

## Genetic Diversity and Early-Stage Drought Tolerance Studies in Maize Genotypes Across Contrasting Moisture Regimes

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### ABSTRACT

Accelerating genetic gains in maize breeding programs aimed at moisture-limited regions requires an understanding of the degree of genetic variability for early-stage drought tolerance. This study investigates the genetic diversity and drought tolerance using 13 agro-morphophysiological traits in 103 Maize (*Zea mays* L.) genotypes under two contrasting moisture regimes for mid-altitude locations, at forestry Research Institute of Nigeria-Federal College of Forestry Teaching and Research Nursery, Jos Plateau located in the Guinea Savannah agro ecological zone of Nigeria. Data collected were subjected to Analysis of Variance (ANOVA) and Hierarchical Cluster Analysis (HCA). Mean Square Estimates under well-watered conditions showed no significant difference ( $P \leq 0.05$ ) for all traits but revealed a statistically significant difference for seedling plant height ( $0.46^*$ ) only and showed a statistically significant difference ( $P \leq 0.05$ ) for all traits under water deficit condition. Diversity analysis revealed substantial phenotypic variation and grouped genotypes into seven distinct clusters each, reflecting broad genetic divergence. Under non-water stress conditions, the largest cluster was comprised 62 genotypes, suggesting a relatively narrow genetic base, whereas the presence of small and singleton clusters indicated highly divergent and potentially valuable drought-responsive genotypes. Under water deficit conditions, a significant reconfiguration occurred, with Cluster I including 67 genotypes and the remaining genotypes scattered across smaller clusters, demonstrating remarkable variability in drought response mechanisms. These results indicated that there were significant genetic variability and diversity among the genotypes and supports the incorporation of root-shoot traits for water-use efficiency studies in drought maize breeding programs.

**Keywords:** Genetic Diversity, Maize, Drought Tolerance, Climate-Smart Agriculture, Agro-Ecology, Characterization, Food Security, Staple food.

## INTRODUCTION

Agriculture underpins human survival by providing food and feeds through integrated biological, chemical, and physical processes, yet it is increasingly vulnerable to climate-related risks, including biotic stressors, abiotic extremes, and socio-economic pressures (El Chami and El Moujabber, 2024). In Nigeria, climate change has evolved from a projected threat into a present systemic risk. Regional temperatures are expected to rise by 1.5-6.5 °C by the end of the century, depending on emissions pathways. These trends are already evident in Nigeria's Guinea Savanna, a critical transition zone between forest and Sahelian systems, where rising temperatures, erratic rainfall, delayed planting seasons, and frequent intra-seasonal dry spells have been widely observed (Sultan and Gaetani, 2016; IPCC, 2023).

Climate vulnerability in the various agro ecological zones of the county is further amplified by shallow soils, limited irrigation infrastructure, and heavy reliance on rain-fed agriculture. These constraints have increased production instability, disrupted food supply chains, and intensified competition over land and water resources, reinforcing socio-political fragility. Climate stress thus operates as a feedback mechanism, linking environmental shocks to food system performance, rural livelihoods, and governance capacity (Sultan and Gaetani, 2016). The forecasted delays of arrivals of rain and the spatial redistribution of rainfall across the continents of Africa are expected to worsen agricultural production capacity for smallholder farmers (IPCC, 2021). In 2022, approximately 27 million people required emergency food assistance, while over 38 million people were at risk of hunger and malnutrition across West Africa and the Sahel due to conflict, climate shocks, and rising food prices (RPCA, 2022).

Within this context, maize (*Zea mays* L.) is strategically critical as a major staple food, a key source of calories and protein-particularly in Nigeria-maize strongly influences food prices, rural incomes. Significantly, average yields remain well below potential due to climatic stress, genetic limitations, and suboptimal management (FAOSTAT, 2025). Improving maize yield stability through climate-resilient traits such as drought tolerance and phenological flexibility is therefore central to strengthening food system resilience and advancing sustainable, food security development pathways. Evidently, the existence of large diversity is the key foundation for to any successful selection for any drought tolerance breeding programme in maize as in the absence of such, breeding progress would stagnate, and resilience to environmental stress could continue to be limiting factor.

