RESEARCH ARTICLE Legume Research- An International Journal, Volume 49 Issue 8: 1277-1288 (August 2026)

Phenotypic and Molecular Diversity of Commercial Yardlong Bean [*Vigna unguiculata* (L.) Walp. Subsp. *sesquipedalis*] Hybrids for Breeding Utilization

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10.18805/LRF-939

ABSTRACT

Background: Yardlong bean [*Vigna unguiculata* (L.) Walp. subsp. *sesquipedalis*] is a significant vegetable legume in Southeast Asia, where breeding programs focus on pod length, yield and uniformity. Prolonged selection for uniform commercial traits may progressively reduce genetic variability within hybrid germplasm if not systematically monitored.

Methods: Twenty commercial F_1 yardlong bean hybrids were evaluated using seventeen morphological traits. Multivariate analyses (correlation, UPGMA and principal component analysis) were performed to assess phenotypic structure. Genetic diversity was examined using nuclear *ITS*, chloroplast *psbA-trnH* and RAPD markers.

Result: Morphological differentiation was moderate and primarily driven by pod-related quantitative traits, particularly pod length and pod weight. DNA barcoding confirmed species identity with complete sequence conservation among hybrids. RAPD analysis revealed substantial polymorphism (79.42%) and genetic similarity coefficients ranging from 0.46 to 0.96, indicating detectable genetic variation among hybrids. However, RAPD is a dominant marker system with limited reproducibility; therefore, validation using co-dominant markers, such as SSR or SNP, is recommended. Hybrids Y15, Y16 and Y18 combined superior pod traits with high genetic similarity, while Y12 and Y20 were genetically distinct. The results indicate that phenotypic similarity does not necessarily reflect genomic similarity. A combined analytical framework integrating phenotypic evaluation with molecular data provides a more reliable basis for germplasm characterization and breeding decisions.

Key words: Breeding, DNA barcoding, Germplasm, Pod traits, RAPD, Yardlong bean.

INTRODUCTION

Yardlong bean [*Vigna unguiculata* (L.) Walp. subsp. *Sesquipedalis*] is widely cultivated throughout tropical and subtropical agroecosystems, where it serves as an important vegetable crop in both subsistence and market-oriented production systems. In Southeast Asia, it represents an important vegetable crop with strong market demand, where consumer preferences strongly favor pod length, tenderness and visual quality (Widyawan *et al*., 2021).

Yield variation in yardlong bean is primarily associated with pod-related quantitative traits, particularly pod length and pod weight, which are consistently prioritized in breeding programs targeting market acceptance (Tantasawat *et al*., 2010; Widyawan *et al*., 2021). Genetic studies have demonstrated that both additive and non-additive gene effects contribute to the inheritance of yield-related traits in cowpea, highlighting their importance in breeding programs (Singh *et al*., 2016).

Conserved genomic regions, such as *ITS* and *psbA-trnH,* are frequently used for species-level identification because their sequences are relatively stable across taxa (Kress *et al*., 2005; Stefanović *et al*., 2009). In contrast, RAPD markers are effective for detecting genome-wide polymorphism and have been widely applied in cowpea and yardlong bean diversity studies (Pidigam *et al*., 2019; Widyawan *et al*., 2021).

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How to cite this article: Truong, H.T.H., Ho, N.T.H. and Ho, H.N. (2026). Phenotypic and Molecular Diversity of Commercial Yardlong Bean [*Vigna unguiculata* (L.) Walp. Subsp. *sesquipedalis*] Hybrids for Breeding Utilization. *Legume Research*. **49(8):** 1277-1288. doi: 10.18805/LRF-939.

Submitted: 25-02-2026 **Accepted:** 30-03-2026 **Online:** 11-04-2026

The incorporation of molecular markers alongside morphological traits enables a more comprehensive assessment of genetic structure and diversity within breeding materials. Recent advances in cowpea genetics and breeding highlight the importance of integrating phenotypic and molecular approaches for sustainable crop improvement (Kim *et al*., 2025). Therefore, this study aimed to (i) evaluate phenotypic variation among commercial hybrids, (ii) assess genetic diversity using multiple marker systems and (iii) examine the relationship between phenotypic and molecular patterns for breeding utilization.

MATERIALS AND METHODS

Plant materials and experiment design

Twenty commercial F_1 hybrids (labeled Y1-Y20) were sourced from seed companies in Vietnam, Thailand and China (Table 1). The experiment was conducted from January 2025 to May 2025 using a completely randomized design with three replications at the Institute of Biotechnology, Hue University, Vietnam. Each plant was grown individually under consistent conditions to minimize environmental variation.

Morphological analysis

Seventeen traits were evaluated using standard descriptors (Bioversity International, 2007), including 10 qualitative and 7 quantitative characters. Quantitative traits included leaf length, leaf width, petiole length, pod length, pod diameter, pod weight and Brix.

Data were analyzed using correlation analysis, UPGMA clustering and principal component analysis (PCA). All multivariate analyses were conducted using PAST software version 4.03 (Hammer *et al.*, 2001).

Molecular analysis

DNA extraction and sequencing analysis

Genomic DNA was extracted using a CTAB-based protocol (Truong *et al*., 2024). PCR amplification and sequencing were performed following standard procedures. Sequence alignment and phylogenetic analysis were conducted in MEGA 12 (Kumar *et al*., 2024) using the Neighbor-Joining method with 1000 bootstrap replicates. The sequences related to this study were submitted to the NCBI and are available at https://submit.ncbi.nlm.nih.gov/subs/?search=SUB16004427.

ITS and trnH-psbA analysis

PCR amplification was performed using the ITS_{u1}-ITS_{u4} primer pair following the protocol described by Rasphone *et al.* (2022). Additionally, variation in chloroplast DNA was assessed with the *trnH–psbA* intergenic spacer (Vir *et al.*, 2023).

