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Evaluating Drought Tolerance in Common Beans using Drought Indices and Molecular Markers

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ABSTRACT

Background: Drought stress is a major abiotic constraint affecting common bean (*Phaseolus vulgaris* L.) production in Vietnam, highlighting the need to identify drought-tolerant genotypes for sustainable cultivation.

Methods: This study assessed drought tolerance in 11 imported genotypes using morphological indices and SSR markers. Plants were grown under control (100% field capacity) and drought stress (30% field capacity) conditions, with tolerance evaluated based on the drought susceptibility index (DSI), drought tolerance efficiency (DTE) and grain weight (GW). Molecular analysis using 20 SSR markers.

Result: DR1, DR2 and DR6 demonstrated the highest drought tolerance (DSI<1, DTE>54%), whereas DR9, DR11 and DR12 (CVR VN2) were drought-sensitive. Molecular analysis using 20 SSR markers identified 81 alleles (4.05 alleles per locus), indicating moderate genetic diversity (PIC= 0.50, Jaccard's index= 0.56). Cluster analysis (UPGMA) grouped the genotypes into two main clusters, with drought-tolerant genotypes forming a distinct sub-cluster. These results reveal a strong correlation between morphological drought tolerance and genetic diversity, emphasizing the potential of DR1, DR2 and DR6 for cultivation in drought-prone regions. Further marker-assisted selection (MAS) and hybridization strategies are recommended to develop high-yielding, drought-tolerant common bean varieties, ensuring climate-resilient production in Vietnam.

Key words: Drought indices, Genetic diversity, *Phaseolus vulgaris* L, SSR.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) belongs to Fabaceae family, is widely grown in Vietnam, America and parts of Africa, is one of the most significant food legume (Mladenov *et al.*, 2023). This crop serves as an affordable and abundant source of essential nutrients, including crude protein (20.69 to 25.81%), carbohydrates (57 to 72,42%) and 5.83 mg of iron (0.5%) (Kuldeep *et al.*, 2025). It is the second most important source of human dietary proteins and the third most important source of calories (Sundharaiya *et al.*, 2023). Moreover, common beans contain bioactive compounds with numerous health-promoting properties, such as antioxidant, anticancer and anti-diabetic effects (Mladenov *et al.*, 2023).

Common bean production faces serious challenges from drought stress, a key factor contributing to global food insecurity. Moderate to severe drought episodes, particularly during the grain-filling stage, have caused significant yield reductions (Sánchez-Reinoso *et al.*, 2020) range from 60% to 99%, especially when water shortages occur during flowering and post-flowering stages (Manjeru *et al.*, 1995). This bean survived in short periods of drought due to their ability to increase the proline content under water-limiting conditions, making it an adaptable crop to climate change (Del Rosario *et al.*, 2025).

In Vietnam, legume crops frequently encounter drought stress during the dry winter season, underscoring the necessity of developing drought-tolerant common bean varieties. While plants adopt various strategies to mitigate drought stress including morphological changes, modified growth patterns and biochemical and physiological adjustments breeding programs have historically prioritized yield-related traits (Quat *et al.*, 2024). Consequently, many high-yielding common bean cultivars remain highly susceptible to drought, whereas landraces and wild relatives often exhibit strong drought tolerance but lower yields. This trade-off poses a significant challenge for breeding programs aiming to combine drought resilience with high productivity.

Marker-assisted selection provides a promising approach for enhancing drought tolerance in common bean (Liu *et al.*, 2007). Among the available molecular markers, simple sequence repeats (SSRs) are widely used for

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genetic diversity analysis and the identification of traits associated with drought tolerance (Assefa *et al.*, 2019). SSR markers linked to drought responses enable breeders to facilitate the selection of genotypes with enhanced yield potential and improved resilience under water-limited conditions (Mir *et al.*, 2012).

This study aims to evaluate drought tolerance in 11 imported varieties through the use of drought indices and SSR markers. By integrating morphological and molecular screening techniques, this research provides a robust framework for identifying elite genotypes suitable for breeding programs aimed at enhancing drought resilience in common beans.

MATERIALS AND METHODS

The present study was carried out at Field Crops Research Institute and Agricultural Genetics Institute, Vietnam Academy of Agricultural Sciences during 2023-2024. The experimental material for this study consisted of 12 common bean accessions, included 11 imported genotypes and one high-yield variety, which is widely cultivated in Vietnam and distributed by Sao Viet Company (Table 1).

