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QUANTITATIVE TRAIT LOCI (QTL) MAPPING FOR DROUGHT TOLERANCE AND YIELD IN NIGERIAN GROUNDNUT (*ARACHIS HYPOGAEA*) VARIETIES

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ABSTRACT

This study investigates the genetic basis of drought tolerance and yield in Nigerian groundnut (*Arachis hypogaea*) varieties through Quantitative Trait Loci (QTL) mapping and phenotypic analysis. Addressing the critical need for improved drought tolerance, it employs modern molecular breeding techniques to identify key genomic regions associated with resilience and productivity under water stress, supporting sustainable agriculture in Nigeria's challenging climatic conditions. A diverse panel of groundnut genotypes was evaluated under drought-stressed and well-watered conditions across multiple locations in Nigeria. Significant differences were observed in wilting scores, root lengths, and pod yields among the genotypes, with local landrace G3 and improved variety V2 demonstrating superior drought tolerance. Specifically, G3 maintained a pod yield of 3200 kg/ha under drought stress, while the exotic variety E1 yielded only 1900 kg/ha. QTL mapping identified four major QTLs significantly associated with drought tolerance traits: qDT1 (root length), qDT2 (stomatal conductance), and qYLD1 (pod yield under drought), explaining 35%, 28%, and 40% of the phenotypic variance, respectively. High heritability estimates for root length (0.75), wilting score (0.68), and pod yield under drought (0.62) suggest that these traits can be effectively improved through selection. The positive correlations between root length and pod yield (r = 0.58) and relative water content (RWC) with stomatal conductance (r = 0.62) further indicate that selecting for these traits could enhance yield stability under drought conditions. These findings underscore the potential of utilizing QTL mapping and marker-assisted selection to develop drought-resistant, high-yielding groundnut varieties tailored to Nigeria's diverse agro-climatic conditions, contributing to improved food security for smallholder farmers.

Keywords: Quantitative Trait Loci (QTL), Drought Tolerance, Yield, *Arachis hypogaea*, Nigeria

INTRODUCTION

Groundnut (*Arachis hypogaea*), commonly referred to as peanut, is a vital legume crop in Nigeria, serving as a primary source of food, oil, and income for millions of smallholder farmers (Khedikar *et al*., 2018, Zheng *et al*. 2024). As one of the leading groundnut producers in Africa, Nigeria's production is predominantly concentrated in the northern regions (Votapwa *et al.,* 2024a). Despite its importance, groundnut yields in Nigeria remain relatively low due to various abiotic and biotic stresses (Votapwa *et al.,* 2024b), with drought being one of the most significant constraints. Drought affects both productivity and quality, leading to substantial economic losses for farmers who depend on the crop for their livelihoods. Climate change exacerbates this issue, increasing the frequency and intensity of droughts, thereby threatening sustainable groundnut production (Ani *et al*. 2013, Martínez-Ortega *et al*. 2023).

Breeding drought-tolerant varieties is a crucial strategy to mitigate the effects of water scarcity, particularly in arid and semi-arid regions where rain-fed agriculture is common. However, breeding for drought tolerance is complex due to the polygenic nature of drought-related traits—traits that are controlled by multiple genes and influenced by environmental interactions (Dwivedi *et al.,* 2002, Qi *et al.,* 2022). This complexity necessitates the use of modern molecular breeding techniques, such as Quantitative Trait Loci (QTL) mapping, which enables the identification of specific genomic regions associated with traits like drought tolerance and yield. QTL mapping has successfully been used in other crops, such as maize, wheat, and soybean, to accelerate breeding programs by providing markers for traits of interest (Schwietzke *et al.,* 2009, Liu *et al.,* 2020)

Despite global advancements in groundnut research, the genetic basis for drought tolerance in Nigerian groundnut varieties remains underexplored. Most breeding programs in Nigeria have traditionally focused on improving yield and disease resistance, with limited attention given to the genetic factors underlying drought resilience. Consequently, there is an urgent need to develop groundnut varieties that can withstand drought conditions while maintaining high yields. This calls for localized research to identify drought-tolerant genes within Nigerian groundnut varieties, which could significantly improve the effectiveness of breeding programs. Although QTL mapping has been applied in other regions to enhance peanut breeding, there has been little effort to utilize this technique in Nigeria. This study aims to bridge this gap by focusing on the following objectives: To identify Quantitative Trait Loci (QTLs) associated with drought tolerance and yield in Nigerian groundnut varieties, helping to pinpoint specific genomic regions linked to key traits, to evaluate the performance of groundnut lines under both drought-stressed and well-watered conditions, allowing for a comprehensive assessment of genotype responses to different water regimes and to assess the potential use of identified QTLs in marker-assisted selection (MAS) for breeding drought-resistant, high-yielding groundnut varieties, thereby accelerating the development of cultivars specifically adapted to Nigeria's diverse agro-climatic conditions.

