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ASSESSING GENETIC DIVERSITY AND DROUGHT TOLERANCE IN IMPORTED GROUNDNUT CULTIVARS USING SSR MARKERS

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ABSTRACT:

Groundnut (*Arachis hypogaea* L.), commonly known as peanut, is an essential legume crop widely cultivated in semi-arid regions, contributing significantly to global food security and agricultural economies. However, environmental stresses, particularly drought, often reduce its productivity, affecting both yield and quality. This study aimed to evaluate the genetic diversity and drought tolerance of groundnut genotypes using SSR markers associated with drought tolerance traits. The SSR analysis identified 365 alleles across 40 markers, averaging 3.38 alleles/ locus. Polymorphism information content (PIC) values ranged from 0.22 to 0.82, indicating substantial genetic variation. Cluster analysis revealed an average genetic similarity of 0.61 among genotypes, indicating significant genetic diversity. The study also assessed the impact of drought stress during full flowering on yield traits. Under well-watered conditions, LC3 and LC2 varieties performed best, while drought stress significantly reduced pod formation and individual plant yield across all genotypes. Reductions reached up to 42.64% for filled pods/plant (LC5) and 38.85% for individual yield (LC7), demonstrating considerable variability in drought responses among genotypes. These findings highlight the genetic basis of drought tolerance in groundnuts and underscore the potential of marker-assisted selection in breeding programs to enhance drought resilience. This research supports the development of drought-resistant peanut varieties, contributing to sustainable agriculture and global food security initiatives.

Keywords: Arachis hypogaea L., drought stress, genetic diversity, SSR

1. INTRODUCTION

Groundnut (*Arachis hypogaea* L.), commonly known as peanut, is a crucial legume crop cultivated extensively in semi-arid regions. It plays a vital role in global food security and agricultural economics (Dwivedi et al., 2003). However, environmental stresses, particularly drought, often hinder its productivity, significantly affecting both yield and quality (Puppala et al., 2023).

Drought remains a significant constraint in groundnut production, especially in drought-prone areas (Abady et al., 2019). It profoundly impacts the flowering stage of groundnut plants, a critical phase for determining crop yield (Jongrungklang et al., 2013; Sarma & Sivakumar, 1989). Drought disrupts various physiological and biochemical processes, ultimately affecting flowering and reproductive success (Oguz et al., 2022; Wahab et al., 2022).

Enhancing drought tolerance in groundnut cultivars is a primary goal for breeding programs focused on increasing crop resilience and promoting sustainable agriculture. Understanding the genetic basis of drought tolerance in groundnut is crucial for developing drought-resistant varieties through marker-assisted selection (Holbrook et al., 2016; Jyostna Devi et al., 2019). Many studies have mapped Quantitative Trait Locus (QTLs) linked to drought tolerance traits using phenotypic evaluations, molecular marker analysis, and genetic mapping (Gautami, 2012; Pandey et al., 2020; Ravi et al., 2011; Varshney, Bertioli, et al., 2009). These investigations have revealed QTLs related to traits like leaf area index, root characteristics, water use efficiency, and yield factors. Understanding these QTLs is essential for developing molecular markers that facilitate marker-assisted selection in breeding programs. Further exploration of genes regulating root systems, including main tap and finer roots, is necessary (Wasaya et al., 2018). Additionally, certain parental lines and mapping populations of peanuts exhibit significant variation in shoot and root traits, highlighting the potential for genetic advancements..

In Vietnam, the diversification of groundnut varieties has been significantly enriched through strategic exchanges of genetic resources. This has led to a broader repository of cultivars with increased adaptability, higher yield, and superior quality. Despite this progress, the selection of groundnut varieties from imported genetic resources has primarily focused on those from China, ICRISAT, and Australia (Mai et al., 2017).

