

Unveiling the Genetic Diversity of Winged Bean (*Psophocarpus tetragonolobus* (L.) DC.) Genotypes: A Phenotypic and Genotypic Assessment for Crop Improvement

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Abstract

The winged bean (*Psophocarpus tetragonolobus*), a highly nutritious yet underutilized legume, exhibits significant genetic diversity across different agro-climatic regions. However, more attention is needed to fully exploit its potential due to lack of adequate knowledge of the existing genetic diversity in the available winged bean germplasm. To bridge this gap, the study evaluates 28-winged bean genotypes collected from various districts of Kerala, Tamil Nadu, Manipur, India and NBPGR. The research aimed to assess the phenotypic variability of these genotypes to support future breeding programs. A randomized block design (RBD) was used for the experiment, with observations recorded for 12 quantitative traits, including vine length, pod yield, seed characteristics, and germination parameters. The results revealed significant variability among genotypes in traits such as pod length (14.31 cm to 46.18 cm), pod width (2.14 cm to 3.63 cm), and number of seeds per pod (5.34 to 22.68). Genetic component analysis indicated high heritability for most traits, particularly the number of seeds per pod (98.98%) and pod length (96.84%), suggesting their strong genetic influence. Principal component analysis (PCA) demonstrated that four principal components accounted for 74.67% of the total variance, with pod- and seed-related traits contributing the most to diversity. Cluster analysis grouped the genotypes into three clusters based on yield and growth characteristics, with Cluster I genotypes exhibiting the highest pod yield per plant (742.15 g). The findings highlight the potential of selected genotypes for genetic improvement, emphasizing the need for further hybridization programs to enhance productivity and adaptability.

1. Introduction

The winged bean (*Psophocarpus tetragonolobus* L.) is a neglected and underutilized perennial legume that thrives well in hot, humid, equatorial countries that belongs to the family Fabaceae (Ojuederie et al. 2020). The diploid chromosome number is $2n = 18$, and belongs to the subfamily Papilionoideae (Harder 1992). It is native to the tropical regions of Southeast Asia and the Pacific Islands, where it is predominantly grown in Indonesia, the Philippines, and Thailand. It is also grown in parts of West and East Africa, where it contributes to food security and diversifies agricultural systems (Sriwichai et al. 2021). In India, particularly in states like Tamil Nadu and Kerala, its cultivation is gaining popularity due to its nutritional benefits and adaptability (Laosatit et al. 2022). The Winged bean thrives in various agro-climatic conditions and is characterized by its distinctive winged pods, which contribute to its name. Its economic worth is derived from the pods, seeds, leaves, flowers, and tubers, all of which have a variety of culinary uses and nutritional advantages. It is a very nutritious legume that is one of the greatest sources of plant-based protein, with 25–40% protein content that offers substantial health advantages (Mohanty et al. 2013). It also contains large amounts of vital minerals including calcium, iron, phosphorus, and potassium, as well as vital vitamins like A and C. As a tropical legume, its seeds contain high amounts of protein and oil (Wan et al. 2014), and it is often referred to as the ‘soybean of the tropics’ (Amoo et al. 2006). The immature pods contain 1–3% protein, as well as several vitamins and minerals (Tanzi et al. 2019). The winged bean’s mature seeds contain protein levels of 28–45% (Bepary et al. 2023), oil of roughly 14–19%, and carbohydrates of 34–40% (Lepcha et al. 2017). Moreover, its raw tubers contain 12–19% protein and 1–4% fat (Lepcha et al. 2017). Because of its high dietary fiber content, it helps control blood sugar levels and supports digestive health (Esan et al. 2020). Despite the nutritional potential of the winged bean, very little attention is paid to exploiting the presence of anti-nutritive factors, including chymotrypsin inhibitors, hemagglutinins, and trypsin inhibitors (Ekpenyong 1978).

The genetic diversity of winged beans is extensive. The diversity was found in the colour characteristics of the flowers, pods, and seeds (Kuswanto et al. 2024). Several studies have reported that a day and night temperature of 27°C and 20°C is optimal for plant growth (Wong and Schwabe 1979; Herath and Ormrod 1979; Wong 1983; Schiavinato and Vaio 1996).

They can be planted at any time within a photoperiod of 11 to 13 hours, as they develop well during the vegetative phase. Under short-day conditions, they typically bloom within 8 to 10 weeks. However, some winged bean varieties exhibit lower sensitivity to short days and can produce pods even during long-day periods (Prasanna 2007). Herath and Ormrod (1979) reported that none of the six Sri Lankan and fifteen PNG accessions flowered under a 30/25°C temperature regime, regardless of whether the photoperiod was 11 or 14 hours. However, flowering occurred under an 11-hour photoperiod when the temperature was reduced to 25/20°C. The number of lateral branches in winged beans varies significantly among germplasm from different regions, as previously reported (Eagleton et al. 1985). This trait could serve as a key target for developing ideal plant architecture ideotypes. However, there remains a lack of comprehensive studies examining the growth, development, and interrelationships among plant architecture traits such as lateral branching, stem length, node number, internode length, and leaf count per plant.

Additionally, research is still needed to understand how these traits influence yield-related factors, such as the number of pods per plant. Studies have shown that a thorough and well-documented evaluation of phenotypic and morphological variation through quantitative traits is a fundamental step in crop improvement programs (Lubberstedt 2000; Adebayo 2005). Multivariate techniques, such as principal component analysis (PCA) and complete cluster linkage analysis, have been effectively used for the phenotypic characterization of underutilized legumes, including winged bean (Mohanty et al. 2013), lima bean (Asante et al. 2008), and the wild cowpea relative *Vigna ambacensis* (Adebayo et al. 2005). However, the limited information on the agronomic, morphological, and physiological attributes of available winged bean germplasm has significantly hindered improvement efforts using genetic, cytogenetic, and molecular approaches. Therefore, this study aims to assess the phenotypic variability in selected genotypes of winged beans for the needs of combinative breeding.

2. Materials and methods

2.1. Genetic materials, Experimental locations, and Experimental Design

The genetic materials used in this study comprised twenty-eight-winged bean genotypes sourced from the different districts of Kerala, Tamil Nadu, Manipur, and NBPGR, India (Table 1). Out of 17 genotypes collected from Tamil Nadu, one genotype was purple winged bean and one genotype was green with purple spotted winged bean. This study was conducted at Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India. The site was located at an altitude of 41m above mean sea level, 11° N latitude and 76.9°E longitude. The experiment was conducted from October 2024 to March 2025 with 180 days of crop duration.