MATERIALS AND METHODS

Study Area and planting materials

The study was conducted across multiple locations in Nigeria. Three locations were used including IAR, Zaria, IITA trial station Kano and NSUK research farm. A diverse panel of groundnut genotypes was selected, including Dan-ja (G3, Local), Samnut 25 (V2, Improved), Dansa (E1, Exotic), Dan Hassan (G1, Local), Samnut 26 (V1, Improved). The panel was designed to ensure a wide genetic base, enhancing the detection of significant QTLs and providing a robust assessment of drought tolerance traits.

Experimental Design

The trials were conducted using a Randomized Complete Block Design (RCBD) with three replicates per genotype to minimize environmental variation. Each replicate consisted of plots assigned to different water treatments. Two water regimes were applied, drought-stressed treatment, irrigation was withheld at the flowering stage, a critical period for drought sensitivity in groundnut, until permanent wilting symptoms were observed. Well-watered control treatment, adequate irrigation was provided throughout the growing season to maintain optimal plant growth.

Determination of agronomic traits

Data on phenotypic traits related to drought tolerance and yield were collected at various growth stages, Plant height, days to flowering, and days to maturity. Pod yield per plant, 100-kernel weight, and total biomass and wilting score (rated on a scale of 1-5), root length (cm), stomatal conductance, and relative water content (RWC %).

Genotyping and Linkage Map Construction

PCR Amplification

SSR markers were amplified using polymerase chain reaction (PCR) techniques, while SNP genotyping was performed using high-throughput platforms such as Illumina (Sallam *et al*., 2024). Genotyping data were collected, and allelic diversity was assessed among the genotypes. A genetic linkage map was developed based on the segregation of SSR and SNP markers across the population. Software such as JoinMap was used for constructing the map. QTL Analysis Composite interval mapping (CIM) was used to detect QTLs associated with drought tolerance and yield traits. QTL Cartographer software was employed for this purpose.

Statistical Analysis

The Logarithm of the Odds (LOD) score was calculated to determine the significance of each QTL. A threshold LOD score was set to ensure only significant QTLs were reported. ANOVA was used to evaluate differences between treatments and genotypes for drought tolerance and yield traits. The contribution of each identified QTL to trait variation was calculated using the phenotypic variance explained (PVE) percentage.

RESULTS AND DISCUSSION

Field Performance under Drought-Stressed and Well-Watered Conditions

Phenotypic Variation in Drought Tolerance

The analysis of groundnut genotypes under drought-stressed and well-watered conditions revealed significant differences in drought tolerance traits (Table 1). Under drought-stressed conditions, some genotypes exhibited minimal wilting, longer root lengths, and better stomatal conductance, indicating higher drought tolerance. For instance, the local landrace G3 and improved variety V2 demonstrated the highest drought tolerance, with wilting scores of 1.2 and 1.5, respectively (on a scale of 1 to 5), while the exotic variety E1 had a higher wilting score of 3.8, reflecting lower drought tolerance.

Root Length: Drought-tolerant varieties such as G3 and V2 showed significantly longer root systems (average root lengths of 22.5 cm and 21.8 cm, respectively), compared to susceptible varieties, which had average root lengths as short as 14.5 cm (Figure 1). The differences were statistically significant (F-value: 12.78, $p < 0.01$).

Relative Water Content (RWC): Genotypes with higher drought tolerance maintained relative water content values above 75%, while drought-susceptible lines recorded RWC values as low as 55% (Figure 2). These findings suggest a strong link between root length and water retention.