Cuba, with its unique agro-environmental conditions and rich agricultural heritage, has given rise to groundnut varieties with potentially distinctive genetic features, especially those related to drought tolerance (Beebe et al., 2013; Galford et al., 2018). These regionally adapted varieties represent a valuable genetic asset for international breeding programs. Exploring their genetic diversity and identifying drought tolerance traits are critical steps toward developing new cultivars capable of thriving in arid conditions

Assessing the genetic diversity of groundnut cultivars using molecular markers, especially SSR (Simple Sequence Repeat) markers linked to drought tolerance, is essential for breeding programs aimed at developing drought-tolerant varieties. This study aims to evaluate the genetic diversity of groundnut varieties imported from Cuba by using SSR markers associated with drought tolerance and assess their drought tolerance based on yield traits. The findings will provide insights into the genetic richness and potential of Cuban groundnut varieties, supporting the development of improved cultivars with enhanced drought tolerance. Ultimately, this contributes to advancing drought-resistant groundnut breeding in Vietnam.

2. MATERIALS AND METHODS

Genetic materials

In this study, genetic material from seven groundnut genotypes imported from Cuba was utilized. Additionally, a red groundnut variety (DBG), commonly cultivated in Bac Giang, Vietnam, served as a reference (Table 1). Leaves from seedlings aged 14 days were sampled for DNA extraction, with strict precautions taken to prevent contamination.Genetic analysis was performed at Agricultural Genetics Institute.

Table 1. Groundnut genotypes used in the study

No.	Groundnut genotypes	Source
1	DBG	Bac Giang, Vietnam
2	LC1	Cuba
3	LC2	Cuba
4	LC3	Cuba
5	LC4	Cuba
6	LC5	Cuba
7	LC6	Cuba
8	LC7	Cuba

DNA isolation and SSR marker analysis

Genomic DNA was isolated from bulk young leaves of ten plants from each genotype. The extraction followed the Cetyl Trimethyl Ammonium Bromide (CTAB) method described by (Doyle & Doyle, 1987). Forty pairs of SSR markers related to major QTLs for drought tolerance-related traits were selected from published references as shown in table 2 (Gautami, 2012; Pandey et al., 2020; Ravi et al., 2011). The PCR reaction was carried out in a 20 µl reaction solution containing: 2 µl of template DNA (50 ng), 2 µl of PCR buffer (10X), 0.4 µl of dNTPs (10 mM), 1 µl of each primer (10 ng), 1 µl of Taq DNA polymerase (5 U/µl). The PCR amplification reactions were performed in a thermal cycler (Cleaver Scientific, United Kingdom) with the following cycle profile: Initial denaturation at 95 °C for 5 min, 35 cycles of 45s denaturation at 95 °C, 60s annealing at 55 °C, 60 °C, or 62 °C (depending on the specific primer), 1 min extension at 72 °C, final extension at 72 °C for 10 min. The PCR products were separated by 2% agarose gels in 1X TAE buffer. The separated bands were visualized under a UV transilluminator.

Table 2. Microsatellite markers linked to major QTLs for drought tolerance-related traits

Marker	Forward (5'-3')	Reverse (5'-3')
GM2246	GCAATTTATGTGCACCCTTTT	CGCTTGACACCAATGAAGTCT
GM660	TCTTTATCCCGATGAATGAAA	CTCCCACAAACACAAACACAC
GM679	GGTGTTATGTATAGCCACCAG	AAATAGTATGGACCAGAAATAATAAG
GM1911	CAGCTTTCTTTCAATTCATCCA	CACTTCGTGTTCTTCCTGCTC
GM694	ATTTGTGCCCTACCACCTTCT	TCCCTCCTAGAGGTTGACTTGA
GM672	GGAGAACCAGTGACGTGACATA	GGATTAATTCTGATACCATGAAAGG
GM690	TGAAAGTAACTCGTTTACAGTTTGAAG	TCACTAAACATGTGGGTAACTAAGAAA
GM626	CATCCAAAGCCAAAGTTCACA	GCTTAGCTTGCTTTGATTAGGG
GM623	CAGGATGAACAGGCACAGAAT	ATGAACAATTGCGATTTGGAC
GM629	CAAGAGGGACGGATAATAGCA	GACGCAAGGAAATGAGCATAC