RAPD analysis

A preliminary RAPD screening was conducted using genomic DNA from two representative accessions (Y2 and Y12) and a set of 100 UBC (Bioneer, Korea) RAPD primers (Supplementary Table 1). To validate the reproducibility and applicability of the selected primers, two additional accessions (Y14 and Y15) were subsequently included, allowing assessment of polymorphism across the broader germplasm set and providing a more reliable estimation of genetic diversity. PCR amplification conditions followed the protocol described by Truong *et al.* (2013).

Marker parameters, including PB, PPB, PIC, MI, Rp and EMR, as well as genetic diversity indices (Na, Ne, h, I), were calculated. Cluster analysis was conducted using UPGMA in PAST software (Hammer *et al*., 2001).

Table 1: List of commercial F_1 hybrids of yardlong bean (*V. unguiculata*) used in this study.

Accession code	Hybrid code	Genbank accession	F_1 hybrid commercial name	Species	Company name	Originated country
HUIB_VU1	Y1	PX998218	Yard long bean	*V. unguiculata*	Thang long Seeds	Thailand
HUIB_VU2	Y2	PX998219	Yard long bean	*V. unguiculata*	Longfen Deeds	China
HUIB_VU3	Y3	PX998220	Yard long bean	*V. unguiculata*	Jinkelifeng	China
HUIB_VU4	Y4	PX998221	Yard long bean	*V. unguiculata*	Longfen Deeds	China
HUIB_VU5	Y5	PX998222	Yard long bean	*V. unguiculata*	Longfen Seeds	China
HUIB_VU6	Y6	PX998223	Yard long bean	*V. unguiculata*	East-West Seeds	Thailand
HUIB_VU7	Y7	PX998224	Yard long bean	*V. unguiculata*	Chua yong Deeds	Thailand
HUIB_VU8	Y8	PX998225	Yard long bean	*V. unguiculata*	Chia Tai-Home garden	Thailand
HUIB_VU9	Y9	PX998226	Yard long bean	*V. unguiculata*	Chia Tai-Home garden	Thailand
HUIB_VU10	Y10	PX998227	Yard long bean	*V. unguiculata*	Chia Tai-Home garden	Thailand
HUIB_VU11	Y11	PX998228	Green dragon No. 9	*V. unguiculata*	Advance Seeds	Thailand
HUIB_VU12	Y12	PX998229	Phum Thong	*V. unguiculata*	Advance Seeds	Thailand
HUIB_VU13	Y13	PX998230	Yard long bean	*V. unguiculata*	Lion Seeds	Thailand
HUIB_VU14	Y14	PX998231	Yard long bean	*V. unguiculata*	Lion Seeds	Thailand
HUIB_VU15	Y15	PX998232	No. 207	*V. unguiculata*	Trung Nong	Vietnam
HUIB_VU16	Y16	PX998233	Yard long bean black seeds	*V. unguiculata*	Phu Nong Seeds	Vietnam
HUIB_VU17	Y17	PX998234	No.8	*V. unguiculata*	C.H. Vietnam	Vietnam
HUIB_VU18	Y18	PX998235	CN66	*V. unguiculata*	Canh Nong Seeds	Vietnam
HUIB_VU19	Y19	PX998236	PN04 (OP)	*V. unguiculata*	Phu Nong Seeds	Vietnam
HUIB_VU20	Y20	PX998237	Yard long bean	*V. unguiculata*	Phu Nong Seeds	Vietnam

Supplementary Table 1: List of RAPD primers used for primary screened in this study.