Despite decades of maize improvement research, significant challenges persist in enhancing productivity and drought resilience. Maize remains vulnerable to drought, parasitic weeds, low soil fertility, salinity, and temperature extremes, resulting in substantial yield losses (Badu-Apraku *et al.*, 2010; Ogunniyan and Olakojo, 2015). Climate change is expected to intensify drought frequency and severity, increasing evapotranspiration and yield instability across maize growth stages (Li *et al.*, 2015).

Most breeding strategies largely emphasize yield improvement under optimal conditions, often underrepresenting stress-response traits and genotype x environment interactions which is critical for climate-exposed systems (Bänziger *et al.*, 2000). Consequently, the functional link between genetic diversity, trait stability, yield resilience and root architecture under drought remains poorly understood. This gap

risks policy and breeding priorities that only favours short-term yield gains over long-term system stability, representing a critical constraint for climate-resilient agriculture and sustainable food governance in Africa.

In most countries of African, drought is amongst abiotic factors linked to persistent constraint on maize production, and disproportionately affecting smallholder farmers, yield instability and livelihood vulnerability (Li *et al.*, 2015). Drought stress at critical growth stages significantly reduces maize yield (Széles *et al.*, 2022).

Ecological theories, (including the Stress-Gradient Hypothesis-SGH), shows that under abiotic stress, early developmental stages disproportionately determine final productivity through resource limitation effects (Kitajima and Fenner, 2000; De La Cruz *et al.*, 2008). Early stress responses remain underrepresented in maize breeding due to phenotyping constraints. However, Seedling-stage screening provides a scalable, cost-effective approach for detecting genetic variation under controlled stress (Hafiz *et al.*, 2014; Wang *et al.*, 2015). This early-stage investigation was designed to Characterize selected germplasm under simulated environmental conditions at seedling-stage focusing on drought resilience traits for future breeding work in Maize.

## **Materials and Methods**

### **Study location**

This study was carried out at the nursery and screen house of the Forestry Research Institute of Nigeria- Federal College of Forestry, Jos, located in the Northern Guinea Savanna ecological zone of Nigeria on 09°56'N, 08°53'E at an altitude of 1,217M above sea level.

### **Sources of Experimental Material**

One hundred and three (103) maize genotypes were obtained from farmers in maize growing areas of Katsina, Kebbi, Bauchi, Nasarawa, Plateau, Oyo and Yobe states. Seeds were also sourced from some research institutes such as the National Centre for Genetic Resources and Biotechnology (NACGRAB) Ibadan, Oyo State, Institute of Agricultural (IAR) Zaria in Kaduna and Savanna Seeds and Livestock Limited a license seed production company in Nigeria (Appendix 1).

### **Screen-house-evaluation and Data Collection**

A total of 103 maize genotypes representing diverse genetic backgrounds were evaluated under controlled screen-house conditions to characterize seedling responses to variable moisture regimes. Six seeds per genotype were sown in uniformly cut polyvinyl chloride (PVC) pipes measuring 45 cm in length and 10 cm in diameter, each containing 4.0 kg of loamy soil. Pots were irrigated to field capacity for three consecutive days prior to sowing to ensure uniform moisture conditions. Following emergence, seedlings were thinned to four per pot and arranged in a Completely Randomized Design (CRD) with three replications. During the establishment phase, pots received 0.6 L of water daily for 10 days to enable uniform seedling growth. Thereafter, irrigation was withheld to impose moisture stress, simulating early-season drought conditions. Morphological and physiological responses were monitored over a 45-day period following the cessation of watering. Fortnightly data were recorded on number of leaves (NOL) and number of shed leaves (NSL), while at 45 days after stress imposition, seedling height (SHT), leaf area (LA), measured according to McKee (1964), and plant collar girth (PCG) were

