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RESEARCH ARTICLE

Genetic diversity assessment of black pepper (*Piper nigrum* L.) cultivars of Western Ghats

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Abstract

The Western Ghats of Kerala, the centre of origin of black pepper (*Piper nigrum* L.), hold a rich repository of landraces and local cultivars, that farmers have maintained over centuries. A survey conducted in these regions identified 21 genotypes with superior yields and distinct characteristics, reflecting the diverse genetic makeup of black pepper across agroclimatic conditions in the state. Moderate variability was observed among these genotypes in qualitative morphological traits, but it was still significant enough to differentiate them. Using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), the genotypes were grouped into 15 clusters at 73 % genetic similarity, indicating shared characteristics and unique differences. Biochemical evaluation revealed variations in their piperine (3.0 –5.6 %), essential oil (3.0–5.6 %) and oleoresin (6.3–13.2 %), which could have implications for flavour, aroma and medicinal properties. Principal Component Analysis (PCA) identified 9 main and independent components explaining 86.59 % of the total variance, with yield-related and physiological traits contributing significantly to the overall diversity. Genotypes G16, G15, G21, G1, G5 and G4 exhibited considerable genetic divergence and desirable qualities. This highlights the potential for leveraging these genotypes to develop improved black pepper varieties with enhanced traits such as yield, quality and resilience to various environmental conditions. The study provides insights into the genetic and biochemical diversity of black pepper germplasm in the Western Ghats of Kerala, offering a foundation for future breeding efforts to enhance the productivity and sustainability of black pepper cultivation.

Keywords

genetic diversity; germplasm; Piper; principal component analysis; quality

Introduction

Black pepper (*Piper nigrum* L.), glorified as the 'King of Spices' or 'Black Gold,' belongs to the family Piperaceae and originated in India in the submountainous tracts of the Western Ghats. It is one of human history's earliest and most commonly used spices. The alkaloid piperine is considered primarily for its distinct flavour and pungency (1). Black pepper is cultivated for its fruits (berries) and is a commercial crop with numerous domestic and industrial applications. It is used as a flavouring agent and a preservative in food and beverages, for preparing essential oils and by the pharmaceutical and perfumery industries (1). The total commercial production of black pepper worldwide is 793817.98 MT (2). Indian black pepper has gained popularity in international markets due to GI-tagged varieties such as Malabar pepper (3). Globally, India contributes the second-highest share to the black pepper cultivated area, reported as 131711 ha, after Indonesia (188817 ha) in 2021 (2). According to the Food and Agriculture Organization (2), the total black pepper cultivated area in India has not shown any significant change. However, in terms of production, pepper faced year-to-year instability, a deteriorated growth rate and widened production gap in the current scenario. Black pepper production in India is 64,815.81 MT (2), with Karnataka and Kerala accounting for 36000 and 22000 MT respectively (4). The decline in black pepper production in India was influenced by various factors such as frequent attacks of diseases, inadequate pest control measures, limited fertiliser and water facilities and the nature of soil and climatic conditions (5). Therefore, considering these circumstances, efforts are required to boost black pepper production and productivity. The first step toward boosting black pepper production is to offer superior varieties.

Black pepper is a woody, perennial climbing vine with different cultivars that differ in yield and morphology (6). Black pepper cultivars exhibit significant leaf shape and size diversity, shoot tip colour, spike length, spike composition (unisexual or bisexual), number of fruits per spike, yield and berry size, shape and quality. The range of black pepper consists of over a hundred cultivars, which contributing to a significant genetic diversity of black pepper in India (7), most of which are of superior quality, with a high level of oil and pungent components. Kerala, occupying a considerable portion of the Western Ghats, is a rich repository of wild forms and diversity of the genus *Piper*. Cultivars with good berry sets, pungency and other desirable characteristics contribute to breeding and conservation programs (9). Observation and recording of morphological traits have been a standard method for identifying and describing distinct black pepper germplasm (6, 9, 10). Many natural resources, including crop genetic resources, are facing extinction, primarily in areas where cropping patterns are transforming at an alarming rate (11).

Indian spices are known for their taste, flavour, aroma and texture, with black pepper cultivars rich in piperine, oleoresin and essential oils especially valued in the value-added produce industry (7). The quality of black pepper, particularly in local Kerala cultivars, is high, though yields are comparatively low. Identifying high-quality cultivars is essential to meet industrial and global demand and to support breeding efforts for improved black pepper varieties. The main breeding goal is to develop high-yielding varieties with quality traits resilient to biotic and abiotic stresses, which significantly impacts production (11). Access to specific high-quality genotypes is crucial for advancing breeding programs for superior black pepper varieties. Kerala, with its rich repository of wild forms and diversity of *P. nigrum*, allows the discovery of local black pepper germplasm that has the potential to provide national and regional-specific superior varieties. The indigenous cultivars and landraces also act as a gene bank for developing cultivars resistant to biotic and abiotic challenges and developing certain agronomic features of commercial value, particularly in light of changing climatic conditions. The loss of genetic diversity produced by intensive artificial selection and breeding resulted in bottlenecks favouring alleles that control major agronomic traits. This scenario can be remedied by creating and releasing high-yielding, premium quality varieties of black pepper with wide adaptability to the growing conditions. In light of the above facts, the present study was conducted to study the diversity of black pepper germplasm at the morphological, biochemical and physiological levels.