No.	Primer name	Primer sequence	No.	Primer name	Primer sequence	No.	Primer name	Primer sequence	No.	Primer name	Primer sequence
1	UBC#501	CGGATATACC	26	UBC#526	AACGGGCACC	51	UBC#551	GGAAGTCCAC	76	UBC#576	CACCTAATGG
2	UBC#502	GCATGGTAGC	27	UBC#527	CTTCAACGTG	52	UBC#552	CTAAATGGCG	77	UBC#577	GTCTGATGTG
3	UBC#503	ATCGTCCAAC	28	UBC#528	GGATCTATGC	53	UBC#553	TCCGAGATCG	78	UBC#578	GGTGTCCACT
4	UBC#504	ACCGTGCGTC	29	UBC#529	CACTCCTACA	54	UBC#554	TCATCCAGGG	79	UBC#579	TGGAATCGTG
5	UBC#505	CCCTTTACAC	30	UBC#530	AATAACCGCC	55	UBC#555	GTGAACAGCA	80	UBC#580	GCGATAGTCC
6	UBC#506	CCTTTCCCGA	31	UBC#531	GCTCACTGTT	56	UBC#556	ATGGATGACG	81	UBC#581	CCCGTTAAGG
7	UBC#507	AGACGTACTC	32	UBC#532	TTGAGACAGG	57	UBC#557	GTGTAGAGCC	82	UBC#582	GGTATAGACG
8	UBC#508	CGGGGCGGAA	33	UBC#533	GCATCTACGC	58	UBC#558	CGATATCCGG	83	UBC#583	GTATTTGCGC
9	UBC#509	ACAGAGACTG	34	UBC#534	CACCCCCTGC	59	UBC#559	GAGAACTGGC	84	UBC#584	GCGGGCAGGA
10	UBC#510	CGCATCTCTT	35	UBC#535	CCACCAACAG	60	UBC#560	CACTGCTGTC	85	UBC#585	CCCGCGAGTC
11	UBC#511	GAATGGTGAG	36	UBC#536	GCCCCTCGTC	61	UBC#561	CATAACGACC	86	UBC#586	CCGGTTCCAG
12	UBC#512	GGGTGGACAT	37	UBC#537	CGAAAGGACT	62	UBC#562	CAAAGTAGCC	87	UBC#587	GCTACTAACC
13	UBC#513	TATACGACCC	38	UBC#538	TGACCTCTCC	63	UBC#563	CGCCGCTCCT	88	UBC#588	CAGAGGTTGG
14	UBC#514	CGGTTAGACG	39	UBC#539	CTTACGTCAC	64	UBC#564	CGGCGTTACG	89	UBC#589	GACGGAGGTC
15	UBC#515	GGGGGCCTCA	40	UBC#540	CGGACCGCGT	65	UBC#565	GGTCGATTTC	90	UBC#590	CCGGCATGTT
16	UBC#516	AGCGCCGACG	41	UBC#541	GCCCCTTTAC	66	UBC#566	CCACATGCGA	91	UBC#591	TCCCTCGTGG
17	UBC#517	GGTCGCAGCT	42	UBC#542	CCCATGGCCC	67	UBC#567	AGACACCTGA	92	UBC#592	GGGCGAGTGC
18	UBC#518	TGCTGGTCCA	43	UBC#543	CGCTTCGGGT	68	UBC#568	ACCTGTTCTC	93	UBC#593	CGAGCTTTGA
19	UBC#519	ACCGGACACT	44	UBC#544	TAGAGACTCC	69	UBC#569	CGAATTGCTG	94	UBC#594	AGGAGCTGGC
20	UBC#520	TGCGCAGCCC	45	UBC#545	ACGTTGAGAC	70	UBC#570	GGCCGCTAAT	95	UBC#595	GTCACCGCGC
21	UBC#521	CCGCCCCACT	46	UBC#546	CCCGCAGAGT	71	UBC#571	GCGCGGCACT	96	UBC#596	CCCCTCGAAT
22	UBC#522	TCGTCTAGCA	47	UBC#547	TATGACCTGG	72	UBC#572	TTCGACCATC	97	UBC#597	TGGTTCCCGA
23	UBC#523	ACAGGCAGAC	48	UBC#548	GTACATGGGC	73	UBC#573	CCCTAATCAG	98	UBC#598	ACGGGCGCTC
24	UBC#524	CGGTTACTAG	49	UBC#549	CCGGCTTATG	74	UBC#574	GCCAGACAAG	99	UBC#599	CAAGAACCGC
25	UBC#525	GCTGGTTGGA	50	UBC#550	GTCGCCTGAG	75	UBC#575	GGAGATGTAC	100	UBC#600	GAAGAACCGC

RESULTS AND DISCUSSION

Morphological characterization

Morphological variation among the evaluated hybrids was moderate, with differentiation largely associated with pod-related traits rather than vegetative characteristics (Table 2, Table 3; Fig 1, Fig 2). Leaf size varied moderately, while petiole length exhibited a narrow range, indicating weaker selection pressure on vegetative traits (Widyawan *et al*., 2021). In contrast, pod traits displayed substantial variation, with pod length (26.18-40.19 cm), diameter (0.74-0.88 cm) and weight (11.11-14.17 g) contributing most to phenotypic diversity and yield (Table 3), consistent with previous findings in vegetable cowpea (Sathish *et al*., 2023). Similar studies have also highlighted the importance of pod diameter, number of pods per plant and yield-related traits

Table 2: Qualitative morphological characterization of twenty F_1 commercial hybrids of *V. unguiculata*.

Hybrid code	GH	SC	PeC	LC	LSh	LSt	LV	FC	PoC	PoS
Y1	1	1	3	1	1	1	1	1	1	2
Y2	1	2	2	1	1	1	1	1	2	1
Y3	1	2	2	2	2	1	1	1	2	1
Y4	2	1	3	1	1	1	1	1	1	1
Y5	2	1	1	2	1	1	1	1	1	2
Y6	1	1	1	1	2	1	1	1	1	1
Y7	1	1	1	2	1	1	1	2	1	1
Y8	1	1	3	2	1	1	1	1	1	1
Y9	1	1	3	1	1	1	1	1	1	1
Y10	2	2	2	2	1	1	1	1	2	1
Y11	2	1	1	1	2	1	1	1	1	1
Y12	2	1	1	2	2	1	1	1	1	1
Y13	1	1	1	1	1	1	1	1	1	1
Y14	1	1	3	1	1	1	1	1	1	1
Y15	1	1	3	1	1	1	1	1	2	1
Y16	1	1	3	2	1	1	1	1	1	1
Y17	1	1	3	2	1	1	1	1	1	2
Y18	1	1	3	1	1	1	1	1	2	1
Y19	1	1	3	1	2	1	1	1	1	1
Y20	1	1	1	1	1	1	1	2	1	1

GH (Growth habit): Pole type (1), Spreading (2).
SC (Stem color): Green (1), Purple (2).
PeC (Petiole color): Green (1), Light purple (2), Purple-green (3).
LC (Leaf color): Green (1), Dark green (2).
LSh (Leaflet shape): Rhombohedric (1), Ovate (2).
LSt (Leaf structure): Trifoliate (1), Others (2).
LV (Leaf venation): Reticulate (1), Others (2).
FC (Flower color): Purple (1), White (2).
PoC (Pod color): Green (1), Purple (2).
PoS (Pod shape): Straight (1), Curved (2).