recorded. Root-related traits were assessed using modified procedures of Harrington *et al.* (1994) and Obeng-Bio *et al.* (2011). To extract intact roots, each pot was immersed in a 2000-L container filled with water, and roots were carefully separated from the soil, followed by further cleaning in a 25-L container to ensure complete sand removal. Roots were cut off from shoots at the cotyledonary node to determine primary root length (LPR, cm) using a meter rule. Fresh and dry biomass of shoots and roots were measured using a sensitive digital Mettler balance. Dry weights were obtained by oven-drying samples at 80°C to constant weight. Moisture content was calculated as the difference between fresh and dry weights. Seedling vigour and general appearance on plant were visually scored on a scale of 1 to 9, according to Akinwale *et al.* (2018) and Nwadike *et al.* (2022) : Scale 1-No visible symptom of stress-(Vigorous plants, no wilting, no dead leaves, no chlorosis, no height reduction, and unrolled turgid leaves); Scale 2-Very mild symptom of stress-(Vigorous plants, no wilting, no dead leaves, no chlorosis, very slight leaf rolling); Scale 3-Mild symptom of stress-(Vigorous plants, no wilting, no dead leaves, no chlorosis, leaf rim start to roll); Scale 4- Mild symptom of stress-(Vigorous plants, no wilting, no dead leaves, slight chlorosis, the leaf has the shape of a V); Scale 5-Moderate symptom of stress-(Vigorous plants, no wilting, 10% dead leaves, slight chlorosis, rolled leaf rim covers part of the leaf blade); Scale 6-Susceptible plants-(Less vigorous plants, moderate reversible wilting, 25% dead leaves, severe chlorosis, the leaf is rolled like that of an onion); Scale 7-Highly susceptible-(Severe irreversible wilting with 50% dead leaves); Scale 8-Highly susceptible-(Severe irreversible wilting with 75% dead leaves, 75% death of seedlings); finally, Scale 9-Very highly susceptible-(Total collapse or 100% death of seedling, dried leaves, and stem), providing a composite measure of drought stress tolerance.

### Data Analysis

Quantitative data collected were subjected to Analysis of Variance (**ANOVA**) using Crop-Stat Analysis Package Software to test for any significant effect of the treatments on traits observed on the experimental entries. The linear additive model for a Completely Randomized Design (CRD) under a fixed-effects framework, as described Gomez and Gomez (1984), was used to evaluate the data. The linear additive model of Completely Randomized Design (CRD) of the form:

$$Y_{ij} = M + t_j + e_{ij}$$

Where:  $Y_{ij}$ : individual performance (observation of the  $i^{\text{th}}$  block / rep in the  $j^{\text{th}}$  genotype).  $M$  : general mean (i.e. of all possible similar experiment).  $e_{ij}$  : experimental error (i.e containing all uncontrolled sources of variation associated with the  $i^{\text{th}}$  block / rep in the  $j^{\text{th}}$  genotype)

Each character's Analysis of Variance was performed as directed, and Fisher and Yates' (1967) test of significance was followed. According to Fisher and Yates (1967), each trait was subjected to a separate ANOVA, and F-tests were utilized to determine the significance of genotype effects at the 5% probability level.

Where significant differences were discovered, treatment means were compared using the Least Significant Difference (LSD) test at  $P \leq 0.05$ . The adequacy of the model was evaluated through residuals diagnosis in order to verify the assumptions of homogeneity of variance and normality.

## Genetic Diversity Analysis

Using Mahalanobis' generalized distance ( $D^2$ ) statistic (Mahalanobis, 1936), which was implemented in SAS (1998), genetic divergence among genotypes was evaluated. Standardized trait means served as the foundation for multivariate analysis, which took inter-trait correlations and variations in measurement scale into consideration. The  $i^{\text{th}}$  and  $j^{\text{th}}$  genotypes generalized squared distance was calculated as follows:

$$D^2_{ij} = P_t = \sum (y_{it} - y_{jt})^2$$

Where:  $D^2_{ij}$  =  $D^2$  between  $i^{\text{th}}$  and  $j^{\text{th}}$  genotypes.  $y_{it}$  : uncorrelated mean value of the  $i^{\text{th}}$  genotype for 't' character.  $y_{jt}$  : uncorrelated mean value of the  $j^{\text{th}}$  genotype for 't' character.

The average Intra cluster distance was done by measuring of intra cluster distances as per Singh and Chaudhary (1977), using the formula  $\sum D^2_{ij}/n$  where,  $\sum D^2_{ij}$  is the sum of distance between all possible combinations and 'n' is number of the genotypes included in a cluster.

The average Inter cluster distance was determine as per Singh and Chaudhary (1977), using the formula  $\sum D^2_{ij}/n_i n_j$ , where  $\sum D^2_{ij}$  is the sum of distance between all possible combinations  $n_i n_j$  of the entries included in the cluster study, where  $n_i$  = number of entries in cluster i and  $n_j$ = number of entries in cluster j.

Prior to analysis, the genotypes were allocated standardized codes as a data management method to improve the accuracy, clarity, and reproducibility of dendrogram-based genetic diversity evaluation analysis.