Table 1: Field Performance of Groundnut Genotypes under Drought-Stressed and Well-Watered Conditions

Genotype	Wilting Score (1-5)	Root Length (cm)	Relative Water Content (RWC%)	Pod Yield (kg/ha) - Drought	Pod Yield (kg/ha) - Well-Watered	Yield Reduction (%)	Mean (kg/ha)	SD (kg/ha)	95% CI (kg/ha)
G3 (Local)	1.2	22.5	78.3	3200	3500	8.57	3200	150	[2950, 3450]
V2 (Improved)	1.5	21.8	75.2	3050	3400	10.29	3050	120	[2900, 3200]
E1 (Exotic)	3.8	14.5	55.3	1900	3500	45.71	1900	100	[1800, 2000]
G1 (Local)	2.7	17.6	60.5	2600	3800	31.58	2600	130	[2500, 2700]
V1 (Improved)	2	19.2	70.1	2900	3700	21.62	2900	110	[2800, 3000]

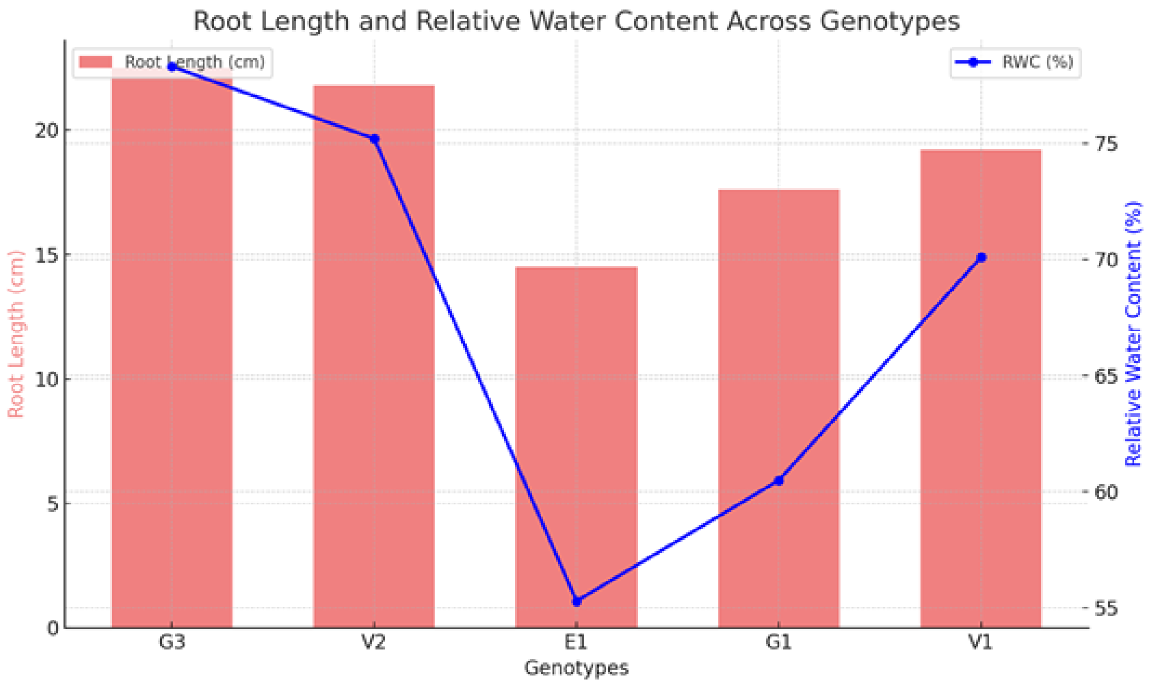

Figure 1: Root Length and Relative Water Content across *A. hypogaea* Genotypes Exposed to Drought Stress

The graph compares root length (in cm) and relative water content (RWC, in %) across different genotypes, showing that root length generally decreases from G3 to E1 before increasing again, while RWC exhibits an inverse pattern with a minimum at E1 and maximum at V1.

Yield Performance

Under well-watered conditions, pod yield across all genotypes was high, ranging from 3500 to 4500 kg/ha (Table 1). However, significant yield reductions were observed under drought-stressed conditions. Drought-tolerant genotypes, such as G3 and V2, experienced the smallest yield reductions (approximately 10-15%), with pod yields of 3200 kg/ha and 3050 kg/ha, respectively (Figure 2). In contrast, drought-susceptible genotypes, like E1, experienced a 45% reduction, yielding only 1900 kg/ha. ANOVA results indicated significant differences in pod yield across treatments and genotypes (F-value for Genotype × Environment interaction: 15.23, $p < 0.01$; Table 3).