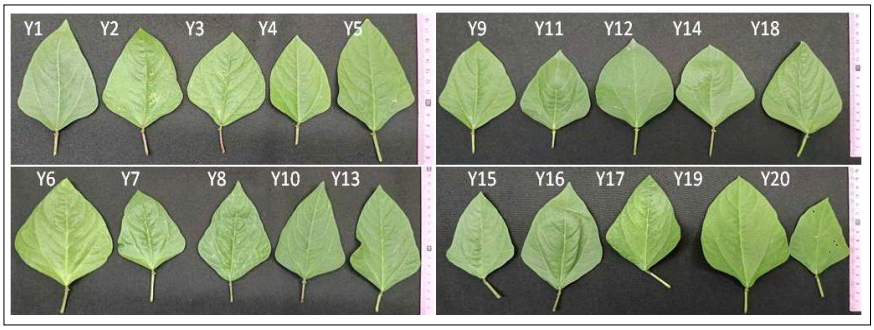


Fig 1: Variation in leaflet morphology among twenty F_1 commercial hybrids (Y1-Y20) of yardlong bean (*V. unguiculata*).

Table 3: Quantitative morphological characterization of F_1 commercial hybrids of *V. unguiculata.*

Hybrid code	LL	LW	PL	PoL	PoD	PoW	BL
Y1	7.55±1.90ef	4.90±1.63e	2.26±0.70ab	35.23±8.19a-d	0.74±0.12d	12.70±3.25a-c	5.65 ±0.92bc
Y2	9.11±1.81a-c	4.97±1.92e	2.23±0.77ab	35±9.22a-d	0.88±0.15a	12.08±2.92a-c	5.30±0.78b-e
Y3	7.98±2.21de	4.94±1.4e	2.35±0.71ab	39.58±8.16ab	0.82±0.15a-d	11.89±3.80a-c	4.94±0.70e
Y4	8.09±1.72c-e	5.19±1.4de	2.42±0.65a	34.38±8.2b-d	0.78±0.11b-d	11.58±3.72bc	5.13±1.07c-e
Y5	8.43±2.03a-e	5.84±1.65a-d	2.35±0.67ab	35.53±9.6a-d	0.79±0.13b-d	12.68±3.26a-c	5.14±0.76c-e
Y6	8.72±2.34a-d	5.33±1.76c-e	2.28±0.72ab	37.12±5.83a-d	0.74±0.19d	14.04±4.57ab	5.43±0.50b-e
Y7	9.41±2.13a	5.9±1.56a-d	2.53±0.78a	38.62±10.31a-c	0.82±0.15a-d	12.46±3.79a-c	5.50±0.75b-d
Y8	9.21±2.32ab	5.65±1.46a-e	2.30±0.71ab	34.2±8.76b-d	0.75±0.14cd	12.29±4.33a-c	5.27±0.58b-e
Y9	8.95±2.08a-d	6.25±1.34a	2.21±0.72ab	38.66±9.77a-c	0.79±0.17b-d	14.09±5.18a	5.27±0.61b-e
Y10	9.36±2.08a	5.51±1.41a-e	2.52±0.69a	35.41±10.68a-d	0.82±0.13a-d	12.04±3.58a-c	6.75±1.53a
Y11	9.30±2.30a	6.17±1.3ab	2.33±0.65ab	40.19±7.99a	0.81±0.17a-d	14.12±6.39a	4.92±0.62e
Y12	8.43±2.19a-e	5.61±1.41a-e	2.27±0.63ab	31.46±11.17d	0.76±0.09cd	11.38±3.25c	5.45±0.76b-e
Y13	7.96±1.75de	5.46±1.34b-e	2.36±0.76ab	33.26±10.08cd	0.80±0.15a-d	11.11±4.02c	5.12±0.67c-e
Y14	8.85±1.87a-d	5.95±1.72a-d	2.31±0.68ab	26.18±9.21e	0.80±0.16a-d	11.42±3.98c	5.53±0.91b-d
Y15	8.67±2.64a-d	5.56±1.41a-e	2.19±0.71ab	35.31±11.23a-d	0.85±0.14ab	12.54±4.13a-c	5.60±0.97bc
Y16	8.54±1.92a-e	6.09±1.36a-c	2.37±0.79ab	36.04±7.67a-d	0.83±0.14a-d	14.17±4.28a	5.65±0.76bc
Y17	8.70±2.05a-d	5.73±1.71a-d	2.05±0.64b	35.06±10.58a-d	0.87±0.15ab	14.12±4.26a	5.80±1.03b
Y18	8.86±2.21a-d	5.97±1.65a-c	2.43±0.77a	35.96±8.8a-d	0.79±0.12b-d	14.40±3.39a	5.32±0.88b-e
Y19	6.88±1.84f	5.75±1.4a-d	2.39±0.55ab	31.6±10.11d	0.83±0.17a-c	12.01±3.38a-c	5.02±0.77de
Y20	8.25±1.90b-e	5.87±1.56a-d	2.23±0.64ab	32.66±8.29d	0.83±0.14a-d	12.04±4.29a-c	7.08±1.37a

LL: Leaf length; LW: Leaf width; PL: Petiole length; PoL: Pod length; PoD: Pod diameter; PoW: Pod weight; BL: Brix level.

as key selection criteria in cowpea improvement programs (Acharya *et al*., 2026). Brix values (4.92-7.08%) indicated variation in quality traits (Choi *et al*., 2024). Correlation analysis indicated strong positive relationships among several pod traits, particularly between pod length and pod weight. The absence of intraspecific variation indicates a high level of sequence conservation within the evaluated material (Fig 3), whereas Brix showed weak relationships, suggesting partial independence between yield and quality traits (Sathish *et al*., 2023; Choi *et al*., 2024). UPGMA clustering grouped hybrids into three clusters (Fig 4), primarily driven by pod traits. At the same time, PCA confirmed their dominant role in variation (Fig 5). Overall, phenotypic variation is moderate and largely governed by pod-related traits.