Seq16C06	TTGCTACTAAGCCGAAAATGAAG	CTTGAAATTAACACATATGCACACA
Seq13B08	GGAGAAAGATCAAACGAGAACA	TTCGAATATCTGACATTTGCTTTT
Seq15C10	ATTCCCATGTCGTCAAGACC	GCGACGGTATTGGCTTTTAG
Seq17E1	TTCGTTGACGTGAGCGTTAC	TTAGGATTGTTCCAAGGCCA
Seq13A10	AACTCGCTTGTACCGGCTAA	AGGAATAATAACAATACCAACAGCA
Seq10D04	ATCCCTGATTAGTGCAACGC	CGTAGGTGGTTTTAGGAGGG
Seq19H03	TGGCAGGCAGTAAACATCAG	TTGAGGACGTGATGAACTGG
Seq2B09	GCAACATGCTCTGAATTTTGAC	TGTGCAACCCAATTCAATAACTT
Seq3B05	CCTCCCTGCTTGATCCAATA	AACTGTAGCGAATGTGTTACATGG
Seq3A06	TGCATCAGCAAGCTACATACG	GCGATTCACCATCAATCTCA
Seq13A7	AATCCGACGCAATGATAAAAA	TCCCCTTATTGTTCCAGCAG
Seq18G09	ATATCAGCGCCAATGACTCC	TCGCTCCTGGCACCTATATC
GA35	CAAAGTTTGCAGTGATTTTGTTG	AAATTTTCAGGTAAATCATTCTT
S108	GCTTACATTACACGTCADCTC	CCGAACTTACAGTTAGGAG
PM499	TCCCTTCTAAACACGAAATGG	ACTGAATGGAGAAAAGAGTGTGG
PM375	CGGCAACAGTTTTGATGGTT	GAAAAATATGCCGCCGTTG
IPAHM108	CTTGTCAAACTCTGTGACTTAGCA	CATGAACAATTACACCCAGTCA
IPAHM287	TCTAACCCTTCGGTTCATGG	TCACTATCCCATCCCTGCTC
IPAHM689	GATGACAATAGCGACGAGCA	GTAAGCCTGCAGCAACAACA
IPAHM105	CAGAGTTTGGGAATTGATGCT	GCCAGATCTGAGCAAGAACC
TC3A12	GCCCATATCAAGCTCCAAAA	TAGCCAGCGAAGGACTCAAT
TC3H07	CAATGGGAGGCAAATCAAGT	GCCAAATGGTTCCTTCTCAA
TC11B04	GATCTGAAGGCTCTGATACCAT	GATCTCAACCAGAACAGTATGC
TC2D08	ATGTGGGGAGGTCGGTAAC	TCACAGGTTTTGTGTGCTCG
TC6E01	CTCCCTCGCTTCCTCTTTCT	ACGCATTAACCACACACCAA
TC9B07	CCATCTCCTTCTTGACTTTAGCC	GTTCTCCAACCTCCTCCTTTTC
TC3E02	TGAAAGATAGGTTTCGGTGGA	CAAACCGAAGGAGGAACTTG
TC2D06	AGGGGGAGTCAAAGGAAAGA	TCACGATCCCTTCTCCTTCA
TC1A02	GCAATTTGCACATTATCCGA	CATGTTCGGTTTCAAGTCTCAA
TC7C06	GGCAGGGGAATAAAACTACTAACT	TTTTCCTTCCTTCTCCTTTGTC

Drought tolerance assessment based on yield traits

In this experiment, we evaluated the drought tolerance of eight groundnut varieties: DBG, LC1, LC2, LC3, LC4, LC5, LC6, and LC7. The experimental procedure followed the method outlined by Thang et al., 2008. The experimental procedure was as follows: Each of the eight groundnut varieties was planted in plastic pots (40 cm in diameter, 30 cm in height). The pots contained 10 kg of alluvial soil, which was air-dried, finely crushed, and mixed with basal fertilizers (0.75 g urea, 5.6 g phosphate, and 1.5 g potassium per pot). Eight seeds were sown in each pot, and soil moisture was maintained at 75-85% during seedling development. After the plants developed two true leaves, thinning was performed to retain five plants per pot. The pots were kept under natural environmental conditions (temperature and humidity).