Evaluating drought tolerance in common bean using drought indices

The experiment water stress treatment was conducted in a greenhouse in 2023, using a completely randomized design with three replications. Seventy-two pots (arranged as 12×3×2) were prepared, each covered with nets to prevent bird interference. All plants were initially cultivated under optimal watering conditions until the introduction of drought stress at the reproductive stage, corresponding to 30-40% full blooming, approximately 50 days after seedling emergence. During this phase, stressed pots were maintained at 30% field capacity, while control pots received water at 100% field capacity. Both groups were grown under identical environmental conditions (Sánchez-Reinoso *et al.*, 2020; Haque *et al.*, 2021). Polythene shades were used to protect pots from unintended rainfall during the drought treatment. Grain weight per plant (GW), drought tolerance efficiency (DTE) and drought susceptibility index (DSI) were assessed as described by Haque *et al.* (2021).

Table 1: List of common bean genotypes used in the study.

Source	Genotype
DR1 (DRV18)	Cuba
DR2	Cuba
DR3	Cuba
DR4	Cuba
DR5	Cuba
DR6	Cuba
DR7	Cuba
DR8	Cuba
DR9	Cuba
DR10	Cuba
DR11	Cuba
DR12 (CVR VN2)	Vietnam

Evaluating drought tolerance in common bean using SSR markers

DNA extraction

The extraction of genomic DNA from 14-day-old green leaves was performed using a modified CTAB protocol, adapted from Doyle and Doyle (1987). A NanoDrop spectrophotometer (Thermo Scientific) was utilized to assess the DNA's purity and concentration. The DNA samples were then diluted to a working concentration of 50 ng/µl for subsequent amplification of SSR markers using an Eppendorf thermocycler.

SSR marker genotyping

A total of 20 SSR markers associated with drought tolerance were used to evaluate drought resistance in a common bean variety (Yu *et al.*, 2000; Özkan *et al.*, 2022; Blair *et al.*, 2012). PCR reactions and conditions were conducted following Cong *et al.* (2023), with modifications to the annealing temperature, which ranged from 55-60°C depending on the specific primer.

Data analysis

The presence or absence of SSR bands in all genotypes was assessed. The data were recorded in a binary matrix, where '1' indicated the presence of an amplification product or band and '0' indicated its absence. This binary matrix was analyzed further. The band scores were used to create a data matrix, which served as the basis for constructing a dendrogram using Jaccard's similarity coefficient and the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in NTSYS-pc version 2.1. Additionally, PowerMarker 3.2 software was employed to compute statistical parameters.

RESULTS AND DISCUSSION

Evaluating drought tolerance in common bean using drought indices

The DSI value is an essential metric for evaluating genotype stability under both optimal and drought-stress conditions. It serves as a criterion to classify common bean genotypes based on their drought tolerance. Genotypes are categorized as tolerant when DSI≤0.5, moderately tolerant when 0.5<DSI≤1 and sensitive when DSI>1 (Rahmah *et al.*, 2020).

Analysis of DSI values for grain weight per plant revealed significant variation, ranging from 0.455 to 1.524, indicating diverse drought responses among the genotypes (Table 2). Based on this classification, DR1 was identified as a drought-tolerant genotype (DSI= 0.455). Genotypes DR2 to DR8 and DR10, with DSI values between 0.5 and 1, were classified as moderately tolerant. In contrast, DR9, DR11 and CVR VN2 (DR12) exhibited DSI values above 1, classifying them as drought-sensitive.

DTE also varied significantly, ranging from 36.824% in CVR VN2 to 69.153% in DR1, further supporting the

observed classification. DR1 exhibited the highest drought tolerance, with a DTE of 69.153% and the lowest DSI (0.455). DR2 (DTE: 64.248%, DSI: 0.872) and DR6 (DTE: 54.795%, DSI: 0.935) demonstrated moderate resilience under water-deficit conditions. Conversely, CVR VN2 (DR12) was the most drought-sensitive genotype, with the lowest DTE (36.824%) and the highest DSI (1.524), accompanied by the lowest grain weight under drought conditions, highlighting its limited adaptation to water stress (Table 2).

The marked yield reduction in CVR VN2 compared to other genotypes may be attributed to inefficiencies in water uptake, underdeveloped root systems, or inadequate osmotic adjustment (Begna, 2022; Dietz *et al.*, 2021). Despite its sensitivity, CVR VN2 could serve as a useful reference genotype in future evaluations of drought tolerance, providing a benchmark for breeding advancements. The variation in DSI and DTE underscores the genetic diversity among the genotypes and highlights the importance of breeding programs aimed at enhancing drought resistance. Investigating physiological mechanisms such as root architecture, osmotic adjustment and antioxidant enzyme activity could provide additional insights into improving resilience in common bean varieties.