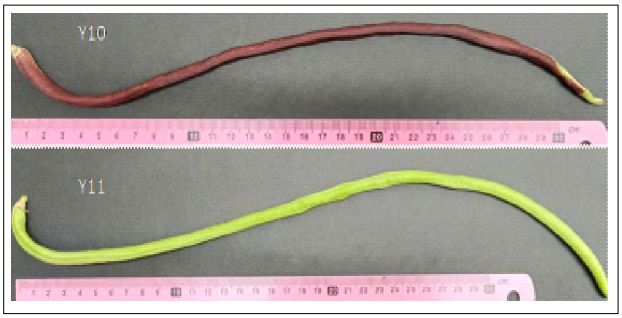


Fig 2: Representative pods showing differences in pod color and length between yardlong bean F_1 commercial hybrids Y10 (upper) and Y11 (lower).

DNA barcoding analysis (*ITS* and *psbA-trnH*)

All twenty F_1 yardlong bean hybrids were successfully sequenced for *ITS* (666 bp) and *psbA–trnH* (334 bp) regions, showing 100% query coverage and identity with *V. unguiculata* reference sequences, with no polymorphic sites detected (Table 4). Phylogenetic analysis revealed that all hybrids clustered with *V. unguiculata* references with strong bootstrap support (100%) in both *ITS* and *psbA-trnH* trees (Fig 6; Fig 7), clearly separating them from other *Vigna* species and confirming their taxonomic identity.

The absence of intraspecific variation indicates strong sequence conservation, which is common in domesticated crops due to founder effects and selection pressure

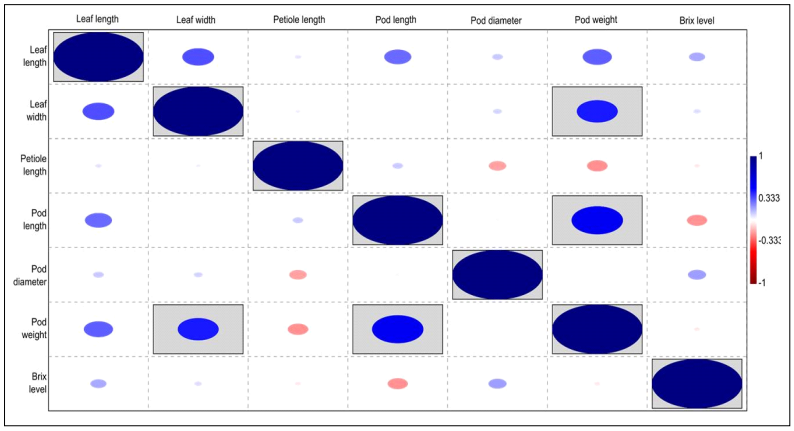


Fig 3: Ellipse-based correlation plot showing relationships among quantitative morphological and quality traits in yardlong bean F_1 commercial hybrids (*V. unguiculata*) corresponding to quantitative traits (Table 3).

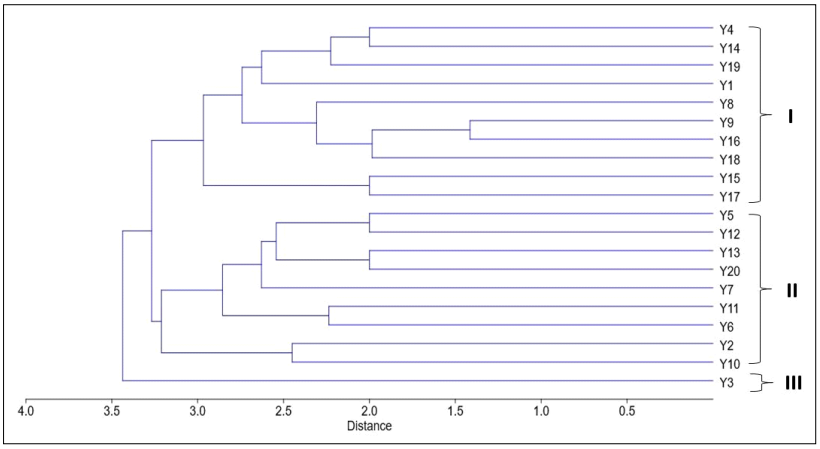


Fig 4: UPGMA dendrogram showing phenotypic relationships among twenty yardlong bean (*V. unguiculata*) F_1 commercial hybrids based on morphological traits.

(Doebley *et al*., 2006; Olsen and Wendel, 2013). Similar low variation in barcode regions has been reported in legumes (Stefanović *et al*., 2009). Among available DNA barcodes, *ITS* has been preferentially applied due to high interspecific divergence (Baldwin *et al*., 1995), while *psbA-trnH* is an effective chloroplast marker for distinguishing taxa (Kress *et al*., 2005; Shaw *et al*., 2005; Shaw *et al*., 2007). The congruent phylogenetic patterns confirm species identity but indicate limited resolution for intraspecific diversity.

RAPD analysis

RAPD markers detected considerable levels of genome-wide polymorphism among the hybrids, indicating the presence of measurable genetic diversity, consistent with