The experimental design was a Randomized block design (RBD) with two replications. The experimental unit consisted of nine rows per replication in the plot with a single-row plot of 15m long with a spacing of 1m between rows. The basal dose of 2.5: 4.0: 2.5 kg NPK/ 4046 m² area was applied while sowing, where one seed was sown per hole, and holes were spaced 1m within a row. The trial was conducted under irrigated conditions using a drip irrigation system, delivering water at a rate of 2 litres per hour. Irrigation was applied on alternate days for the first 30 days, and subsequently, once every three to five days, depending on soil moisture levels. Weeding was performed manually to obtain a clean field throughout the experiment. Staking was given three weeks after planting (WAP) at the inception of twinning to support the plants due to their vigorous vining nature and to obtain optimum growth and yield. At 4 WAP, 2.5: 4.9: 2.5 kg NPK was top-dressed to meet the required nutrients. Other necessary management practices, such as regular weeding, twinning, and insect-pest control were carried out whenever required.

The observations were taken randomly from five selected plants in each replication for various quantitative traits viz., Vine length (cm), Stem diameter (cm), Primary branches per plant, Days to fifty percent flowering, Days to pod maturity (days), Pod length (cm), Pod width (cm), Number of pods per cluster, Number of pods per plant, Single pod weight (g), Number of seeds per pod, Pod weight per plant (g), FDG- First day of germination(days), LDG- Last day of germination(days), FGP- Final germination percentage (%), MGT- Mean germination time to study genetic diversity.

Data analyses

The quantitative traits data were subjected to Analysis of Variance (ANOVA) using the 'lme4' package in R software (Bates et al. 2020). The software R version 3.4.3 was utilized to conduct Principal Component Analysis (Kaiser 1957 and Jeffers 1967). Pearson's correlation using KAU GRAPES software (Prateesh et al. 2020). Finally, clustering analysis was carried out using STAR v. 2.0.1 software to group the accessions with similarity in their agro-morphological traits based on the ward minimum variance method (Ward et al. 1963).

Table 1
List of the Winged bean genotypes used for this study and location details

Sl.no	Genotype	Collection site	District	Altitude (m)	Latitude (°N)	Longitude (°E)
1.	CBE- PT- 01	Palakad	Palakad (KL)	2,383	10.77	76.65
2.	CBE- PT- 02	Thrissur	Thrissur (KL)	3,032	10.53	76.21
3.	CBE- PT- 03	Chavakad	Thrissur (KL)	3,091	10.57	76.05
4.	CBE- PT- 04	Gudalur	Niligiris (TN)	1,100	11.50	76.49
5.	CBE- PT- 05	Nellakotta	Niligiris (TN)	1,320	11.41	76.68
6.	CBE- PT- 06	Salem West	Salem (TN)	278	11.32	77.40
7.	CBE- PT- 07	Yercaud	Salem (TN)	272	11.53	78.14
8.	CBE- PT- 08	Valapady	Salem (TN)	264	11.49	78.44
9.	CBE- PT- 09	Salem south	Salem (TN)	261	11.39	78.64
10.	CBE- PT- 10	Lamphelpat	Imphal (Manipur)	790	24.82	93.90
11.	CBE- PT- 11	Wangoi	Imphal (Manipur)	790	24.81	93.95
12.	CBE- PT- 12	Senapati	Imphal (Manipur)	790	24.39	25.45
13.	CBE- PT- 13	Odddanchatram	Dindigul (TN)	300	10.48	77.75
14.	CBE- PT- 14	Vyrichettipalayam	Trichy (TN)	194	11.28	78.48
15.	CBE- PT- 15	Chrompet	Chengalpattu (TN)	49	12.95	80.14
16.	CBE- PT- 16	Kamalapuram	Salem (TN)	134	11.28	78.48
17.	CBE- PT- 17	Kadayampatti	Salem (TN)	418	11.87	78.11
18.	CBE- PT- 18	Gobichettipalayam	Erode (TN)	213	11.45	77.43
19.	CBE- PT- 19	Sathyamangalam	Erode (TN)	245	11.50	77.24
20.	CBE- PT- 20	Kodumudi	Erode (TN)	144	11.07	77.88
21.	CBE- PT- 21	Kanjikoil	Erode (TN)	232	11.36	77.59
22.	CBE- PT- 22	Namakkal	Namakkal (TN)	218	11.21	78.16
23.	CBE- PT- 23	Sankarapuram	Kallakurichi (TN)	163	11.88	78.91
24.	EC0038955	PNG	Papua New Guinea	667	12	120
25.	EC0038959	PNG	Papua New Guinea	667	12	120
26.	EC142667	Nigeria	Nigeria	200	14	15
27.	IC0041980	Maharastra	Maharastra	1200	22	80
28.	EC0114273	PNG	Papua New Guinea	667	12	120

EC- Exotic collection, IC- Indigenous collection, TN- Tamil Nadu, KL- Kerala, PNG- Papua New Guinea

2.2. Methods used for assessment of germination

To find the germinability of each selected genotypes, germination study was conducted. For this study, mature seeds were collected from different locations. From the earlier study, it was reported that germination percentage is decreased by storing the seeds for more than 45 days of harvest (Kamal et al. 2023). For this study, the collected seeds were stored for more than three months and seed treatment was not done, to find the germination percentage of each genotype. Germination parameters like first day of

germination (FDG), last day of germination (LDG), final Germination percentage (FGP), and mean germination time (MGT) were calculated using the following equations.

- First day of germination (FDG) = Day on which the first germination seen
- Last day of germination (LDG) = Day on which the last germination seen
- Final Germination percentage (FGP) = Final number of seeds germinated in seed lot *100
- Mean germination time = $\sum fx / \sum f$ (Orchard *et al.*, 1977)

where, f = seed germinated on day x

x = day of germination

2.3. Statistical analysis

The phenotypic coefficient of variation, genotypic coefficient of variation, broad-sense heritability, and genetic advance were calculated by the formulas given by Burton *et al.* 1953 and Johnson *et al.* 1955.

PCV = (Phenotypic Standard Deviation / Mean) * 100

GCV = (Genotypic Standard Deviation / Mean) * 100

Heritability (h^2) = (Genotypic Variance / Phenotypic Variance) * 100

3. Results and Discussion

3.1. Assessment of germination

To find the germination of selected genotypes, the study was conducted. The observations were recorded daily by counting the number of seeds germinated in each replication. Germination parameters like first day of germination (FDG), last day of germination (LDG), final germination percentage (FGP) and mean germination time (MGT) were recorded.