## RESULTS

### Genotypic Response Variation Under Contrasting Moisture Stress Condition

Mean Square Estimates from Analysis of Variance (ANOVA) for One Hundred and Three Genotypes of Maize was carried out under well-watered Conditions Table 2 showed no significant difference ( $P \leq 0.05$ ) for most traits but revealed a statistically significant difference for plant height ( $0.46^*$ ) only. While, the mean square table of Analysis of Variance (ANOVA) results in Table 3 under water deficit conditions showed a statistically significant difference ( $P \leq 0.05$ ) for all traits studied.

**Cluster Analysis of 103 Maize Genotypes for Drought Tolerance Traits at Seedling Stage Under Contrasting Moisture Stress Condition**  
**Cluster Analysis Summary Under Well-Watered Conditions**  
Cluster Analysis Result of 103 Genotypes of Maize at the Seedling Stage for Drought Tolerance Traits in Jos, 2019 under well-watered Conditions. Figure 1 displays the dendrogram cluster analysis for 103 genotypes of maize at the seedling stage evaluated for drought tolerance features in Jos under non-moisture stress circumstances. Cluster 1 has 62 genotypes, Cluster 2 has 9 genotypes, Cluster 3 has 12 genotypes, Cluster 4 has 8 genotypes, Cluster 5 has 5 genotypes, Cluster 6 has a single genotype, and Cluster 7 has 6 genotypes.

### **Cluster Analysis Summary Under Water-Deficit Conditions**

Figure 2 displays the Dendrogram Cluster result for the 103 maize genotypes at the seedling stage that were examined for traits related to drought tolerance under water deficit conditions: Cluster 1 has a wide range of 67 genotypes; Cluster 2 has three genotypes; Cluster 3 has thirteen genotypes; Cluster 4 has five genotypes; Cluster 5 has just four genotypes; Cluster 6 has seven genotypes; and Cluster 7 has four genotypes.

**Table 2: Analysis of Variance (ANOVA) Mean Squares for Seedling Traits of 103 Maize Genotypes Under Well-Watered Conditions at Jos.**

| Source of Variation | D. F | G%           | SPH    | LA     | PCG    | NL     | NSL    | FSW    | DSW     | FRW    | DRW    | LPR    | RV      | SA     |
|---------------------|------|--------------|--------|--------|--------|--------|--------|--------|---------|--------|--------|--------|---------|--------|
| <b>Genotype</b>     | 102  | 603.44<br>ns | 0.46 * | 2.07ns | 6.55ns | 0.18ns | 0.46ns | 0.05ns | 0.01 ns | 0.07ns | 1.65ns | 0.10ns | 0.01 ns | 0.00ns |
| <b>Error</b>        | 206  | 25.91        | 0.51   | 1.91   | 5.24   | 0.14   | 0.15   | 0.10   | 0.01    | 0.03   | 0.06   | 0.14   | 0.01    | 0.00   |
| <b>Total</b>        | 308  |              |        |        |        |        |        |        |         |        |        |        |         |        |

\*= significant at  $P \leq 0.05\%$  probability level, \*\* = significant at  $P \leq 0.01\%$  probability level, ns = not significant.