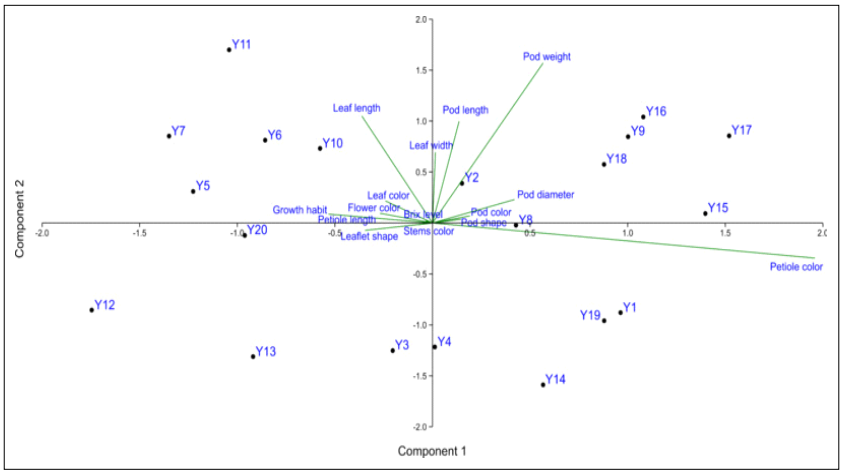
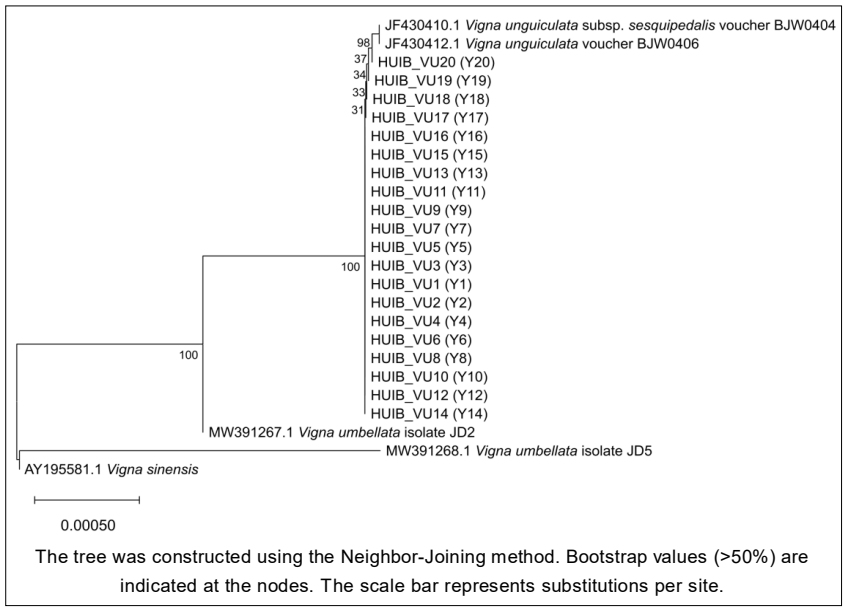


Fig 5: PCA biplot showing the relationships between morphological traits and the distribution of twenty yardlong bean (*V. unguiculata*) F_1 commercial hybrids along the first two principal components.



The tree was constructed using the Neighbor-Joining method. Bootstrap values (>50%) are indicated at the nodes. The scale bar represents substitutions per site.

Fig 6: Phylogenetic relationships of 20 F_1 commercial hybrids of *V. unguiculata* inferred from *ITS* sequences.

Table 4: Sequencing performance and GenBank similarity of *ITS* and *psbA-trnH* regions in 20 F_1 commercial hybrids of *V. unguiculata*.

Regions	Successful sequencing rate (%)	Size (bp)	Number of variable nucleotide	Query cover (%)	e-value	Per cent identity (%)	Reference sequence in GenBank
ITS	100	666	0	100	0	100	JF430410.1 (*V. unguiculata*)
psbA-trnH	100	334	0	100	3.00E-172	100	KJ468104.1 (*V. unguiculata*)

previous reports in cowpea under different agro-climatic conditions (Kumar *et al*, 2017; Pidigam *et al*., 2019; Widyawan *et al*., 2021). Of 100 primers screened, eight produced clear and reproducible polymorphic patterns (Table 5; Fig 8). These generated 4-15 bands per primer (mean 9.50), with 79.42% polymorphism, consistent with previous studies (Pidigam *et al*., 2019; Saha *et al*., 2020). PIC values (0.15-0.30) and resolving power (1.0-4.3) indicated moderate marker informativeness (Botstein *et al*., 1980), while MI and EMR confirmed marker efficiency (Widyawan *et al*., 2021; Shubha *et al*., 2022).

Genetic similarity ranged from 0.46 to 0.96 (Table 6), reflecting both closely related and divergent genotypes. UPGMA clustering grouped hybrids into four clusters (Fig 9), indicating moderate differentiation despite a shared commercial background. Genetic diversity indices (Na = 1.8289; h = 0.2669; I = 0.3999) further supported this pattern (Table 7).

Comparison with morphology showed both concordance and incongruence. Hybrids Y15, Y16 and Y18 clustered consistently across analyses (Fig 4, 5 and 9), while others showed divergence, indicating convergent selection (Sathish *et al*., 2023). DNA barcoding showed no variation (Fig 6 and 7), confirming species identity but limited diversity (Doebley *et al*., 2006). Overall, RAPD analysis revealed genetic variation not apparent from morphological observations alone.

Concordance and incongruence between phenotypic and molecular patterns

The integration of morphological traits, DNA barcoding and RAPD markers highlighted both agreement and discrepancy