Germination was first observed in CBE-PT-10 after 8.74 days of sowing, the earliest among all genotypes, while EC0038959 took the longest at 27.24 days, with an average first day of germination (FDG) of 16.19 days. The last day of germination varied significantly, with EC0038959 taking the longest at 59.35 days and CBE-PT-03 the shortest at 15.35 days, averaging 33.21 days across all genotypes. The final germination percentage (FGP) ranged from 59.67–97.45%, with EC0038955 showing the lowest germination rate. The mean germination time (MGT) varied between 1.86 to 2.67 days, with EC0038955 germinating the fastest (1.86 days) and CBE-PT-01 the slowest (2.67 days). These findings align with the research of Kamal *et al.*, 2023.

Table 2
Assessment of germinability of seeds

Genotype	FDG (days)	LDG (days)	FGP (%)	MGT (days)
CBE- PT- 01	12.54	24.01	97.45	2.67
CBE- PT- 02	18.54	32.12	92.46	2.51
CBE- PT- 03	9.24	15.35	93.09	2.53
CBE- PT- 04	15.46	24.67	90.34	2.49
CBE- PT- 05	11.87	28.19	88.12	2.31
CBE- PT- 06	21.12	34.08	84.09	2.37
CBE- PT- 07	17.36	31.34	63.34	2.12
CBE- PT- 08	10.98	32.40	69.26	2.29
CBE- PT- 09	9.16	22.12	94.67	2.58
CBE- PT- 10	8.74	19.00	69.18	2.09
CBE- PT- 11	11.37	36.93	58.09	1.99
CBE- PT- 12	14.01	37.21	60.15	1.99
CBE- PT- 13	15.94	29.04	72.35	2.13
CBE- PT- 14	22.91	32.35	77.87	2.22
CBE- PT- 15	25.35	37.21	82.27	2.45
CBE- PT- 16	12.13	29.09	75.12	2.48
CBE- PT- 17	24.25	40.23	92.12	2.53
CBE- PT- 18	19.64	38.12	94.36	2.57
CBE- PT- 19	10.17	28.14	90.16	2.49
CBE- PT- 20	24.38	39.35	88.48	2.47
CBE- PT- 21	16.09	29.09	91.18	2.48
CBE- PT- 22	15.71	30.23	88.28	2.46
CBE- PT- 23	19.04	32.87	63.34	1.98
EC0038955	22.71	52.20	59.67	1.86
EC0038959	27.24	59.35	64.54	1.99
EC142667	18.16	39.08	73.81	2.13
IC0041980	15.23	37.12	79.21	2.19
EC0114273	16.19	39.26	75.02	2.24

FDG- First day of germination, LDG- Last day of germination, FGP- Final germination percentage, MGT- Mean germination time

3.2. Assessment of quantitative traits of the selected genotypes

In this study, totally sixteen quantitative characters were selected and these characters showed significant variation among the genotypes. The twelve vegetative, reproductive characters and four seed characters are represented in Tables 3 and 4.

3.2.1. Assessment of Vegetative and Reproductive growth characteristics

The significant morphological diversity in growth habit, pod characteristics, leaf structure, and flower traits are as shown in Fig. 1, and Fig. 2.

The vegetative traits such as vine length (VL), stem diameter (SD), and primary branches per plant (PBP) were recorded after the final harvest. The vine length among genotypes ranged from 418.64 cm to 729.76 cm, with EC142667 having the shortest vine (418.64 cm) and CBE-PT-17 the longest (729.76 cm), averaging 572.58 cm. The stem diameter varied between 2.02 cm and 2.82 cm, with CBE-PT-06 having the smallest (2.02 cm) and CBE-PT-20 the largest (2.82 cm), with a mean of 2.42 cm. The average number of branches recorded was 9.76. Fifty percent flowering was observed earliest in CBE-PT-01 (52.85 days) and latest in EC0038955 (78.95 days) after sowing, with an average of 65.39 days. The earliest 50% flowering was in CBE-PT-01 (52.85 days), while EC0038959 flowered the latest at 78.95 days. The days to pod maturity ranged from 99.4 days to 118.95 days, with CBE-PT-14 maturing the earliest (99.4 days) and CBE-PT-22 the latest (118.95 days), with an average of 108.19 days. These results agree with the previous findings of authors on diversity assessment studies of winged bean (Kamal et al. 2023).

The variability of winged bean pods is shown in Fig. 3. The pod length ranges from 14.31 cm to 46.18 cm (Fig. 1). The average pod length was 23.25 cm. The highest pod length was recorded in CBE- PT- 17 (46.18) and the lowest was in CBE- PT- 11 (14.31). The highest pod width was observed in CBE- PT- 17 (3.63 cm) and the lowest was in EC0114273 (2.14 cm) with a mean value of 2.74 cm. The number of pods per cluster was recorded highest in CBE- PT- 14 (7.63) with an increased number of pods, whereas, the lowest was in EC142667 (4.06). The mean value of the number of pods per cluster in the plant was 5.59. Number of pods per plant ranged from 20.44 to 28.82 with mean value of 24.27. The highest number of pods was recorded in CBE- PT- 04 (28.89) and lowest in EC142667 (20.44). The single pod weight per plant was observed maximum in CBE- PT- 17 (51.17 g), however, the pod weight was minimum in CBE- PT- 23 (22.22 g). The mean value recorded for single pod weight was 31.86. Similarly, pod yield per plant was recorded in the range of 466.12 to 929.55. The maximum yield of the pod was observed in CBE- PT- 17 (929.55 g) and minimum pod yield in EC142667 (466.12 g). The results have supported the earlier studies on the evaluation of winged beans (Bhadmus et al. 2023).

Table 3
Average performance of winged bean genotypes with respect to vegetative and reproductive traits

