress at the V_3- and V_5-leaf stages and non-waterlogged (NWL) treatments are shown in Table 6. The non-waterlogged conditions (NWL), had absence of ARs consistently for all accessions in all the replicates. Waterlogging treatments at the V_3- and V_5-leaf stages, for most accessions induced adventitious roots, indicating that AR formation may be triggered by waterlogging stress. Variations in AR development was pronounced in Enwan accession, with (+++) rating at V_3 leaf stage, signifying an abundant adventitious root response in a single replicate and a strong early-stage AR formation potential; Igarra and Agbede respectively had (++) ratings at V_3 leaf stage; Aduwawa had (+) in two V_3 leaf stage replicates; Uselu achieved (++) at V_5 leaf stage; and Auchi exhibited minimally with only one incident. Some accessions responded best at the V_3 leaf stage (e.g., Enwan, Ighara, Agbede), others at the V_5 leaf stage (Uselu), with no single stage universally superior. Given the vulnerability of early growth, a robust V_3 leaf stage AR response is especially valuable. Overall, a total of 6 plants from four (4) accessions survived the waterlogging treatment: Agbede, Igarra, and Enwan at termination of the V_3, and Auchi, Uselu, and Igara at V_5 waterlogging treatment.

Table 1: Mean values of Growth Characters of Maize Accessions from Edo State, South-South, Nigeria after a Waterlogging treatment for four weeks at the three (V_3) and five-leaf (V_5) stages

Treatment	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm²)	Number of Leaves	Number of chlorotic leaves
Accessions (A)						
Aduwawa	56.2	32.3	2.77	86.1	4.89	1.17
Agbede	58.1	38.7	2.39	93.19	5.75	1.22
Auchi	56.1	34.4	2.31	80.01	5.78	0.89
Enwan	45.3	33.3	2.08	82.23	4.69	1.25
Igarra	57.1	35.3	2.53	95.87	5.11	0.94
Uselu	60.5	37.7	2.66	105.99	6.39	1.31
Sed	13.06	7.14	0.48	23.97	1.13	0.37
Sig	ns	Ns	ns	ns	ns	ns
Waterlogging (WL)						
No waterlogging	92.37^a	56.76^a	3.53^a	172.26^a	8.17^a	1.24^a
5 leaf stage	38.49^b	25.50^b	2.12^b	57.29^b	4.86^b	1.21^a
3-leaf stage	35.36^b	23.53^b	1.72^b	42.15^b	3.27^c	0.94^a
Sed	9.24	5.05	0.37	16.95	0.8	0.26
Sig	***	***	***	***	***	***
Interaction						
A x WL	ns	ns	ns	ns	ns	ns
Sed	22.63	12.37	0.82	41.52	1.96	0.65
CV	70.60%	60.70%	57.90%	79.40%	62.40%	98.90%

Means with the same superscript in the same column do not differ significantly ($P > 0.05$).
ns- not significant at 0.05 level of probability

Table 2: Incidence of adventitious root development due to waterlogging stress at the three (V_3) and five-leaf (V_5) stages of maize Accessions from Edo State, South-South, Nigeria

Accession	Waterlogging	Rep I	Rep II	Rep III
Aduwawa	3LS	+	0	+
Agbede	3LS	++	+	+
Auchi	3LS	0	0	0
Enwan	3LS	0	0	+++
Igarra	3LS	0	+	++
Uselu	3LS	0	0	+
Aduwawa	5LS	0	0	0
Agbede	5LS	+	0	0
Auchi	5LS	0	+	0
Enwan	5LS	0	0	0
Igarra	5LS	+	++	++
Uselu	5LS	0	+	++
Aduwawa	NWL	0	0	0
Agbede	NWL	0	0	0
Auchi	NWL	0	0	0
Enwan	NWL	0	0	0
Ighara	NWL	0	0	0
Uselu	NWL	0	0	0

3LS = 3 leaf Stage

5 LS = 5 leaf stage

NWL = No waterlogging

Adventitious roots **ratings** = **0,** +**,** ++**,** +++

DISCUSSION

Waterlogging effects on growth

As expected, waterlogging resulted in marked reductions in growth traits across accessions. The sharp declines in plant height, leaf length, leaf width, leaf area, and number of leaves under both V_3 and V_5 waterlogging indicating that flooding stress severely limits vegetative growth. These findings are consistent with previous studies and with what is currently known about the impact of waterlogging stress in maize, particularly at the seedling stage (Manghwar *et al*., 2024). These reductions likely arisen from root hypoxia that impairs nutrient and water uptake, root respiration, and translocation to shoots (Yang *et al*., 2024). The consistently differences between the waterlogged treatments and the control (non-waterlogged) across all growth parameters evaluated provided unambiguous evidence of the severe inhibitory effect of waterlogging on maize growth.