The drought tolerance of the groundnut varieties was assessed during the full bloom period. Two treatments were applied:

Treatment 1: Plants were watered adequately throughout the growing period, maintaining soil moisture at 70-85%.

Treatment 2: Plants were watered adequately (maintaining moisture at 70-85%) until the peak flowering period. Watering was then stopped until 70% of the plants exhibited wilting (defined as the complete loss of turgor pressure in the leaves). At that point, watering was resumed
Evaluation criteria included the number of pods per plant, the number of filled pods per plant, and individual plant yield (g/plant) according to QCVN01-57:2011 (Ministry of Agriculture and Rural Development, 2011). The study was conducted in the glasshouse of the Legumes Research and Development Center, Field Crops Research Institute, Vietnam Academy of Agricultural Sciences.

Data analysis
Alleles corresponding to SSR markers were recorded as 1 for presence and 0 for absence. These binary scores were compiled into a computer file to create a matrix, which served as the foundation for genetic diversity analysis. We calculated the Simpson diversity index, also known as the polymorphism information content (PIC), for each SSR marker. The PIC value for each marker was determined using the method suggested by Cong et al., 2023 as follows:

$$\mathbf{PICj} = \mathbf{1} - \sum_{i=1}^{n} \boldsymbol{Pij^2}$$

Where, (i) represents the (i)th allele of the (j)th marker, (n) is the total number of alleles at the (j)th marker, (P) denotes allele frequency.
Genetic relationships among the genotypes were determined using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis. A similarity matrix, based on shared fragments, was constructed following the approach outlined by Nei & Li, 1979. The analysis was performed using the software package NTSYS 2.1, and genetic similarity was calculated using the Jaccard coefficient from the 0-1 scores obtained from SSR markers.
Additionally, for agronomic traits, we conducted experimental analyses using a completely randomized design with a minimum of three replications. Subsequently, we applied Duncan's multiple-range test ($P < 0.05$) to analyze the data

3. RESULTS

Characteristics of SSR markers
All 40 SSR primers produced reproducible bands, with 33 primers (82.5%) suitable for genetic diversity assessment of the eight groundnut varieties, while the remaining seven were monomorphic. A total of 365 alleles were detected from the 40 SSR markers across all eight genotypes. The number of alleles per locus ranged from one for all monomorphic markers (Seq13A10, Seq16C06, Seq13B08, Seq15C10, GA35) to six for certain polymorphic markers (GM1911, Seq19H03, Seq10D04, IPAHM108, TC7C06), with an average of 3.38 alleles/locus (Table 3).
The polymorphism information content (PIC) value measures allelic differentiation. The mean PIC value for the 33 SSR markers was 0.62, with values ranging from 0.22 for primer PM499 to 0.82 for primer Seq19H03 (Table 3).
There were 46 unique fragments that could distinguish the genotypes (table 3): 5 in DBG, 1 in LC1, 21 in LC2, 1 in LC3, 10 in LC4, 3 in LC5, 2 in LC6, and 3 in LC7. The primers produced distinguishable fragments as shown in table 3: TC7C06 gave 3 fragments (1 each for LC2, LC4, LC7), IPAHM108 gave 3 fragments (1 each for LC2, LC4, LC5), GM626 gave 3 fragments (1 each for LC2, LC5, LC7), GM629 gave 1 fragment (for LC2), Seq10D04 gave 2 fragments (for LC2), Seq19H03 gave 2 fragments (1 each for LC2, LC5), Seq2B09 gave 2 fragments (1 each for LC2, LC4), Seq3B5 gave 3 fragments (1 each for DBG, LC2, LC4), Seq13A7 gave 2 fragments (1 each for LC3, LC4), TC3H07 gave 4 fragments (1 each for DBG,

LC2, LC4, LC7), TC11B04 gave 2 fragments (for LC2), TC2D08 gave 2 fragments (1 each for DBG, LC2), PM499 gave 1 fragment (for LC2), GM1911 gave 2 fragments (1 each for LC2, LC4), PM375 gave 1 fragment (for LC7), IPAHM689 gave 1 fragment (for LC1), IPAHM105 gave 4 fragments (1 each for DBG, LC2, LC4, LC6), Seq17E1 gave 1 fragment (for LC4), Seq18G09 gave 1 fragment (for LC2), TC3E02 gave 1 fragment (for LC2), TC2D06 gave 3 fragments (1 each for DBG, LC2, LC6), and S108 gave 2 fragments (1 each for LC2, LC4).