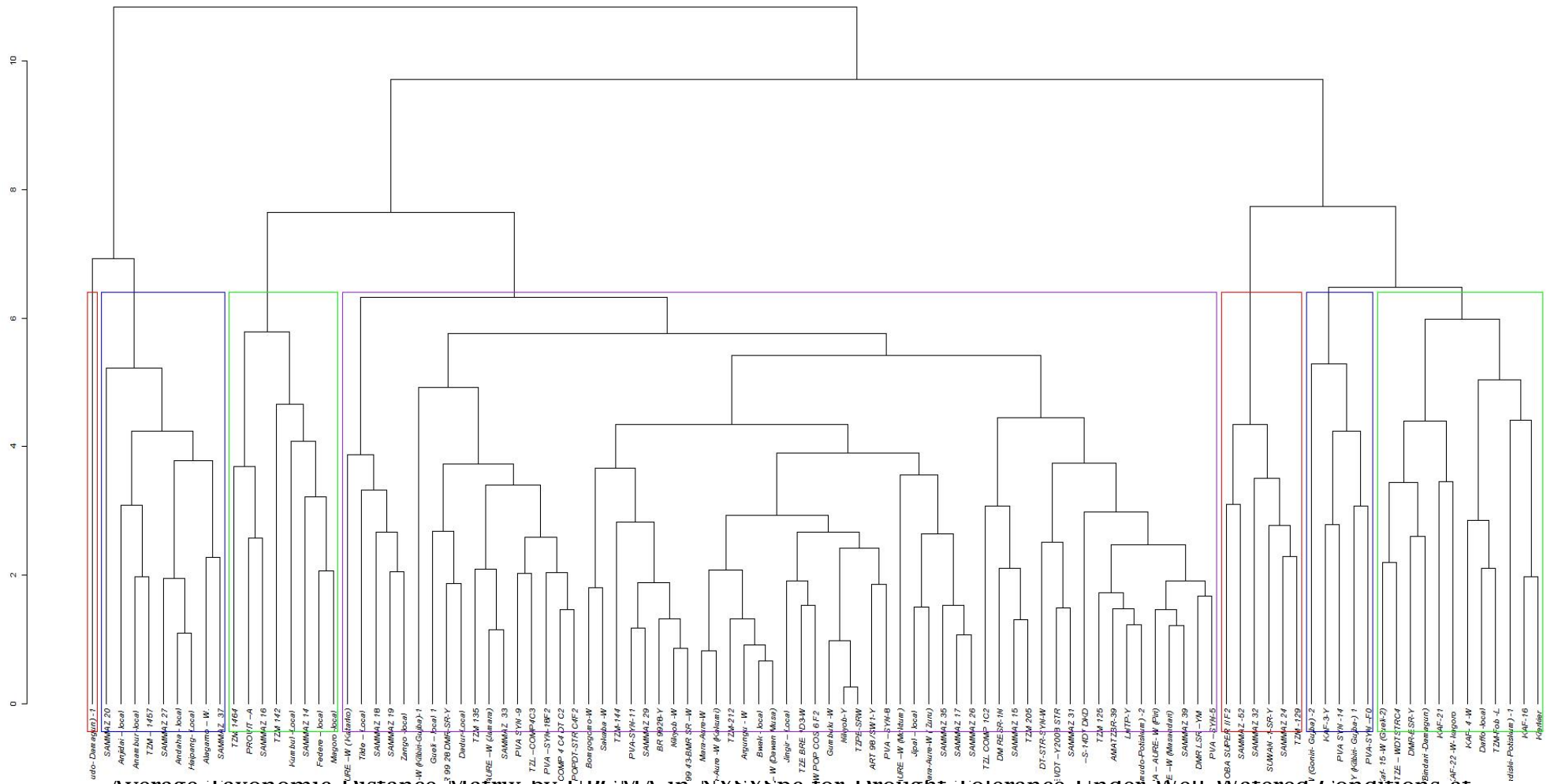
Germination percentage (G%), Plant Seedling Height (PSH), Leaf Area (LA), Plant Collar Girth (PCG), Number of Leaf (NL), Number of Shade Leaf (NSL), Fresh Shoot Weight (FSW), Dry Shoot Weight (DSW), Fresh Root Weight (FRW), Dry Root Weight (DRW), Length of Primary Root (LPR), Root Volume (RV) and Seedling Aspect (SA).

**Table 3: Analysis of Variance (ANOVA) Mean Squares for Seedling Traits of 103 Maize Genotypes under Water-Deficit Conditions at Jos.**

| Source of Variation | D. F | G%      | SPH      | LA     | PCG    | NL      | NSL    | FSW      | DSW    | FRW     | DRW    | LPR      | RV      | SA     |
|---------------------|------|---------|----------|--------|--------|---------|--------|----------|--------|---------|--------|----------|---------|--------|
| <b>Genotype</b>     | 102  | 590.91* | 323.131* | 5.912* | 5.427* | 12.058* | 4.533* | 778.620* | 4.571* | 28.036* | 1.645* | 106.930* | 16.144* | 7.495* |
| <b>Error</b>        | 206  | 68.62   | 0.861    | 4.908  | 2.039  | 0.323   | 0.016  | 14.150   | 0.025  | 0.422   | 0.055  | 31.270   | 5.244   | 0.000  |
| <b>Total</b>        | 308  |         |          |        |        |         |        |          |        |         |        |          |         |        |