Genotype	VL	SD	PBP	D50F	DPM	PL	PW	PC	NPC	NPP	SPW	NSP	PYP
CBE- PT- 01	523.05	2.57	7.21	52.85	106.55	16.3	2.59	Green	4.38	21.23	23.26	6.11	673.47
CBE- PT- 02	544.12	2.52	7.78	55.78	103.8	21.49	3.23	Green	4.18	24.73	35.71	10.56	715.18
CBE- PT- 03	643.89	2.41	6.98	63.93	118.75	16.68	2.67	Green	5.36	26.5	31.11	6.66	563.49
CBE- PT- 04	541.28	2.61	7.48	64.44	104.8	21.97	3.4	Green	5.49	28.82	41.02	12.38	745.45
CBE- PT- 05	654.02	2.07	12.45	57.85	111.4	19.19	2.35	Green	6.62	27.64	25.85	6.43	813.11
CBE- PT- 06	574.67	2.02	11.34	64.45	107.81	44.19	3.4	Green	4.95	22.94	45.57	19.34	722.82
CBE- PT- 07	444.76	2.3	9.94	65.64	106.4	21.04	3.18	Green	5.11	23.34	37.54	10.95	637.33
CBE- PT- 08	578.34	2.43	8.34	64.25	104.15	19.91	2.97	Green	5.49	23.12	23.49	6.51	625.54
CBE- PT- 09	723.57	2.12	8.12	64.15	102.85	19.56	2.68	Green	6.58	25.33	26.9	6.01	510.03
CBE- PT- 10	536.57	2.32	10.01	64.95	105.3	16.46	2.45	Green	5.62	25.81	22.9	7.05	599.47
CBE- PT- 11	583.09	2.52	11.94	66.25	102.75	14.31	2.15	Green	4.39	26.63	24.79	5.61	603.43
CBE- PT- 12	666.89	2.44	12.09	69.15	114.9	19.7	2.35	Green	6.09	27.88	25.32	8.05	592.55
CBE- PT- 13	521.98	2.62	11.56	63.54	108.3	18.44	2.43	Green	6.12	26.47	26.9	6.34	566.64
CBE- PT- 14	621.89	2.12	10.29	57.73	99.4	16.19	2.21	Green with purple spots	7.63	23.09	24.04	6.73	478.64
CBE- PT- 15	654.19	2.33	9.67	65.52	108.05	44.29	3.47	Green	6.95	20.63	47.94	7.24	712.11
CBE- PT- 16	542.96	2.22	8.83	69.62	107.35	18.55	2.41	Green	5.53	25.41	25.37	5.93	762.16
CBE- PT- 17	729.76	2.71	12.98	62.92	111.45	46.18	3.63	Green	5.99	26.52	51.17	22.68	929.55
CBE- PT- 18	567.35	2.64	8.98	66.87	104.15	23.55	3.07	Purple	6.37	20.7	34.01	11.23	633.73
CBE- PT- 19	678.34	2.71	10.59	66.34	113.8	17.41	2.16	Green	5.17	26.23	26.88	6.86	661.27
CBE- PT- 20	592.55	2.82	11.34	74.95	115.65	45.82	3.53	Green	6.86	22.44	49.33	19.12	605.04
CBE- PT- 21	623.47	2.32	10.23	64.35	104.15	19.69	2.47	Green	5.21	25.73	28.98	6.66	574.89
CBE- PT- 22	598.32	2.34	10.82	72.85	118.95	18.37	2.28	Green	4.87	21.32	28.82	6.08	686.33
CBE- PT- 23	421.94	2.76	11.92	62.68	105.25	36.52	3.63	Green	5.03	23.57	48.66	19.09	578.06
EC0038955	653.89	2.72	10.22	78.95	114.65	21.98	2.96	Green	5.96	26.23	38.91	12.15	499.03
EC0038959	442.89	2.45	9.95	66.43	106.4	17.62	2.17	Green	6.03	20.51	22.22	6.89	716.53
EC142667	418.64	2.33	6.02	75.92	110.78	17.31	2.56	Green	4.06	20.44	27.43	5.34	466.12
IC0041980	437.45	2.3	7.21	64.85	107.35	18.39	2.37	Green	5.06	24.39	23.68	6.09	547.75
EC0114273	512.56	2.45	9.15	63.86	104.35	19.95	2.14	Green	5.61	22.13	24.55	5.94	577.35

VL- Vine length, SD- stem diameter, PBP- Primary branches per plant, D50F- Days to fifty percent flowering, DPM- Days to pod maturity, PL- Pod length, PW- Pod width, PC- Pod colour, NPC- Number of pods per cluster, NPP- Number of pods per plant, SPW- Single pod weight (g), NSP- Number of seeds per pod, PYP- Pod yield per plant (g)

3.2.2. Assessment of seed characteristics

The seed length ranged from 8.86 mm to 10.33 mm, with CBE-PT-21 having the longest seeds (10.33 mm) and EC0038959 the shortest (8.86 mm), averaging 9.70 mm. For seed width, CBE-PT-08 recorded the widest seeds (7.24 mm), while CBE-PT-13 had the narrowest (6.72 mm), with an average of 6.99 mm (Fig. 2). The hundred-seed weight was highest in CBE-PT-05 (21.34 g) and lowest in

CBE-PT-09 (19.02 g). The seed yield per plant varied significantly, with CBE-PT-17 producing the highest yield (312.34 g) and CBE-PT-14 the lowest (99.34 g), with an average yield of 167.32 g per plant. These findings align with previous research on genotype evaluation for pod yield and nutritional quality (Bhadmus et al. 2023).

Table 4
Average performance of seed traits

Genotype	SL	SW	HSW	SYP
CBE- PT- 01	10.18	7.03	20.13	130.23
CBE- PT- 02	9.36	6.78	19.98	212.03
CBE- PT- 03	8.94	7.14	20.32	145.89
CBE- PT- 04	9.9	6.97	19.02	263.87
CBE- PT- 05	9.83	7.05	21.34	110.68
CBE- PT- 06	10.01	6.99	19.55	301.09
CBE- PT- 07	9.44	7.13	20.98	250.89
CBE- PT- 08	9.47	7.24	20.24	99.87
CBE- PT- 09	8.93	6.89	19.02	132.08
CBE- PT- 10	10.2	7.13	20.87	129.84
CBE- PT- 11	9.62	6.98	19.34	115.93
CBE- PT- 12	9.45	7.17	21.33	197.34
CBE- PT- 13	10.08	6.72	19.87	111.21
CBE- PT- 14	9.6	6.85	19.76	99.34
CBE- PT- 15	9.54	6.97	19.04	104.56
CBE- PT- 16	9.83	6.95	19.24	122.34
CBE- PT- 17	10.2	7.22	20.03	312.34
CBE- PT- 18	9.09	6.86	19.77	186.34
CBE- PT- 19	9.74	6.97	19.66	115.56
CBE- PT- 20	10.23	7.03	20.95	226.89
CBE- PT- 21	10.33	6.95	19.13	144.67
CBE- PT- 22	9.97	7.02	20.33	122.78
CBE- PT- 23	9.6	6.98	19.68	114.43
EC0038955	9.77	7.02	20.22	276.29
EC0038959	8.86	6.88	19.55	298.38
EC142667	9.76	7.02	20.33	99.78
IC0041980	10.23	6.99	19.75	134.53
EC0114273	9.48	6.97	19.97	125.78

SL- Seed length (mm), SW- Seed width (mm), HSW- Hundred seed weight, SYP- Seed yield per plant

3.3. Estimation of genetic components

In a population, the degree of variability is important for the effective selection process. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were mainly used to measure the variation in the population. The total variation present in

the population is the total of genotypic and environmental effects. The phenotypic coefficient of variation for all the traits was higher than the genotypic coefficient of variation (Table 5). This supports the previous findings of the winged bean (Kamal et al. 2023). T.N. Khan (1978) reported that the coefficient of variation in winged bean ranges from 30–83%. In his further study, he recorded variations in seed weight (21%), shelling percentage (25%), number of seeds per pod (27%), and pod length (64%). In the present study, the highest genotypic and phenotypic coefficient of variation were observed in the trait number of seeds per pod (47.20, 54.51), followed by pod length (36.08, 41.66), and the lowest was found in the trait days to pod maturity (3.99, 4.61). Therefore, it is recognized that phenotypic characters within the trait were controlled by genetic factors over environmental factors (Prashanth et al. 2016).