Table 3. Number, unique alleles and PIC value of the amplified SSR markers in groundnut

No.	Marker	Number of alleles	Unique alleles	PIC
1	GM2246	4	-	0.69
2	GM660	2	-	0.44
3	GM679	3	-	0.65
4	GM1911	6	2	0.81
5	GM694	2	-	0.38
6	GM672	2	-	0.49
7	GM690	2	-	0.38
8	GM626	5	3	0.72
9	GM629	4	1	0.61
10	Seq17E1	3	1	0.65
11	Seq10D04	6	2	0.79
12	Seq19H03	6	2	0.82
13	Seq2B09	5	2	0.78
14	Seq3B5	4	3	0.56
15	Seq3A6	3	-	0.63
16	Seq13A7	5	2	0.74
17	Seq18G09	3	1	0.59
18	S108	3	2	0.41
19	PM499	2	1	0.22
20	PM375	4	1	0.72
21	IPAHM108	6	3	0.78
22	IPAHM287	4	-	0.72
23	IPAHM689	4	1	0.69
24	IPAHM105	5	4	0.69
25	TC3H07	5	4	0.69
26	TC11B04	5	2	0.77
27	TC2D08	3	2	0.41
28	TC6E01	3	-	0.66
29	TC9B07	2	-	0.50
30	TC3E02	5	1	0.73
31	TC2D06	4	3	0.61
32	TC1A02	2	-	0.28
33	TC7C06	6	3	0.74
	Total	128	46	
	Mean	3.8		0.62

Genetic similarity and clustering analyses

Using the NTSYS software, the average genetic similarity among the eight groundnut accessions was 0.61, with individual values ranging from 0.47 (between LC2 and LC1) to 0.76 (between LC6 and LC7).

The UPGMA cluster analysis, based on the 34 polymorphic loci, produced a phenogram that grouped the accessions into two main clusters at a genetic similarity value of 0.52. Cluster I included seven accessions (DBG, LC1, LC3, LC4, LC5, LC6, and LC7), while Cluster II contained only cultivar LC2, making it the most differentiated among the eight genotypes.

Within Cluster I, two subclusters were identified with a genetic similarity of 0.60. Sub-cluster I-1 consisted of six accessions (DBG, LC1, LC3, LC5, LC6, and LC7), while Sub-cluster I-2 included only the LC4 accession. At a genetic similarity of 0.64, the DBG accession was differentiated from the remaining genotypes, which originated from Cuba (Fig. 1).