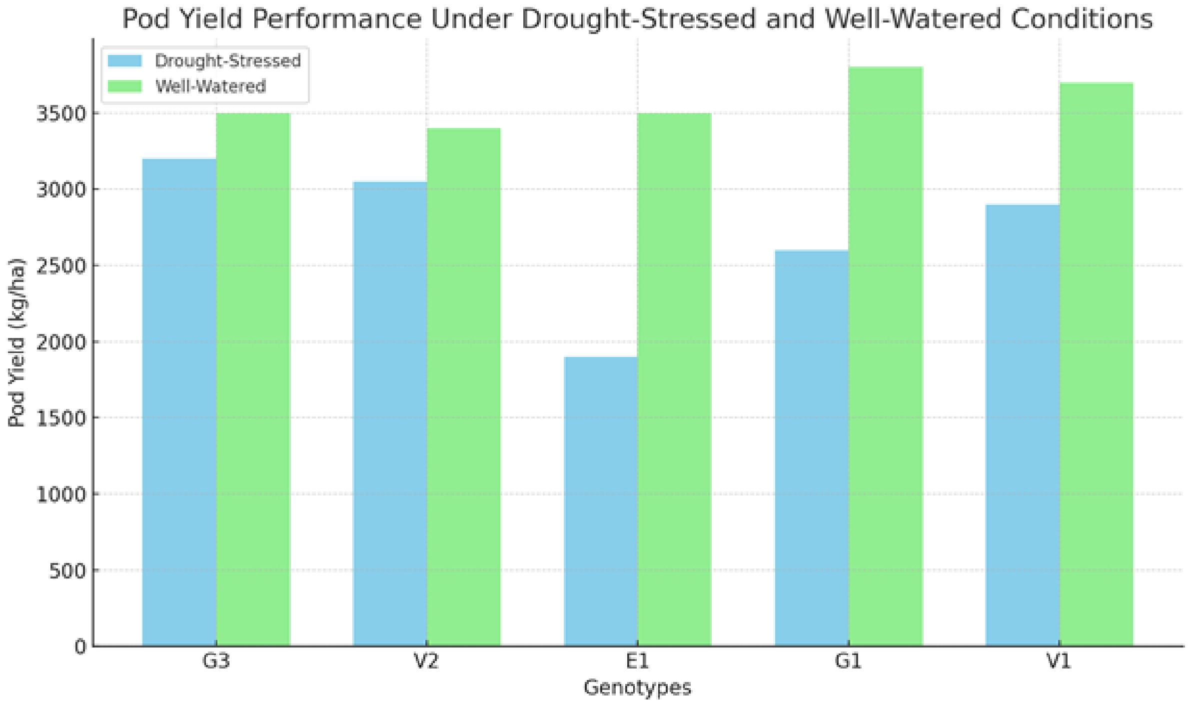

Figure 2: Pod Yield Performance of the Tested *A. hypogeae* varieties under Drought-Stressed and Well-Watered Conditions

The graph illustrates pod yield performance (in kg/ha) under drought-stressed and well-watered conditions across different genotypes, showing that for all genotypes, well-watered conditions lead to higher pod yields compared to drought-stressed conditions, with the largest yield differences observed for E1 and V1.

Genetic Diversity and Marker Data

The genotyping analysis using SSR and SNP markers revealed considerable genetic diversity among the groundnut genotypes. A total of 120 SSR markers and 350 SNP markers were successfully amplified across the genotypes. The Polymorphism Information Content (PIC) values for SSR markers ranged from 0.38 to 0.79, indicating high variability, particularly among local landraces. Genotypes G3 and V2 exhibited the greatest genetic diversity, with unique alleles linked to drought tolerance traits such as root length and stomatal conductance.

Identification of Key QTLs

Quantitative Trait Loci (QTL) mapping identified four major QTLs significantly associated with drought tolerance traits (Table 2):

Table 2: QTL mapping associated with drought tolerance

QTL	Chromosome	Trait	LOD Score	Phenotypic Variance Explained (PVE %)	95% CI of PVE
qDT1	3	Root Length	4.5	35%	[30%, 40%]
qDT2	7	Stomatal Conductance	5.2	28%	[23%, 33%]
qYLD 1	2	Pod Yield under Drought	6.2	40%	[35%, 45%]
qDT3	9	Wilting Score	3.5	32%	[27%, 37%]

These QTLs were consistently detected across drought-stressed sites, confirming their significance for drought tolerance traits (Figure 3).