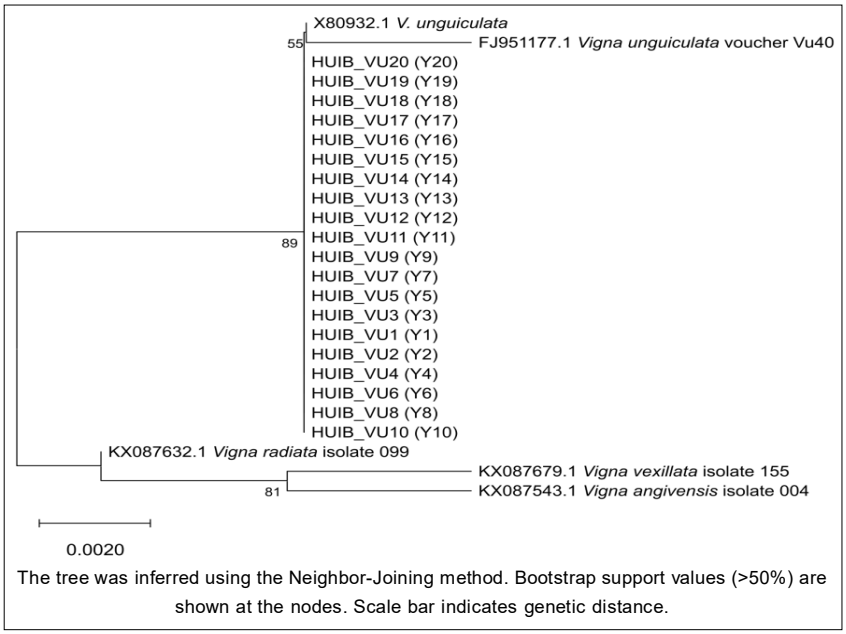


Fig 7: Phylogenetic relationships of 20 F_1 commercial hybrids of *V. unguiculata* based on *psbA-trnH* sequences.

Table 5: Polymorphic RAPD primers obtained from the initial screening were used for subsequent genetic diversity analyses of twenty *V. unguiculata* F_1 commercial hybrids.

Primer	Band size (bp)	TB	PB	PPB (%)	PIC	Rp	MI	EMR
UBC#429	350-1450	10	10	100	0.18	2.00	1.80	10.00
UBC#447	350-2200	15	15	100	0.15	2.60	2.20	15.00
UBC#459	380-2400	12	8	66.67	0.24	4.30	1.26	5.33
UBC#460	280-1900	10	7	70.00	0.23	3.40	1.11	4.90
UBC#462	400-2500	9	7	77.78	0.24	3.10	1.31	5.44
UBC#483	1100-3000	4	2	50.00	0.15	1.00	0.15	1.00
UBC#486	600-1750	5	4	80.00	0.30	2.30	0.94	3.20
UBC#487	280-1300	11	10	90.91	0.32	5.20	2.88	9.09
Mean	300-2500	9.50	7.88	79.42	0.22	2.99	1.46	6.75

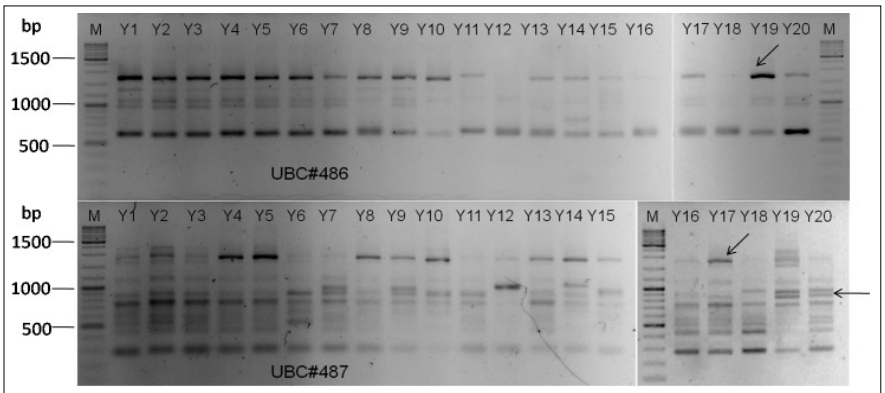
TB: Total number of bands; PB: Number of polymorphic bands, PPB: Percentage of polymorphic bands; PIC: Polymorphic information content; EMR: Effective multiplex ratio; MI: Marker index; Rp: Resolving power.

between phenotypic traits and molecular data. DNA barcoding revealed complete sequence conservation in the *ITS* and *psbA-trnH* regions, with 100% identity to *V. unguiculata*, confirming species identity but indicating limited variation at these loci. The lack of detectable polymorphism in these regions is consistent with patterns reported in domesticated crops, where historical bottlenecks and intensive selection have constrained variation in conserved loci (Doebley *et al*., 2006; Olsen and Wendel, 2013).

In contrast, RAPD analysis detected moderate polymorphism (79.42%) and genetic differentiation among hybrids, consistent with previous studies in yardlong bean and cowpea (Pidigam *et al*., 2019; Widyawan *et al*., 2021). Concordance was observed in hybrids Y15, Y16 and Y18, which showed both phenotypic similarity and high genetic relatedness, reflecting selection for pod-related traits such as pod length and weight (Sathish *et al*., 2023). These findings highlight the importance of integrating multiple marker systems to capture both conserved and variable genomic regions.

However, incongruence was evident in hybrids such as Y1 and Y3, which were morphologically similar but genetically distinct, suggesting convergent selection acting on different genetic backgrounds. The distinct position of Y12 further confirmed the presence of divergent genetic materials. These findings suggest that similar phenotypic expression does not necessarily correspond to underlying genetic similarity. Thus, integrating morphological evaluation with molecular data offers a more robust approach for germplasm characterization and breeding applications.

These results inform breeding strategies. Hybrids Y15, Y16 and Y18 are suitable elite parents for yield improvement but offer limited variability. In contrast, divergent genotypes such as Y12 provide valuable sources for broadening the genetic base. Crosses between elite and divergent lines



Lane M: DNA size marker (bp); lanes Y1-Y20 represent individual F_1 commercial hybrids of *V. unguiculata*. Arrows indicate representative polymorphic bands contributing to genetic differentiation among hybrids.

Fig 8: PCR-RAPD amplification profiles of twenty *V. unguiculata* F_1 commercial hybrids generated using primer UBC#486 and UBC#487.