The degree of inheritance of characters from parents to offspring is called heritability. The high heritability traits are less effective with the environmental factors and might be directly improved by the selection process (Erskine 1981). The high heritability was observed in the trait number of seeds per pod (98.98), followed by vine length (98.46), whereas, low heritability was recorded in stem diameter (89.41). The high heritability traits can be improved by two or three repeated selections followed by a single seed descent method of breeding (Mohamadali et al. 2007). The genetic advance as percent mean recorded maximum in number of seeds per pod (84.20), followed by pod length (64.35), and minimum in stem diameter (14.19).

Table 5
Genetic components of selected genotypes

Traits	PV	GV	GCV	PCV	H ² B	GAM
VL	8372.27	6279.20	13.55	15.65	98.46	24.16
SD	0.05	0.04	7.95	9.18	89.41	14.19
PBB	3.20	2.40	16.03	18.51	97.06	28.60
D50F	35.68	26.76	8.03	9.28	93.33	24.33
DPM	25.39	19.04	3.99	4.61	95.71	27.12
PL	100.73	75.55	36.08	41.66	96.84	64.35
PW	0.24	0.18	14.51	36.76	90.77	35.90
NPC	0.82	0.62	14.05	16.22	95.71	25.08
NPP	5.31	3.98	8.55	9.87	93.33	15.25
SPW	90.72	68.04	25.83	29.83	92.50	46.12
NSP	21.61	16.21	47.20	54.51	98.98	84.20
PYP	10862.93	8147.20	14.74	17.02	95.71	26.31

VL- Vine length, SD- stem diameter, PBP- Primary branches per plant, D50F- Days to fifty percent flowering, DPM- Days to pod maturity, PL- Pod length, PW- Pod width, PC- Pod colour, NPC- Number of pods per cluster, NPP- Number of pods per plant, SPW- Single pod weight, NSP- Number of seeds per pod, PYP- Pod yield per plant, PV- Phenotypic variance, GV- Genotypic variance, GCV- Genotypic coefficient of variation (%), PCV- Phenotypic coefficient of variation (%), H²B- Heritability, GAM- Genetic advance as percent mean (%).

3.4. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a widely used statistical method for accessing the genetic diversity within the genotypes and to identify the key variables that contribute to the particular variation (Price et al. 2006). The variability within the genotypes was distributed across 14 principal components (PCs). Out of 14 PCs, four had Eigen values more than one. The PCA based on 12 vegetative and reproductive traits revealed that the first four PCs accounted for 74.67% of the total observed variation for yield and its attributing traits among the genotypes (Table 6). It was in accordance with the findings of Tiwari and Singh (2019) and Dewangan et al. (2022). This implies that the traits with high coefficients in the principal components can be used to characterize other winged bean genotypes (Olanrewaju et al. 2021). Further, these traits could be considered for genetic improvement in breeding programs (Olomitutu et al. 2021).

The first, second and third accounted for 64.74% of the total variances observed. The biplot revealed that PL, NSP, SPW, and PW had positive contributions to the first PC. The major contributing traits to the second PC were Vine length (VL), Primary branches per plant (PBB), Number of pods per cluster (NPC), Number of pods per plant (NPP) and Days to pod maturity (DPM) with 16.33% variation. However, PC1 is dominated by pod and seed related traits and PC2 is related to vegetative growth traits.

In a scree plot graph, eigen values that are associated with a factor were plotted in descending order against the number of PCs to illustrate the proportion of variance attributed to these components. In Fig. 4, PC1 (First Principal Component) explains the most variance of 34.7%, followed by a steep drop to PC2 (16.3%) and PC3(13.7%). As PC1 and PC2 contribute to the wide genetic diversity, choosing genotypes based on traits from PC1 and PC2 would be advantageous. The first principal component (PC1) genotypes (CBE-PT-17, CBE-PT-04, CBE-PT-02) contributed to the diversity in traits such as stem diameter (0.428), pod length (0.92), pod width (0.851), single pod weight (0.946), and pod yield per plant (0.474). Key traits contributed to PC2 genotypes (CBE-PT-03, CBE-PT-05) are VL (0.797), PBB (0.545), and NPC (0.528). The third PC (EC0038955, CBE-PT-22) contributed traits are D50F (0.86), and DPM (0.729). Meanwhile, the fourth PC (CBE-PT-14, CBE-PT-15) contributed roughly to the trait NPP (0.528).

Table 6
Principal components of 28 genotypes based on quantitative traits

Traits	PC1	PC2	PC3	PC4
VL	0.266	0.797	0.008	-0.096
SD	0.428	-0.183	0.397	0.36
PBB	0.486	0.545	-0.021	0.001
D50F	0.15	-0.044	0.86	-0.237
DPM	0.266	0.278	0.729	0.167
PL	0.92	-0.107	-0.097	-0.228
PW	0.851	-0.344	-0.15	-0.015
NPC	0.225	0.528	-0.128	-0.683
NPP	0.04	0.637	-0.011	0.528
SPW	0.946	-0.186	0.004	-0.047
NSP	0.918	-0.153	-0.055	0.076
PYP	0.474	0.205	-0.402	0.404
Eigen values	4.167	1.96	1.64	1.19
Percent variance (%)	34.729	16.332	13.681	9.93
Cumulative variance (%)	34.729	51.06	64.74	74.67

PC- Principal components, VL- Vine length, SD- stem diameter, PBB- Primary branches per plant, D50F- days to fifty percent flowering, DPM- days to pod maturity, PL- pod length, PW- pod width, NPC- number of pods per cluster, NPP- number of pods per plant, SPW- Single pod weight (g), NSP- number of seeds per pod, PWP- pod weight per plant, %- percentage.