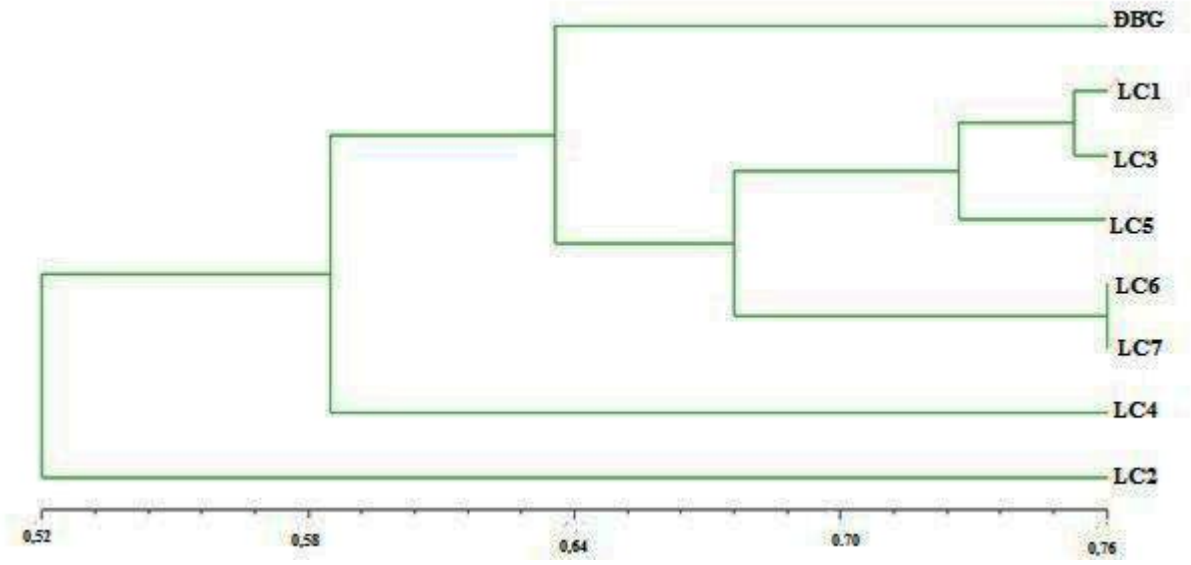

Figure 1. Dendrogram showing diversity among 8 varieties of groundnut based on SSR markers by using NTYSYS 2.1

This analysis highlights the significant genetic diversity within the groundnut accessions and the unique genetic position of the LC2 variety, which could be valuable for breeding programs aiming to introduce new traits.

Effects of drought during the full flowering stage on yield traits of groundnut varieties

Some yield traits of eight groundnut genotypes were evaluated under both well-watered and drought stress conditions (Table 4). In well-watered conditions, LC3 and LC2 exhibited the highest number of pods per plant, mature pods per plant, and individual plant yield, while DBG showed the lowest yield traits among the genotypes. Among the imported varieties, LC1 displayed the lowest number of mature pods per plant and individual plant yield under well-watered conditions.

Under drought stress at the flowering stage, varieties LC2, LC3, and LC4 demonstrated the highest yield traits, whereas the remaining varieties had similarly low individual plant yields, without statistically significant differences ($P<0.05$).

Table 4. Effects of drought during the full flowering stage on yield traits of groundnut varieties

Condition	Variety	Number of pods/plant (pods/plant)	Number of filled pods/plant (pods/plant)	Individual yield (g/plant)
Well-watered	LC1	8.75d	4.92a	9.3a
	LC2	10.11e	6.84d	11.8d
	LC3	10.58f	6.8d	11.7d
	LC4	10.2e	5.5b	10b
	LC5	6.38b	5.75bc	9.99b
	LC6	8.42c	5.6b	10.42c
	LC7	8.85d	5.96c	10.66c
	DBG	5.83a	5.1a	9.16a
Drought stress	LC1	5.3a	3.5a	6.57a
	LC2	6.63e	4.53d	7.55c
	LC3	6.28d	4.67d	7.48bc
	LC4	6.91e	4.36cd	7.2b
	LC5	5.5ab	3.3a	6.33a
	LC6	5.83bc	4.12bc	6.51a
	LC7	6.01cd	3.89b	6.52a
	DBG	5.53ab	3.33a	6.35a

Note: Different letters indicate statistically significant differences ($p < 0.05$).

Examining the impact of drought on yield traits, LC3 showed the most pronounced effect on pod formation, with a 40.65% decrease in the number of pods per plant. DBG was the least affected, with a decrease of 5.15%, while LC5 experienced a decrease of 13.79%. The number of mature pods per plant was most severely impacted in LC5, decreasing by 42.64%, whereas LC4 showed the least reduction at 20.75%. DBG exhibited a decrease of 34.72% in the number of mature pods per plant. For individual plant yields, LC4 experienced the smallest decrease at 28.01%, whereas LC7 was the most affected, with a reduction of 38.85%. DBG's individual plant yield decreased by 30.68%, with LC2 and LC3 decreasing by 36.02% and 36.07%, respectively (Figure 2).