Drought stress introduced at the reproductive stage is critical for determining yield stability and resilience, as this phase is highly sensitive to water limitations. In this study, drought stress was imposed at 30-40% full blooming, approximately 50 days after seedling emergence-a stage characterized by active reproductive organ development and grain formation. Water scarcity during this period can severely affect flower retention, pollen viability, pod setting and grain filling, resulting in significant yield reductions (Gusmao *et al.*, 2012). The variation in DSI and DTE values among genotypes reflects their differing abilities to cope with reproductive-stage drought stress, reinforcing the need for selecting genotypes with enhanced physiological and biochemical adaptation mechanisms.

Table 2: Selected parameters of drought tolerance in common bean under drought treatment.

Genotye	GW (under drought condition)	DTE (%)	DSI
DR1	12.538	69.153	0.455
DR2	11.832	64.248	0.872
DR3	10.331	59.790	0.978
DR4	10.431	62.245	0.919
DR5	5.817	54.770	0.910
DR6	11.234	54.795	0.935
DR7	8.927	61.987	0.925
DR8	9.832	59.081	0.885
DR9	6.118	58.270	1.014
DR10	10.231	59.082	0.995
DR11	9.754	62.093	1.031
CVR VN2 (Reference)	8.626	36.824	1.524

Reproductive-stage drought stress directly impacts pollen sterility, ovule fertilization and pod development. Under limited water conditions, reduced pollen viability results in poor fertilization rates and lower pod set (Khatun *et al.*, 2021). This may explain the significant yield reduction observed in CVR VN2 (DR12), which exhibited the highest DSI (1.524) and lowest DTE (36.824%), indicating its inability to maintain reproductive function under stress. In contrast, DR1, DR2 and DR6 showed superior drought resilience, likely due to mechanisms such as improved pollen viability, delayed leaf senescence and better resource allocation to reproductive structures. Additionally, genotypes with efficient osmotic adjustment and deeper root systems likely achieved better water uptake during this stage, mitigating the effects of drought on reproductive development.

Hormonal regulation and carbohydrate partitioning also play crucial roles in reproductive-stage drought responses. Drought-induced imbalances in abscisic acid (ABA) and gibberellins can lead to premature flower and pod abortion, reducing yield potential (Khatun *et al.*, 2021). Furthermore, restricted carbohydrate translocation to developing grains under drought conditions may contribute to lower grain weight in sensitive genotypes like CVR VN2 (DR12). In contrast, drought-tolerant genotypes such as DR1 and DR2 likely maintained efficient sugar transport, supporting grain filling and minimizing yield losses.

The genetic diversity in DSI and DTE values observed in this study highlights the importance of incorporating reproductive-stage drought tolerance into breeding programs. Marker-assisted selection and quantitative trait loci mapping for reproductive-stage drought tolerance traits could accelerate the identification of key genes involved in pollen fertility, carbohydrate partitioning and ABA signaling. Integrating field trials with controlled environment experiments would provide a more comprehensive understanding of genotype responses to reproductive-stage water stress across diverse conditions.

Overall, introducing drought stress at the reproductive stage offers a realistic assessment of genotype performance under field-relevant drought conditions. This study underscores the need for breeding strategies targeting reproductive-stage drought tolerance to develop high-yielding, stress-resilient common bean cultivars. Future breeding efforts can enhance reproductive-stage drought tolerance by incorporating physiological, biochemical and molecular strategies, contributing to improved food security and fostering agricultural sustainability in drought-affected regions.

Evaluating drought tolerance in common bean using SSR markers

Overall SSR diversity

This study assessed the usefulness of SSR markers for examining the genetic diversity of 12 common bean genotypes and evaluating their drought tolerance. A total of 20 SSR markers produced 81 alleles, with allele numbers

ranging from 2 (BM153, BM187) to 6 (PVBR 9/DQ185877), yielding an average of 4.05 alleles per marker. The observed polymorphic bands highlighted differences among the genotypes. Previous research, including studies by Özkan *et al.* (2022) and Zhou *et al.* (2021), aligns with these findings. Wang *et al.* (2023) emphasized the importance of polymorphic bands in revealing genetic variability and establishing systematic relationships among genotypes.