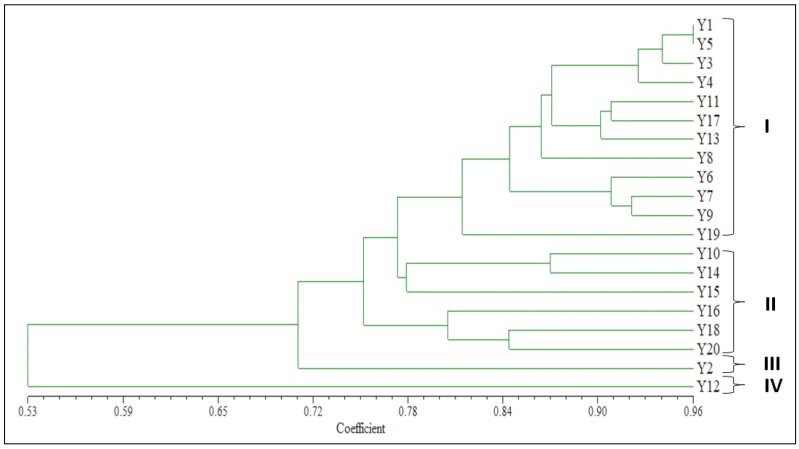


Fig 9: UPGMA dendrogram illustrating the genetic relationships among twenty F_1 commercial hybrids of *V. unguiculata* based on RAPD marker analysis.

Table 6: Pairwise genetic similarity coefficients among twenty F_1 commercial hybrids of *V. unguiculata*.

Hybrids	Y1	Y2	Y3	Y4	Y5	Y6	Y7	Y8	Y9	Y10	Y11	Y12	Y13	Y14	Y15	Y16	Y17	Y18	Y19	Y20
Y1	1.00																			
Y2	0.82	1.00																		
Y3	0.95	0.82	1.00																	
Y4	0.92	0.84	0.92	1.00																
Y5	0.96	0.80	0.93	0.93	1.00															
Y6	0.88	0.75	0.88	0.88	0.92	1.00														
Y7	0.84	0.71	0.84	0.84	0.88	0.91	1.00													
Y8	0.84	0.71	0.84	0.87	0.88	0.86	0.82	1.00												
Y9	0.79	0.66	0.79	0.79	0.83	0.91	0.92	0.84	1.00											
Y10	0.74	0.68	0.76	0.82	0.75	0.80	0.74	0.82	0.79	1.00										
Y11	0.86	0.70	0.83	0.86	0.89	0.87	0.88	0.88	0.88	0.80	1.00									
Y12	0.46	0.57	0.46	0.46	0.50	0.55	0.57	0.54	0.64	0.54	0.61	1.00								
Y13	0.86	0.70	0.86	0.86	0.87	0.82	0.83	0.86	0.80	0.75	0.89	0.50	1.00							
Y14	0.68	0.58	0.71	0.71	0.72	0.78	0.76	0.79	0.84	0.87	0.83	0.62	0.78	1.00						
Y15	0.71	0.58	0.71	0.71	0.75	0.80	0.82	0.76	0.87	0.74	0.80	0.59	0.80	0.82	1.00					
Y16	0.72	0.59	0.72	0.75	0.76	0.71	0.75	0.80	0.75	0.67	0.84	0.53	0.87	0.72	0.78	1.00				
Y17	0.89	0.74	0.89	0.89	0.88	0.83	0.82	0.87	0.82	0.82	0.91	0.51	0.91	0.79	0.74	0.80	1.00			
Y18	0.72	0.62	0.70	0.78	0.74	0.68	0.72	0.64	0.67	0.64	0.71	0.47	0.76	0.59	0.67	0.82	0.70	1.00		
Y19	0.83	0.72	0.83	0.80	0.82	0.84	0.80	0.80	0.83	0.80	0.79	0.50	0.76	0.78	0.70	0.66	0.83	0.63	1.00	
Y20	0.80	0.70	0.78	0.86	0.82	0.82	0.86	0.75	0.83	0.75	0.84	0.50	0.84	0.70	0.78	0.79	0.80	0.84	0.79	1.00

Table 7: Genetic diversity indices of twenty F_1 commercial hybrids of *V. unguiculata* revealed by RAPD analysis.

Indices	na	ne	h	I
Mean	1.8289	1.4617	0.2669	0.3999
SD	0.3791	0.3765	0.1947	0.2658

na: Observed number of alleles; ne: Effective number of alleles; h: Nei's gene diversity; I: Shannon's information index; SD: Standard deviation.

are recommended to enhance variation and exploit heterosis (Boukar *et al*., 2019; Kim *et al*., 2025).

CONCLUSION

This study provides an integrated analysis of phenotypic and molecular variation in commercial yardlong bean hybrids. Morphological analysis revealed moderate variation mainly governed by pod-related traits, particularly pod length and weight, reflecting strong yield-oriented selection. DNA barcoding confirmed species identity with complete sequence conservation, whereas RAPD markers detected moderate polymorphism and grouped hybrids into distinct clusters, indicating underlying genetic differentiation despite phenotypic similarity.

Some hybrids with similar pod performance were genetically distinct, while others showed both phenotypic and molecular similarity, demonstrating that phenotypic uniformity should not be interpreted as evidence of genetic uniformity. Hybrids Y15, Y16 and Y18 combined superior yield traits with high genetic similarity, making them suitable for production. Hybrid Y20 showed potential for quality improvement, while divergent genotypes such as Y12 provide valuable resources for broadening the genetic base. The combined use of phenotypic and molecular analyses offers a more robust framework for breeding-oriented germplasm evaluation.

ACKNOWLEDGEMENT

The present study was supported by the Core Research Program, Hue University (Grant No. NCTB.DHH.2024.03). We thank Dr. Sonexay Rasphone from Savannakhet University, Laos, for sharing plant materials.

Disclaimers

The authors are solely responsible for the content's accuracy; the opinions expressed do not necessarily reflect those of their institutions and no liability is assumed for any consequences resulting from its use.

Conflict of interest

The authors declare no conflicts of interest.

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