The first principal component (PC1) explains the most variance of 34.7%, followed by a steep drop to PC2 (16.3%) and PC3(13.7%). As PC1 and PC2 contribute to the wide diversity, they were plotted graphically to demonstrate the relationship among the winged bean genotypes. For easy visualization, both genotypes and variables were combined into a single biplot graph, that was centered on two primary principal components, namely PC1 and PC2 (Fig. 5). The vector length of each trait within the graph illustrates its contribution to overall divergence. The longest vector length of trait Single pod weight (SPW), indicating substantial contribution to divergence, followed by Pod length (PL), Pod width (PW), Number of seeds per pod (NSP), Vine length (VL), Primary branches per plant (PBB), and Pod yield per plant (PYP).

The angle formed between the trait vectors illustrates the direction of association between the traits (Price et al. 2006). The genotypes, CBE-PT-12 and CBE-PT-05 contributed wider diversity to the VL, CBE-PT-12 to NPP, CBE-PT-05 to PBB, CBE-PT-20 and CBE-

PT-06 contributed to PW and SPW. Similarly, CBE-PT-14 records moderate variability, whereas, CBE-PT-17 and EC0038959 are genetically distinct from others, possibly contributing to a wider variability among the genotypes.

3.5. Correlation analysis

Table 7
Pearson's correlation matrix of selected genotypes

	VL	SD	PBB	D50F	DPM	PL	PW	NPC	NPP	SPW	NSP	PYP
VL	1											
SD	-0.044	1										
PBB	0.342	0.141	1									
D50F	-0.006	0.212	0.057	1								
DPM	0.29	0.235	0.172	0.513**	1							
PL	0.19	0.219	0.395*	0.111	0.158	1						
PW	0.032	0.331	0.068	0.022	0.011	0.802***	1					
NPC	0.464*	-0.1	0.34	0.022	-0.033	0.257	0.056	1				
NPP	0.424*	0.062	0.27	-0.097	0.122	-0.185	-0.078	0.043	1			
SPW	0.141	0.369	0.296	0.164	0.194	0.893***	0.912***	0.126	-0.039	1		
NSP	0.067	0.408*	0.43*	0.085	0.127	0.823***	0.813***	0.076	0.037	0.867***	1	
PYP	0.212	0.039	0.319	-0.286	0.113	0.396*	0.316	-0.008	0.142	0.335	0.361	1

plant, SPW- Single pod weight (g), NSP- number of seeds per pod, PWP- pod weight per plant (g)

*** Correlation is significant at 0.001 (two-tailed)

** Correlation is significant at 0.01 (two-tailed)

* Correlation is significant at 0.05 (two-tailed)

The correlation matrix provides insights into how different traits in a plant breeding study are related (Cohen et al., 2009). In this study, the correlation between growth and yield-related traits such as PL and PW ($r = 0.802^*$), NPC and PYP ($r = 0.464^*$) records a strong positive correlation. PL and SPW ($r = 0.893^*$), show a highly significant relationship. Similarly, very strong correlation exists between PL and NSP ($r = 0.823^*$), PW and SPW ($r = 0.912^*$), PW and NSP ($r = 0.813^*$), SPW and NSP ($r = 0.867^*$), D50F and DPM ($r = 0.513^*$). The yield component correlation traits such as PW and PYP ($r = 0.316$) show a positive correlation, whereas, a moderate correlation exists between SPW and PYP ($r = 0.335$), and PL and PYP ($r = 0.396^*$). A negative correlation was observed between D50F and PYP ($r = -0.286$). The previous study (Ayomide et al. 2023) reported a positive correlation between D50F and DPM that has supported our study.

3.6. Hierarchical Clustering of selected genotypes

To access the genetic diversity among the selected winged bean genotypes, the dendrogram generated from cluster analysis classified the 28 genotypes into three distinct groups (Fig. 3). Hierarchical clustering determines the optimal number of clusters by grouping similar trait genotypes. The classification was based on their similarity for one or more quantitative traits studied, irrespective of their origin (Nielsen et al. 2016). The first cluster consists of ten genotypes (CBE-PT-05, CBE-PT-17, CBE-PT-15, CBE-PT-19, CBE-PT-04, CBE-PT-16, CBE-PT-02, CBE-PT-06, CBE-PT-01, CBE-PT-22) that are likely associated with higher pod yield per plant (PYP), followed by long pods (PL), and number of seeds per pod (NSP) that has higher Euclidean distance due to wide diversity. The second cluster consists of six genotypes (CBE-PT-03, CBE-PT-21, CBE-PT-09, CBE-PT-14, EC0038955) represents the highest number of pods per plant (NPP), pods per cluster (NPC) with the moderate-yielding and stable-performing genotypes. These genotypes exhibit balanced growth traits, adaptability, and moderate productivity. The third cluster consists of 12 genotypes (CBE-PT-10, CBE-PT-13, EC014273, CBE-PT-20, CBE-PT-11, CBE-PT-08, CBE-PT-18, CBE-PT-07, CBE-PT-23, EC142667, EC0038959, IC0041980) recording

moderate pod yield (PYP), and longer flowering days and maturity period (DPM). This result suggests that genotypes grouped based on their distinct feature could be utilized as parents in the hybridisation program to enhance the yield of winged beans (Ayomide et al. 2023). Furthermore, these traits could be considered for the genetic improvement of winged beans (Olomitutu et al. 2022)

Table 8
Cluster means for the selected quantitative traits

Trait	Cluster- I	Cluster- II	Cluster- III
VL	604.07	655.60	504.84
SD	2.41	2.36	2.50
PBB	9.92	9.66	9.70
D50F	63.26	66.37	66.68
DPM	109.4	109.12	106.74
PL	26.79	18.97	22.44
PW	2.89	2.56	2.72
NPC	5.41	6.14	5.48
NPP	24.55	25.79	23.30
SPW	35.16	29.21	30.46
NSP	10.36	7.71	9.24
PYP	742.15	536.44	596.42

4. Conclusion

The evaluation of 28-winged bean genotypes revealed substantial phenotypic and genetic variation, indicating significant potential for crop improvement. The study identified key traits, such as pod length, pod width, number of pods per cluster, and pod yield per plant, that exhibit high heritability and genetic advance. These traits can serve as effective selection criteria in breeding programs aimed at improving yield and adaptability. The principal component analysis highlighted pod yield-related traits as major contributors to genetic diversity, reinforcing their importance in selection strategies. Cluster analysis categorized the genotypes into three distinct groups, with Cluster I genotypes demonstrating superior yield attributes. Notably, genotypes CBE-PT-17, CBE-PT-04, and CBE-PT-15 were identified as high-yielding, making them prime candidates for breeding initiatives. Conversely, genotypes in Cluster III exhibited moderate yield potential but longer maturity duration, suggesting their suitability for specific agro-climatic conditions. The high heritability of critical traits, such as number of seeds per pod and pod length, underscores the genetic control over these characteristics, making them ideal targets for selection in future breeding efforts. Moreover, the strong correlations observed between pod length, pod width, number of seeds, and pod yield suggest that selecting one trait could positively influence overall productivity. Despite its immense nutritional value, the winged bean remains underutilized in commercial agriculture. The results of this study provide a foundation for breeding programs to develop improved cultivars with enhanced yield, shorter maturity periods, and better adaptability to varying environmental conditions. Future research should integrate molecular approaches to further dissect the genetic basis of yield-related traits, ensuring the sustainable utilization of winged bean in global food security initiatives.