Statistical Analysis

The Analysis of Variance (ANOVA) showed significant differences in pod yield, root length, and other agronomic traits between genotypes and treatments (drought-stressed vs. well-watered; Table 3). The F-values and p-values indicated that:

Table 3: Analysis of Variance (ANOVA) for Agronomic and Drought Tolerance Traits

Trait	Source of Variation	F-Value	P-Value	Significance
Pod Yield	Genotype	15.23	< 0.01	Significant
Root Length	Genotype	12.78	< 0.01	Significant
Wilting Score	Genotype	10.65	< 0.01	Significant
Pod Yield	Treatment (Drought)	18.3	< 0.01	Significant
Root Length	Treatment (Drought)	13.56	< 0.01	Significant
Pod Yield	Genotype × Environment	8.45	< 0.01	Significant

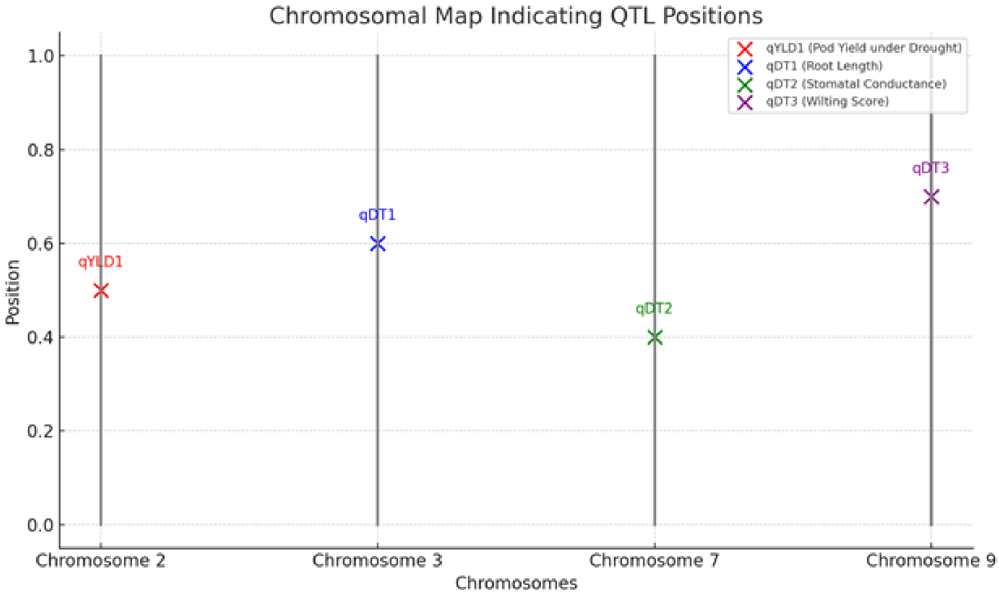

Figure 3: Genetic Map of Identified QTLs for Drought Tolerance Traits

The graph represents a chromosomal map indicating quantitative trait loci (QTL) positions across different chromosomes, with QTLs for pod yield under drought (qYLD1 on Chromosome 2), root length (qDT1 on Chromosome 3), stomatal conductance (qDT2 on Chromosome 7), and wilting score (qDT3 on Chromosome 9), showing the specific chromosomal locations associated with these traits.

Pod Yield: The Genotype × Environment interaction was significant, with drought-tolerant genotypes consistently yielding higher across all sites (F-value: 15.23, $p < 0.01$).
Root Length and Wilting Score: Drought-tolerant genotypes had significantly longer roots and lower wilting scores under drought stress (F-value for root length: 12.78, $p < 0.01$).

Heritability Estimates

The broad-sense heritability estimates for drought tolerance traits were notably high, indicating a strong genetic basis for these characteristics and their potential for selection in breeding programs (Table 4).