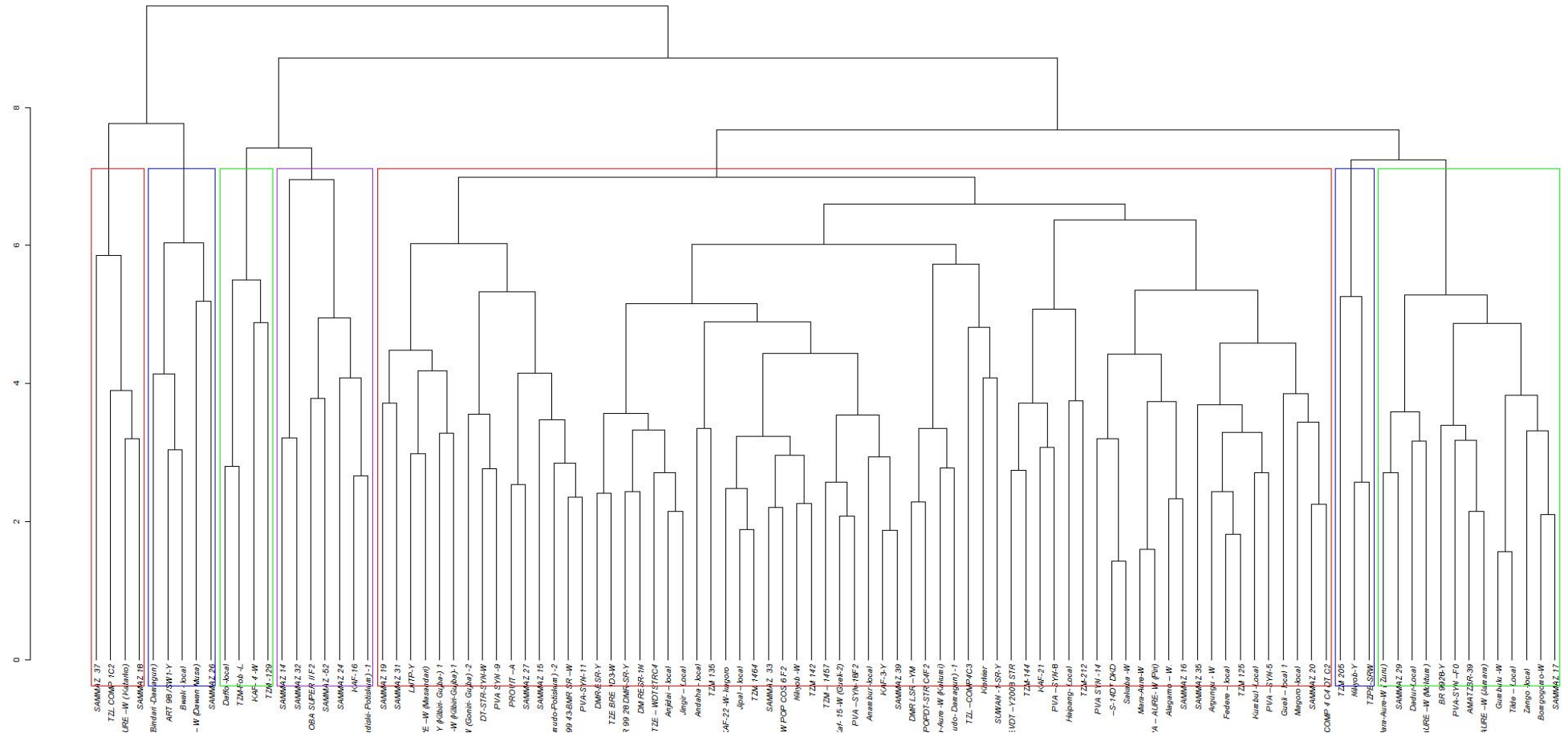
\*= significant at  $P \leq 0.05\%$  probability level, \*\* = significant at  $P \leq 0.01\%$  probability level, ns = not significant.

Germination percentage (G%), Plant Seedling Height (PSH), Leaf Area (LA), Plant Collar Girth (PCG), Number of Leaf (NL), Number of Shade Leaf (NSL), Fresh Shoot Weight (FSW), Dry Shoot Weight (DSW), Fresh Root Weight (FRW), Dry Root Weight (DRW), Length of Primary Root (LPR), Root Volume (RV) and Seedling Aspect (SA).