Declarations

Conflicts of interest:

The authors disclose that there is no conflict of interest. The funders had no role in the design of this study; in the collection, analyses, or interpretation of data; in the writing of manuscript; or in the decision to publish the results.

Author Contribution

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Figures

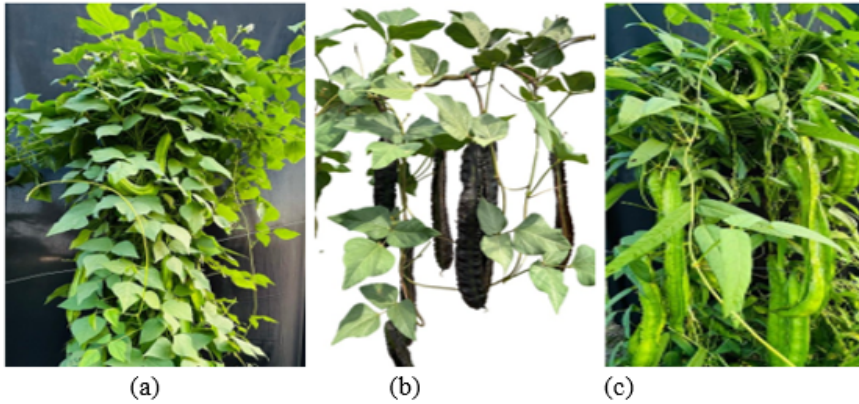


Figure 1

Growth habit and pod variability in winged bean genotypes: (a). Green winged beans (b). Purple winged bean and (c). Long pod green winged bean

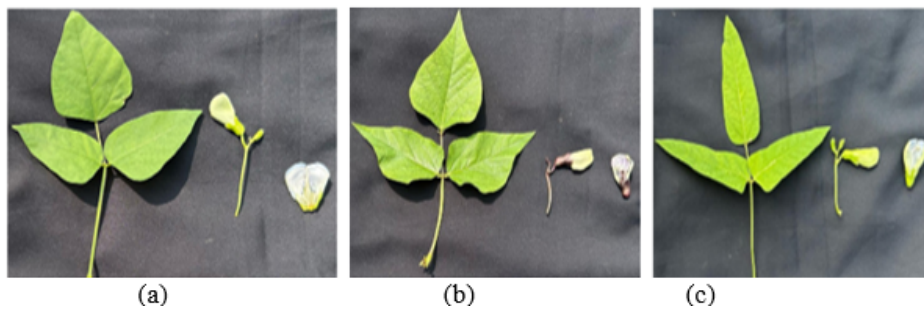


Figure 2

Morphological variation in leaf and flower traits of winged bean genotypes: (a). Green winged beans (b). Purple winged bean and (c). Long pod green winged bean



Figure 3

Pod variability of selected winged bean genotypes



Figure 4

Figure 3. Variability of collected winged bean seeds

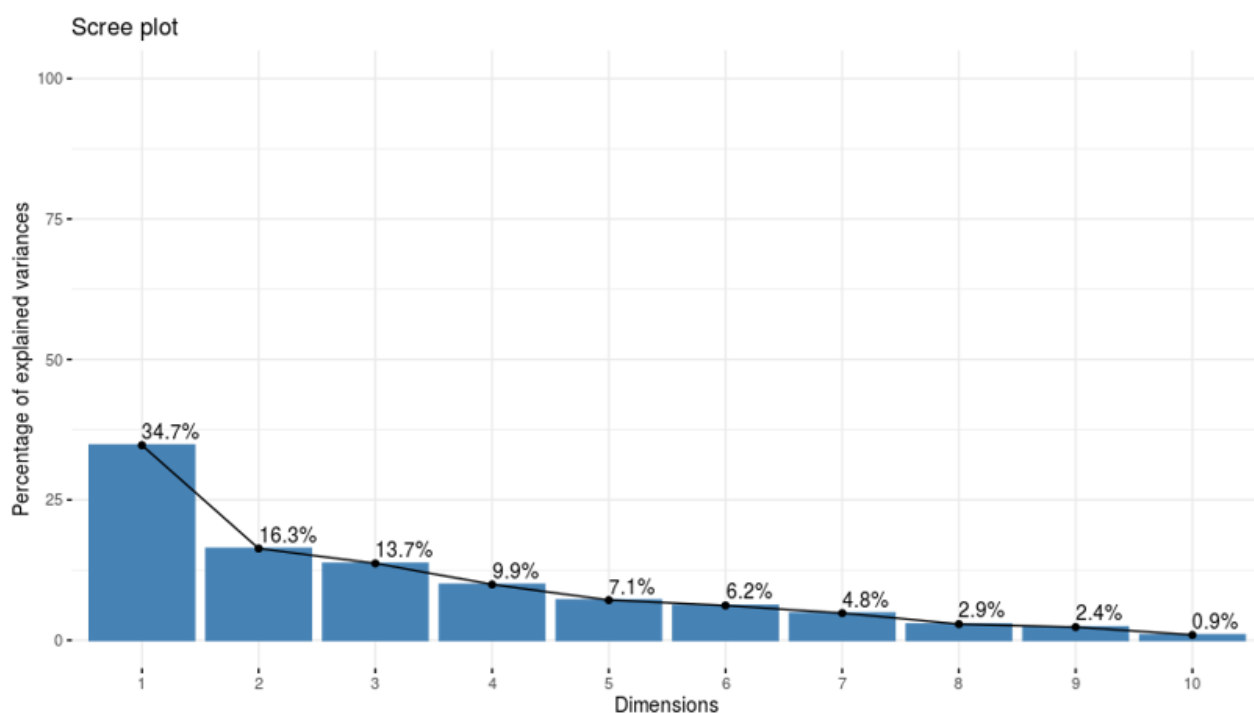


Figure 5

Figure 4. Scree plot showing the contribution of various principal components toward diversity