Materials and Methods

A survey was carried out in 16 farmer's fields in 5 districts of Kerala for 2 years (2019–2021). Black pepper cultivars were selected based on their performance in the agro ecosystem. From a survey of 150 plants, 21 genotypes that were superior in yield with distinct traits were selected for morphological, biochemical and physiological characterisation. Detailed passport information regarding the study location and genetic material has been shown in Table 1.

Morphological characterisation of black pepper cultivars

Six selected plants of each cultivar were used to record data to characterise the selected cultivars at the morphological level. The observations were recorded against critical morphological characters, namely, runner shoot tip colour (using colour charts of Royal Horticulture Society), lateral branch pattern, leaf lamina shape, leaf base shape, leaf size (using standard descriptor), leaf margin, spike twisting, spike setting, berry shape, seed shape, number of nodes per lateral, number of well-developed berries per spike, number of spikes per lateral branch, number of spikes per vine (visual observations), time of harvest maturity (number of days from flowering to maximum maturity), vine column height (using smart measure 1.6.7 for android), vine column diameter (using measuring tape), lateral branch length, leaf length, leaf width, leaf petiole length, spike length, spike peduncle length (using a scale), spike yield per vine, berry yield per vine, hundred berry weight (weighing balance), hundred berry volume (using water displacement method), berry diameter (using Vernier calliper) and dry recovery. The genotypes were described using standard descriptors (12, 13).

Biochemical analysis

Piperine content (%) was determined following the method with slight modification (14). Powdered black pepper was placed in the extraction thimble of a Soxhlet apparatus and extracted with chloroform for 2 h. The resulting solution was then allowed to cool in a beaker and the solvent was removed. About 20 mL of ethanolic potassium hydroxide solution was added to this concentrated residue and allowed to stand for 1 h. This solution was filtered to separate the alcoholic solution from the insoluble residue. The filtrate was left undisturbed overnight in the refrigerator.

Table 1. Passport details of the selected black pepper cultivars.

Genotypes	District	Location	Latitude/longitude	Special characters
G1	Wayanad	Noolpuzha	8°58'78"/ 76°34'20"	Vigorous vine, close setting of berries, bold berries
G2	Wayanad	Noolpuzha	8°58'78"/ 76°34'20"	Good fruit set, bold berries
G3	Wayanad	Noolpuzha	11°60'64"/ 76°34'18"	High spiking intensity
G4	Wayanad	Nenmani	11°65'47"/ 76°26'65"	Close setting of berries
G5	Wayanad	Nenmani	11°65'47"/ 76°26'65"	Vigorous vine, close setting of berries, long spikes
G6	Idukki	Cheppukulam	9°88'62"/ 76°65'16"	High spiking intensity
G7	Idukki	Cheppukulam	9°88'62"/ 76°65'16"	Suitable for higher elevation
G8	Idukki	Cheppukulam	9°44'39"/ 76°17'39"	Regular yield
G9	Idukki	Peringassery	9°86'56"/ 76°83'48"	Vigorous vine, good setting
G10	Idukki	Peringassery	9°86'56"/ 76°83'48"	Regular yielder
G11	Idukki	Uppukunnu	9°84'97"/ 76°87'67"	Early maturity
G12	Idukki	Senapathy	9°83'23"/ 77°06'24"	High yield
G13	Idukki	Senapathy	9°83'62"/ 77°06'15"	High yield
G14	Idukki	Senapathy	9°83'21"/ 77°04'41"	High spiking intensity
G15	Alappuzha	Vallikunnam	9°13'10"/ 76°57'67"	Vigorous vine, long spikes
G16	Alappuzha	Vallikunnam	9°12'14"/ 76°52'61"	Early maturity
G17	Alappuzha	Vallikunnam	9°14'29"/ 76°58'37"	High yield
G18	Trivandrum	Kalliyoor	8°43'15"/ 77°01'44"	Stable yield
G19	Trivandrum	Vellayani	8°42'96"/ 76°98'77"	Staggered flowering, bold berries
G20	Kottayam	Vakathanam	9°51'49"/ 76°57'03"	Stable yield
G21	Kottayam	Vakathanam	9°51'76"/ 76°57'51"	Vigorous vine, bold berries

The following day, long yellow needles of piperine crystals were collected. If no crystals formed, 4-5 mL of water was added and the solution was refrigerated again overnight. The piperine crystals were initially separated from the solution by filtration. The filtered crystals were then washed with 95 % ethanol and air-dried.