Average Taxonomic Distance Matrix by UPGMA in NYSpc for Drought Tolerance Under Well-Watered Conditions at Seedling Stage in Jos.

Dendrogram using Agglomerative Clustering Method



## **DISCUSSION**

### **ANOVA Mean Squares for Seedling Traits Under Contrasting Moisture Regimes**

Analysis of variance revealed contrasting patterns of trait expression under well-watered and moisture-stress conditions. Under well-watered conditions, genotypic effects were significant only for seedling height, while the remaining traits were largely uniform across genotypes. This uniformity suggests physiological stabilization among the evaluated germplasm under optimal moisture, allowing most genotypes to reach near-optimal growth and biomass accumulation. These findings are consistent with earlier studies indicating under a non-limiting moisture availability can mask underlying genetic variability in maize seedlings, particularly for growth-related traits such as shoot length, leaf area, and biomass allocation (Li *et al.*, 2015). This agrees with reports of field observations in tropical maize germplasm, where favourable irrigation conditions minimized phenotypic variations among early-stage seedlings (Sudozai *et al.*, 2013). These results highlight the significance of imposing controlled stress conditions when screening for drought-tolerance traits.

Under moisture-stress conditions, all thirteen measured traits revealed a highly significant genotypic differences in response to water deficit, revealing substantial genetic variation. Stress-induced differences were most pronounced in roots and shoots related traits frequently linked to drought-adaptive mechanisms in maize. The revealed significant genotype x environment interaction highlights that stress conditions effectively amplified existing genetic differences that remain latent under optimal moisture. These patterns support earlier reported findings in some maize germplasm population; for example, extensive phenotypic variation in early-stage drought responses was observed among tropical and subtropical accessions in studies by Vennam *et al.* (2023) and Zhang *et al.* (2025), indicating that germplasm origin and genetic background strongly influence adaptive responses. This consistency across diverse germplasm underscores the reliability of seedling-stage traits as early indicators for drought tolerance.

Breeding programs aimed at drought-prone areas will be significantly impacted by the observed variability under moisture stress. High genotypic variation traits including biomass partitioning, root architecture, and seedling height could be used as trustworthy selection indices to find genotypes resistant to drought (Wang *et al.*, 2025). These results demonstrate how combining these characteristics with additional well-known drought indices, like the Tolerance Index (STI), Stress Drought Susceptibility Index (DSI), and Geometric Mean Productivity (GMP), can help optimize parental selection and achieve much-needed rapid breeding improvements. Importantly, incorporating these stress-responsive traits into selection procedures provides a deliberate way to develop maize cultivars that are more drought-resistant, have consistent yields, and contribute more to food security in more erratic agroclimatic conditions.

### **Genotypic Clustering and Diversity Patterns**

The Clustering pattern revealed unique cluster sizes, large cluster of genetically uniform material, small cluster of distinct genotypes and a single cluster indicates a highly

divergent population. Under well-watered conditions, a large dominant cluster (Cluster I) revealed less differentiation and homogenous group, reflecting physiological stabilization and limited differentiation under optimal moisture condition. This clustering pattern indicated minimal genetic divergence. However, the presence of a single clusters indicated a level of divergence in the population. Under water deficit condition induced substantial reconfiguration, with Cluster I expanding to 67 genotypes while other clusters revealed pronounced diversity in drought response mechanisms. Cluster II, containing the fewest genotypes, likely represents elite drought-avoidance lines based on possible superior root architecture and biomass partitioning-critical for extreme moisture scarcity (Ganesan *et al.*,2010).The stress-induced shift in cluster distribution, accompanied by variation, suggest genotype  $\times$  environment interactions and validates clustering as a tool for identifying possible resilient parental material (Pipatsitee *et al.*, 2022; Zhang *et al.*, 2025).These revealed patterns support targeted selection from divergent clusters to broaden the genetic base for climate-resilient maize for future breeding programme.

## CONCLUSION

This research identified substantial genetic diversity in maize and shows that both above-and below-ground traits are central to drought tolerance and breeding for climate-resilient varieties. The Study also reveals that Maize genotypes could perform similar under optimal moisture but different under moisture stress. Clustering analysis and pattern clustered genotypes under both optimal and water-limited conditions, providing valuable pool of genetic material for future breeding programs for development of climate-resilient maize varieties.

## CONFLICTS OF INTEREST

The author declares no conflicts of interest

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