The level of genetic diversity was assessed through polymorphic information content (PIC). According to Serrote *et al.* (2020), markers with PIC values greater than 0.5 are highly polymorphic, values between 0.25 and 0.5 indicate moderate polymorphism and values below 0.25 reflect low polymorphism. In this study, PIC values ranged from 0.27 (BM221) to 0.68 (BM158, PVBR10/ DQ185878), with an average of 0.5 per marker (Table 3). The PIC values reported here are higher than those in other studies, such as Khdir *et al.* (2023). Conversely, higher PIC averages have been documented by Özkan *et al.* (2022) and Vidak *et al.* (2021). The relatively high PIC values in this study confirm that the SSR markers employed were highly polymorphic. Saghai-Maroof *et al.* (1984) noted that markers with PIC values of 0.5 or higher are particularly effective for genetic studies. SSR markers are considered highly suitable for characterizing genetic diversity in common bean and have been widely applied to advanced breeding materials (Gaballah *et al.*, 2021).

Gene diversity (He)

Gene diversity, or heterozygosity (He), represents the likelihood that an individual in a population is heterozygous at a given locus (Temnykh *et al.*, 2000). A higher He value suggests greater allelic diversity, which enhances its informativeness. The He values for SSR markers in this study ranged from 0.29 to 0.73, with a mean of 0.56 (Table 3). Zhou *et al.* (2021) reported similar findings with a mean He of 0.58 using 71 SSR markers on 303 local common bean genotypes. However, this study showed lower allelic diversity compared to findings by Özkan *et al.* (2022) and Vidak *et al.* (2021). Markers BM158, PVBR10/ DQ185878, BM211 and PVBR 6/ DQ185876 exhibited the highest He values of 0.73, 0.72, 0.69 and 0.67, respectively, making them valuable for further genetic diversity and drought tolerance studies.

Genetic similarity analysis using UPGMA

Analysis based on Jaccard's similarity coefficient and UPGMA clustering highlighted genetic diversity among 12 genotypes, providing insights into their differentiation. Genetic similarity coefficients ranged from 0.614 to 1.00, averaging 0.802, demonstrating significant diversity within the germplasm. The UPGMA dendrogram divided the 12 genotypes into two main clusters at a similarity cutoff of 0.70. Cluster 1 included DR1 through DR11, imported Cuban genotypes known for their drought resilience, while Cluster 2 contained only DR12 (CVR VN2), a high-yielding variety widely cultivated in Vietnam.

Cluster 1 further divided into Sub-cluster 1.1 (DR1, DR2, DR5, DR6, DR7, DR3, DR8), characterized by high genetic similarity (coefficient>0.80), indicating shared breeding history and similar drought tolerance mechanisms. Sub-cluster 1.2 (DR9, DR10, DR11, DR4) showed greater

Table 3: Summary statistics for 20 SSR markers across 12 common bean genotypes.

Marker name	Allele no.	Gene diversity	PIC
DROUGH1	4	0.50	0.45
DROUGH2	4	0.50	0.38
BM153	2	0.38	0.30
BM187	2	0.50	0.50
BM114	3	0.52	0.44
BM152	4	0.50	0.38
BM158	5	0.73	0.68
BM211	4	0.69	0.64
BM221	4	0.29	0.27
BM278	5	0.63	0.56
BM137	4	0.63	0.56
BMd-12	5	0.58	0.53
PVBR2/ DQ185873	4	0.64	0.57
PVBR4/ DQ185874	5	0.65	0.61
PVBR5/ DQ185875	4	0.49	0.36
PVBR 6/ DQ185876	3	0.67	0.59
PVBR 9/ DQ185877	6	0.58	0.53
PVBR10/ DQ185878	5	0.72	0.68
PVBR11/ DQ185879	4	0.56	0.50
PVBR12/ DQ185880	4	0.49	0.42
Mean	4.05	0.56	0.50