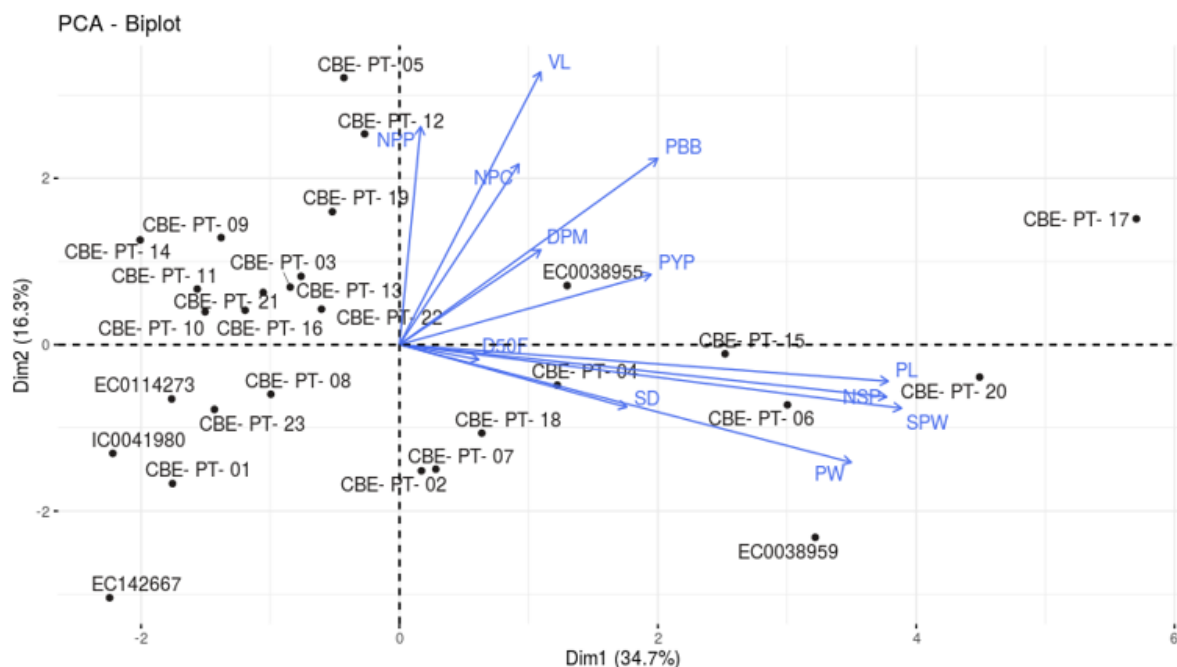


Figure 6

Figure 5. PCA biplot using 12 quantitative traits of selected genotypes.

VL- Vine length, SD- Stem diameter, PBP- Primary branches per plant, D50F- Days to fifty percent flowering, DPM- Days to pod maturity, PL- Pod length, PW- Pod width, NPC- Number of pods per cluster, NPC- Number of pods per cluster, SPW- Single pod weight (g), NSP-

Number of seeds per pod, PYP- Pod yield per plant (g)

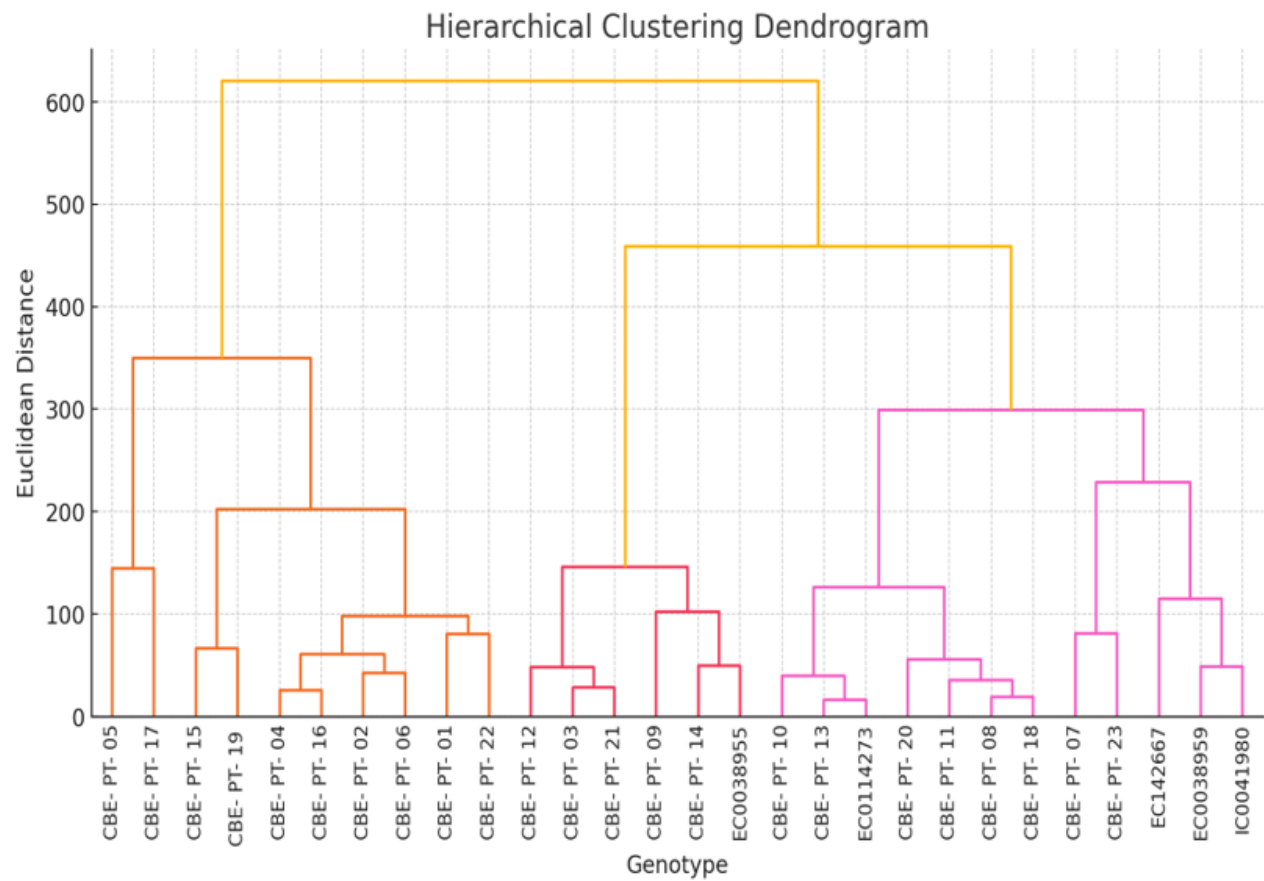


Figure 7

Figure 3. A cluster of the 28 genotypes derived from 12 quantitative traits studied.