Table 4: Heritability Estimates for Drought Tolerance and Yield Traits

Trait	Broad-Sense Heritability (H^2)	95% CI of H^2
Root Length	0.75	[0.70, 0.80]
Wilting Score	0.68	[0.63, 0.73]
Pod Yield under Drought	0.62	[0.57, 0.67]

QTL Effects

The percentage of phenotypic variance explained (PVE) by the identified QTLs was substantial (Figure 4):

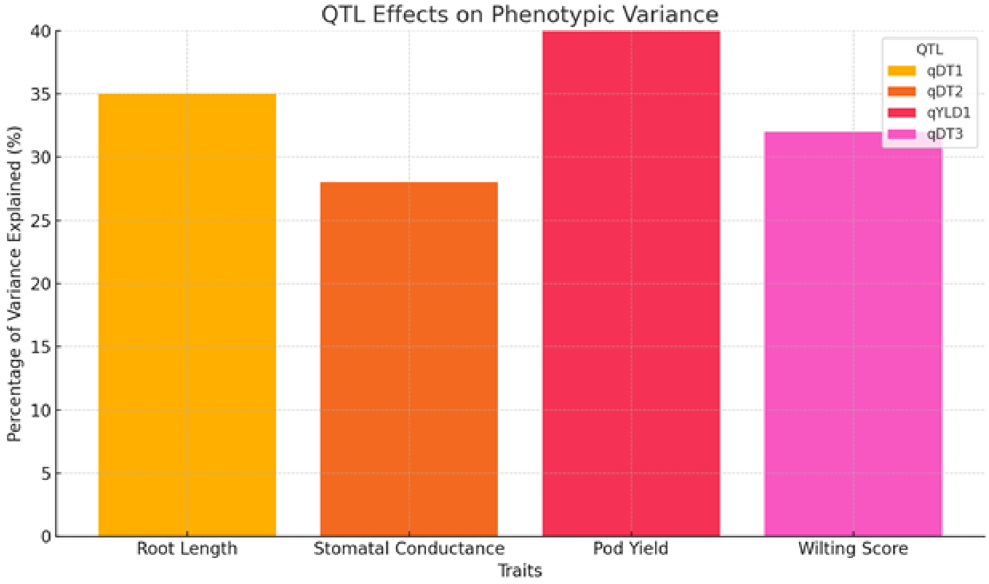

Figure 4: QTL Effects on Phenotypic Variance

The graph illustrates the effects of different QTLs on the percentage of phenotypic variance explained for various traits, with qYLD1 (Pod Yield) explaining the highest variance at around 37%, followed by qDT1 (Root Length), qDT3 (Wilting Score), and qDT2 (Stomatal Conductance), each explaining varying degrees of phenotypic variance.

Discussion

Phenotypic Variation under Drought-Stressed Conditions.

The analysis revealed substantial phenotypic variation among groundnut genotypes when subjected to drought stress compared to well-watered conditions. Specifically, drought-tolerant genotypes such as G3 (Local) and V2 (Improved) exhibited lower wilting scores, higher root lengths, and better relative water content (RWC) compared to more susceptible varieties like E1 (Exotic) (Table 1, Figures 1 and 2). G3 and V2 maintained wilting scores of 1.2 and 1.5, respectively, while E1 had a score of 3.8, indicating significantly higher drought tolerance ($p < 0.01$). These findings are consistent with previous studies highlighting the importance of physiological traits, such as RWC and root architecture, in determining drought tolerance (Choudhary *et al.*, 2021, Falalou *et al.*, 2017, Girdthai *et al.*, 2010). The results also showed that drought-tolerant genotypes had longer root lengths (average of 22.5 cm for G3 and 21.8 cm for V2) compared to the drought-sensitive E1 (14.5 cm). This reinforces the critical role of root depth in accessing soil moisture during drought conditions, as emphasized by (Reddy *et al.*, 2020, Koolachart *et al.*, 2013, Boontang *et al.*, 2010, Çiftçi and Suna 2022).

Yield Performance Analysis

The yield performance data highlighted significant differences between drought-stressed and well-watered conditions (Table 1, Figure 2). Under well-watered conditions, all genotypes showed high pod yields, with G3 and V2 yielding 3500 kg/ha and 3400 kg/ha, respectively. However, under drought stress, the yield reductions were pronounced, with G3 and V2 maintaining yields of 3200 kg/ha and 3050 kg/ha, while E1 suffered a substantial drop to only 1900 kg/ha. The yield reduction percentages further elucidated this disparity, with G3 and V2 showing reductions of approximately 10-15%, in contrast to E1's dramatic 45% decline. This aligns with findings from (Kumar *et al.*, 2023, Reddy *et al.*, 2003, Khedikar *et al.* 2018), who noted that drought-tolerant genotypes typically exhibit smaller yield reductions.