Thus, drought stress negatively impacted the yield traits of all imported groundnut varieties and local Vietnamese varieties, significantly reducing the individual plant yield of all studied genotypes by up to 34.14%.

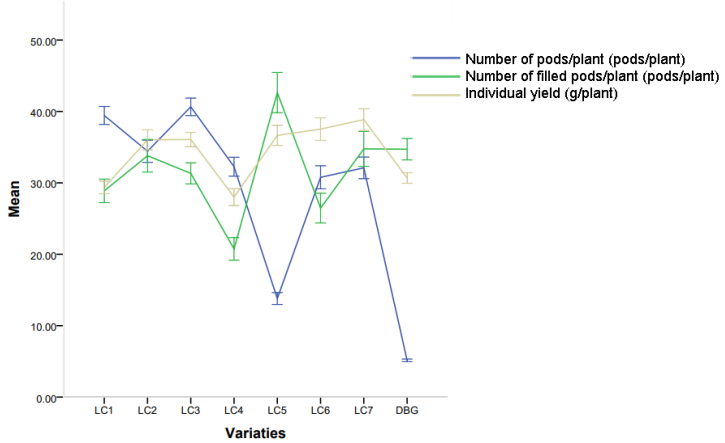

Figure 2. Reduction in the number of pods/plant, filled pods/plant, and individual yields due to drought stress.

4. DISCUSSION

The successful amplification of reproducible bands by all 40 SSR primers indicates a robust set of markers suitable for genetic analysis. The discovery that 33 of these primers (82.5%) are polymorphic and suitable for genetic diversity assessment aligns well with similar studies, reinforcing the utility of SSR markers in groundnut genetics. The total of 365 alleles detected across eight genotypes highlights significant genetic variability, which is crucial for breeding programs aimed at improving traits such as drought tolerance.

The number of alleles/locus ranged from one for monomorphic markers to six for the most polymorphic ones, with an average of 3.38 alleles per locus. This is consistent with findings from previous studies. Pandey et al., 2020 reported an average of 3.5 alleles per locus in their study on groundnut genotypes, which is very similar to our findings. Ravi et al., 2011 found a slightly higher average of 4.2 alleles per locus, which might be due to the inclusion of a more diverse set of genotypes or the use of different SSR markers. Gautami, 2012 observed an average of 3.1 alleles per locus, aligning closely with our results, reinforcing the consistency of SSR markers in detecting genetic variation in groundnut. Markers like GM1911, Seq19H03, Seq10D04, IPAHM108, and TC7C06, which had a higher number of alleles, proved to be particularly informative for distinguishing between closely related genotypes.

The PIC value is an important parameters to measure and estimate genetic diversity in a population; a higher value of PIC indicates a more complex and diverse population structure (Garzón-Martínez et al., 2015). Locus polymorphism is classified as low, medium, or high based on the value of PIC. A value less than 0.25 is considered low, between 0.25 and 0.5 is medium, and greater than 0.5 is high (Serrote et al., 2020). In this study, the mean PIC value of 0.62 indicates a high level of allelic diversity, which is crucial for effective marker-assisted selection.

High PIC values observed for markers indicate their informativeness and ability to detect a large number of alleles. This suggests a substantial genetic variation at these loci, making them particularly valuable for detailed genetic mapping and diversity studies. Researchers prefer high PIC markers because they provide more information about genetic variation and can distinguish between closely related genotypes.

The consistent high PIC values observed in this study align with findings from other studies on groundnut and other crops, reinforcing the reliability of simple sequence repeat (SSR) markers. For instance, Varshney et al. (2009) reported PIC values ranging from 0.25 to 0.87 in their study of SSR markers in groundnuts, which is similar to the range found in our study. Similarly, Ravi et al., 2011 reported PIC values ranging from 0.3 to 0.85, with a mean of 0.65, demonstrating comparable diversity levels. Gautami, 2012 found a mean PIC value of 0.60, with values ranging from 0.25 to 0.80, further supporting the importance of SSR markers in breeding programs.