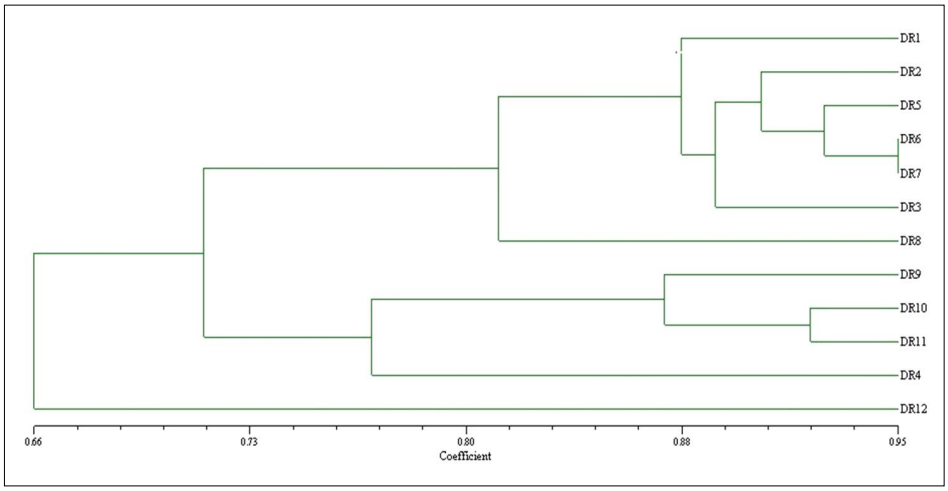


Fig 1: The UPGMA classification tree for 12 common bean genotypes showing the genetic similarity.

genetic variation, with DR4 exhibiting the most distinct genetic profile. Conversely, DR12 (CVR VN2) in Cluster 2 was genetically unique (coefficient ~0.66), reflecting a separate breeding focus on yield improvement rather than drought resilience (Fig 1).

The genetic distinction between DR12 and Cuban genotypes offers potential for hybridization, combining high yield with stress tolerance to address challenges like climate change and poor soil conditions. Conserving Cuban genotypes as a genetic resource is crucial for future breeding programs. Further research involving molecular markers, PCA and hybrid trials could aid in developing superior varieties that balance yield, adaptability and resilience for sustainable agriculture.

The integration of morphological and molecular screening revealed considerable genetic and phenotypic variation among the 12 common bean genotypes, offering valuable insights into mechanisms of drought tolerance.

Morphological evaluation, based on grain weight, drought susceptibility index and drought tolerance efficiency classified DR1, DR2 and DR6 as drought-tolerant genotypes, whereas DR9, DR11 and CVR VN2 (DR12) were identified as drought-sensitive, with CVR VN2 showing the highest susceptibility (DSI= 1.524, DTE= 36.824%). Molecular clustering using Jaccard's similarity coefficient and UPGMA analysis further validated these findings, grouping the genotypes into two primary clusters. Cluster 1 (DR1 - DR11) comprised Cuban-imported genotypes known for their stress tolerance, while Cluster 2 (DR12/CVR VN2) represented a genetically distinct high-yield variety cultivated in Vietnam.

Within Cluster 1, Sub-cluster 1.1 (DR1, DR2, DR5, DR6, DR7, DR3 and DR8) demonstrated high genetic similarity (coefficient >0.80), corresponding to their notable drought tolerance. In contrast, Sub-cluster 1.2 (DR9, DR10, DR11 and DR4) exhibited greater genetic diversity, reflective of moderate to low drought tolerance levels. The genetic distinctiveness of CVR VN2 (coefficient ~0.66) suggests a separate breeding history that emphasized yield enhancement over stress adaptation. This separation highlights the potential for hybridization with Cuban drought-tolerant genotypes to improve both yield and drought resilience.

The strong correlation between genetic clustering and drought tolerance underscores the significance of integrating phenotypic selection with molecular breeding approaches in future crop improvement initiatives. Further research involving molecular marker analysis, QTL mapping and hybrid performance trials is recommended to identify key drought-responsive genes and expedite the development of high-yielding, climate-resilient common bean varieties, supporting sustainable agricultural practices under water-limited conditions.

CONCLUSION

A combined analysis of molecular data and drought tolerance parameters revealed distinct clustering patterns among genotypes based on their physiological performance. DR1, DR2 and DR6 emerged as the most drought-resilient genotypes, while DR9, DR11 and DR12 (CVR VN2) were identified as drought-sensitive. Molecular analysis indicated moderate genetic diversity, with drought-tolerant genotypes forming a clearly defined subgroup. These findings highlight the potential of DR1, DR2 and DR6 for cultivation in drought-prone areas and advocate for further marker-assisted selection (MAS) and hybridization strategies to develop climate-resilient common bean varieties.

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Disclaimers

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Informed consent

All procedures for experiments were approved by the Vietnam Academy of Agricultural Sciences and handling techniques were approved by the Agricultural Genetics Institute.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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