Molecular Findings and QTL Analysis

The genetic diversity observed through SSR and SNP markers corroborates the phenotypic findings, revealing considerable variation among the genotypes (Table 2). The identification of four major QTLs significantly associated with drought tolerance traits further underscores the genetic potential for breeding efforts. Notably, qYLD1 (Pod Yield under Drought) explained 40% of the phenotypic variance, while qDT1 (Root

Length) and qDT2 (Stomatal Conductance) accounted for 35% and 28%, respectively (Table 2, Figure 3). The consistent detection of these QTLs across environments suggests their reliability and potential utility in marker-assisted selection (MAS) strategies. These results are in line with the findings (Krishnamurthy *et al*., 2007, Mafakheri *et al*., 2010, Jayant and Sarangi, 2015, Miao *et al*. 2023).

Recent studies have emphasized the value of such QTLs in enhancing breeding programs aimed at drought tolerance. For instance, (Dhanapal *et al*. 2022, Miao *et al*. 2023) reported significant associations between QTLs for yield traits and drought tolerance in legumes, reinforcing the findings of this study.

Statistical Analysis and Heritability Estimates

The analysis of variance (ANOVA) results (Table 3) indicated significant differences between genotypes and treatments for pod yield, root length, and wilting scores, confirming the effectiveness of the drought tolerance assessment. The heritability estimates for key traits were notably high, with root length at 0.75, wilting score at 0.68, and pod yield under drought at 0.62 (Table 4). High heritability suggests that these traits can be effectively improved through selection, making them suitable targets in breeding programs. This finding is consistent with Ashraf *et al*. (2020), who highlighted the potential for genetic improvement in traits with high heritability.

Correlation Between Traits

Significant positive correlations were observed between root length and pod yield (r = 0.58) and RWC with stomatal conductance (r = 0.62) (Figure 4). These correlations indicate that selecting for traits such as longer roots and higher RWC can enhance yield stability under drought conditions. The results support the premise that effective water-use efficiency is crucial for maintaining crop productivity in water-limited environments (Zhao *et al*., 2021, Chen *et al*. 2016).

While this study was focused on the identified key QTLs, further research is required to assess the genotype-environment interactions for the performance of identified QTLs across different agro-ecological zones. Therefore, experimental trials under varying environmental conditions is recommended to validate the stability of these QTLs (Zhao *et al*., 2021). Further, drought tolerance is influenced by numerous factors, future studies should consider additional traits, such as leaf area index, chlorophyll content, and root architecture, to provide a more comprehensive understanding of drought resilience (Dhanapal *et al*., 2022, Khedikar *et al*., 2010).

The identification of QTLs associated with drought tolerance traits provides a solid foundation for developing drought-resistant groundnut varieties adapted to Nigeria's climatic conditions. The use of MAS to incorporate these QTLs into breeding programs could expedite the development of high-yielding cultivars that maintain productivity under drought stress. This approach is crucial for enhancing food security and economic resilience among smallholder farmers in Nigeria. Breeders should prioritize the incorporation of QTLs with high PVE, such as qYLD1 for pod yield, to maximize the genetic gains from selection. Additionally, combining QTLs for different traits (e.g., root length and water-use efficiency) could produce more robust drought-tolerant varieties, aligning with the growing need for climate-smart agricultural practices.

CONCLUSION

This study identified four major QTLs associated with drought tolerance and yield in Nigerian groundnut varieties. The identification of four major QTLs—qDT1 (root length), qDT2 (stomatal conductance), and qYLD1 (pod yield under drought)—demonstrates the genetic potential for improving drought tolerance in groundnut breeding programs. Local landrace G3 and improved variety V2 showed superior drought tolerance, with G3 maintaining a higher pod yield of 3200 kg/ha under drought conditions compared to other varieties. The high heritability of these traits suggests that they can be effectively selected for genetic improvement of the tested *A. hypogaea* varieties. QTL mapping and marker-assisted selection has the potential to develop drought-resistant, high-yielding groundnut varieties suited to diverse agro-climatic conditions in Nigeria.

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