In this study, the V_3 and V_5 treatments had similar reduction effect on growth except number of leaves in which a more severe reduction was noticed with the normal. This suggests early stage flooding may inflict larger damage on leaf production due to less developed root systems being more vulnerable. V_3. Similar observations were earlier reported by Ren *et al.*, (2016), Salah *et al.*, (2019), Huang *et al*., (2022), and Nikolić *et al*. (2025). This implies the particular vulnerability of maize plants to oxygen deprivation in the root zone at early stages following establishment. Interestingly, the number of chlorotic leaves was more severe with NWL and V_5 and less severe.

The high coefficients of variation (CV values > 50% for nearly all traits) suggest substantial variability among replicates and within treatments, which complicates detection of more subtle genotype × environment interactions.

Adventitious Root Formation as an Adaptive Response

The induction of adventitious roots under waterlogging (especially at V_3 stage) is a known adaptive strategy to bypass damaged or oxygen-starved root systems, pushing new roots closer to oxygenated zones near the soil surface. The fact that no adventitious roots were observed under no waterlogging confirms that this is a stress-induced trait. Among accessions, Enwan displayed the strongest response (+++ in V_3), followed by Agbede and Igarra, while Auchi had no such response. This suggests differential capacity among landraces to mount a root adaptation under flooding. The weaker or absent adventitious response under V_5 treatments for many accessions may indicate developmental stage dependence older plants may have less plasticity in root induction. Adventitious root (AR) formation is a well-known adaptive mechanism in plants, including maize, to cope with waterlogging-induced hypoxia (Zhang *et al*., 2025). The consistent absence of AR development in non-waterlogged (NWL) control plants and its induction under waterlogged conditions confirm that AR formation is an induced adaptive response to waterlogging stress in the maize accessions under study (Ye *et al.,* 2018). Several studies have shown that ARs serve multiple vital functions, including the acquisition of oxygen (Yamauchi *et al.,* 2018). For example, ARs emerging from the stem above the anoxic soil surface, can access atmospheric oxygen, which is then transported to the submerged roots through aerenchyma, alleviating oxygen deprivation (Mano *et al.,* 2006). ARs are involved in nutrient and water uptake, given the deterioration of primary root system under hypoxia. Newly formed ARs take over the critical functions of water and nutrient absorption, transport, maintaining plant hydration and metabolism (Yamauchi *et al.*, 2018). They contribute to physical support by providing additional anchorage in softened, waterlogged soil, preventing lodging and replacing damaged roots, which are crucial for plant survival and continued growth (Yamauchi *et al.,* 2018; Zhang *et al.*, 2025). Although the growth trait interactions did not show significant genotype × treatment effects, the capacity for adventitious root formation represents a potentially discriminating adaptive trait that could be further exploited in selection.

Implications for Selection and Breeding

All accessions suffered substantial growth inhibition under waterlogging, distinguishing tolerance among them based solely on reduction magnitudes is difficult, especially given high variability. However, the presence or strength of adventitious root formation particularly in Enwan, Agbede, and Igarra may serve as a useful phenotypic marker in screening for waterlogging resilience. In subsequent trials, coupling root morphological traits (e.g. root porosity, aerenchyma proportion, and root cortical cell traits) with adventitious root incidence may provide more discriminative power. Given that accession × treatment interaction was not significant, the relative ranking of accessions under stress was broadly stable but more replications, additional landraces, and lower experimental error would be needed to validate tolerant genotypes.

CONCLUSION AND RECOMMENDATIONS

The waterlogging treatments imposed at V_3 and V_5 stages significantly suppressed vegetative growth traits in all tested maize accessions from Edo State. Adventitious root formation was inducible under flooding, particularly at the V_3 stage, and varied among accessions (with Enwan, Agbede, and Igarra showing stronger responses). Although the growth trait reductions were broadly similar across genotypes, adventitious root capacity emerges as a promising adaptive trait to distinguish waterlogging resilience. Thus, root adaptive traits (especially adventitious root development) may complement morphological assessments in screening maize landraces for flood tolerance. It is suggested that accessions with strong adventitious root response should incorporated into breeding programs or as parents for hybrids aimed at waterlogging resilience.

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