The identification of 46 unique fragments across the eight genotypes further underscores the genetic differentiation among these varieties. Notably, LC2 exhibited the highest number of unique fragments (21), suggesting it possesses significant genetic diversity that could be harnessed for breeding programs. This high level of unique fragment generation is consistent with findings from other studies that have explored genetic diversity in groundnuts, such as the study by Varshney et al. (2009)

In the current study, the genetic similarity values ranged from 0.47 to 0.76, which is comparable to the findings of Cuc et al., 2008, who reported genetic similarity values between 0.45 and 0.8 among a diverse set of groundnut accessions. The UPGMA cluster analysis grouped the accessions into distinct clusters, with LC2 standing out as the most genetically differentiated. This differentiation is crucial for breeding programs as it identifies LC2 as a valuable source of unique genetic traits.

Drought stress significantly impacted the yield traits of all eight groundnut genotypes studied, highlighting the sensitivity of groundnut plants to water scarcity, especially during the flowering stage. Drought stress can disrupt the synchronization of male and female flower development, leading to asynchronous flowering and decreased pollination efficiency (Alqudah et al., 2011; Sehgal et al., 2018; Seleiman et al., 2021). Moreover, drought stress can also affect the quality and viability of pollen grains, as well as the receptivity of the stigma, which can result in reduced fertilization rates and lower pod and seed set (Alqudah et al., 2011; Bita & Gerats, 2013). The effects of drought on groundnut flowering can have cascading impacts on the overall yield, as the number and quality of pods and seeds produced are directly linked to the successful completion of the flowering stage (Singh et al., 2013).

This sensitivity was evident in the reduction of pod numbers, mature pods, and individual plant yield under drought conditions compared to well-watered conditions as documented in studies by Mai et al., 2017. The imported varieties exhibited varying responses to drought stress. This diversity suggests that different genotypes have adapted differently to their environments. Investigating the genetic basis of this adaptation could provide insights into the molecular pathways involved in drought tolerance.

The number of pods per plant and the number of mature pods per plant were two key yield traits affected by drought stress. LC5 experienced the most severe reduction in the number of mature pods per plant (42.64%), while LC4 experienced the least reduction (20.75%). These findings suggest that while some genotypes can partially maintain pod formation under drought conditions, the maturation of these pods is still heavily affected by water scarcity.

Individual plant yield showed a similar trend, with reductions ranging from 28.01% (LC4) to 38.85% (LC7). DBG's yield decreased by 30.68%, indicating that even the genotypes with higher drought tolerance still suffered significant yield losses under drought stress. This underscores the critical impact of drought on overall productivity and the need for breeding programs to focus on enhancing drought resilience.

The observed trade-offs between yield components are intriguing. While LC3 and LC2 had higher pod numbers and individual plant yield, they also experienced significant reductions in pod formation during drought stress. This suggests that allocating resources to pod production comes at the cost of drought tolerance. Researchers could explore the underlying physiological mechanisms responsible for these trade-offs in yield components. In addition, based on genetic analysis using SSR markers, crossing imported groundnut varieties with local DBG varieties may yield hybrids with superior drought tolerance, productivity, and local environmental adaptability. This approach could provide valuable insights and potentially lead to the development of high-performing cultivars resilient to drought conditions.

5. CONCLUSIONS

The use of 40 SSR markers in this study to assess the genetic diversity of eight groundnut genotypes demonstrated their reliability for genetic analysis. High PIC values and genetic similarity analysis highlight these markers' potential in identifying drought-resistant traits. Drought stress significantly impacts the yield traits of these genotypes, with considerable variability among them. For farmers in drought-prone regions, selecting appropriate groundnut varieties is crucial. While LC3 and LC2 perform well under optimal conditions, their susceptibility to drought warrants caution. LC4, with a smaller reduction in individual plant yield, might be a more balanced choice. Breeders might consider crossbreeding imported and local varieties to develop high-yield, drought-resistant cultivars suited to local conditions. This study emphasizes the importance of integrating genetic and phenotypic data to enhance breeding programs.

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