Oleoresin (%) was extracted using the solvent extraction process with the Soxhlet apparatus and acetone as the solvent. Dried black pepper berries of 3 g were crushed and loaded into the thimble inside the Soxhlet extractor. Acetone (250 mL) was added to the round bottom flask. The extraction was completed when the solvent in the tube showed no colour. The thimble was removed at the final stage of extraction and the solvent remained in the round bottom flask, along with the oleoresin and was allowed to evaporate.

The essential oil (%) in dried berries was extracted using a modified Clevenger hydro distillation apparatus. Twenty grams of dried black peppercorns were ground and taken in a round bottom flask to which 200 mL of distilled water was added. This mixture was subjected to hydrodistillation to separate the volatile oil. The process continued for 3 h.

Physiological observations

The leaf thickness (mm) of 10 leaves of each genotype was measured using a digital Vernier calliper. Epicuticular wax (mg cm^{-2}) was calculated based on the method described with slight modifications (15). A sample of 10 cm^2 of leaf bits was taken from the middle portion of the leaf, avoiding the midrib and the initial weight was noted. The leaf bits were dipped in 10 mL of chloroform for 30 sec. The test tubes were kept undisturbed until the chloroform evaporated. The final weight of leaf bits was measured. The differences indicated the wax content. Stomatal density refers to the stomata count per square centimeter of leaf, and it was observed under a compound microscope. The relative water content (RWC) of leaves was measured based on the method described (15). Ten leaf discs of about 1 cm diameter were punched from leaves of every genotype and the fresh weight (FW) was recorded. The turgid weight (TW) was measured after the leaf discs were floated for 3 h in distilled water in covered petri dishes until the discs became fully turgid. The discs were oven-dried at 80 °C for 24 h and the dry weight (DW) was determined.

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

…….(Eqn. 1)

The specific leaf area (SLA) was calculated using a method proposed (16). To measure SLA, circular leaf cores of 9 mm diameter were extracted from mature leaves. Care was taken to avoid the main leaf vein during coring. These leaf cores were placed in small parchment bags and transported to the laboratory. Leaves were dried for 48 h at 60 °C and stored in a silica gel desiccator. SLA was computed by dividing the total one-sided fresh leaf area by the dry weight.

Statistical analysis

The qualitative data were considered for cluster analysis using NTSYS (Numerical Taxonomy System) package 2.2. Yield data for the selected genotypes were recorded for 2 consecutive years. Observations on quantitative characters, biochemical traits and physiological parameters were recorded and Principal Component Analysis (PCA) was carried out using KAU - GRAPES 1.0.0.

Results

Morphological characterisation based on qualitative traits

In the present investigation, 21 qualitative characters of black pepper were studied (Table 2). The genotypes used in this study exhibited moderate variability. The shoot tip colour of runner shoots observed in the selected black pepper genotypes was light green (149B), light purple (N77D) and dark purple (N79B). The majority of runners had (76.19 %) light purple shoot tips. The lateral branch patterns observed were semi-erect, horizontal and hanging, with the majority (66.67 %) having horizontal branch habits, followed by hanging types (23.81 %). Expressions of lateral branch length were short (<30 cm), medium (30–40 cm) and long (>40 cm), where many exhibited long lateral branches with few nodes (<20). Most of the studied genotypes had ovate-lanceolate and cordate leaf lamina with cordate base. The observed leaf margin was of 2 types: even and wavy; the majority was even (90.48 %). The leaf size in terms of length and width varied as short (<10 cm), medium (10 to 16 cm) and long (>16 cm) and narrow (<7 cm), medium (7 to 10 cm) and broad (>10 cm) respectively. Most produced medium-sized leaves with medium petiole lengths (2–3 cm).

There was a variation in spike twisting and setting. The spike twisting observed in 2 genotypes was a major characteristic of that cultivar identification. Surprisingly, most genotypes showed a compact setting that denoted a high fruit set. The berry shape was round for all genotypes except for an oval shape for the 2 genotypes, G19 and G20. The genotypes that had oval-shaped fruits had ovate-shaped seeds. Berry size, an important key characteristic determining yield, varied among the cultivars as small (< 3 cm), medium (3 to 4.25 cm) and bold (> 4.25 cm). Most of the genotypes produced medium-sized fruits followed by bold berries. Among the genotypes studied, spike peduncle length varied from short (<1 cm) to long (>2 cm). Most genotypes (61.91 %) showed medium peduncle length (1-2 cm). Spike length varied as short (<10 cm), medium (10-15 cm) and long (>15 cm). Nine genotypes (42.86 %) had short spike lengths, 10 genotypes had medium spike lengths (47.62 %) and 2 genotypes (9.52 %), such as G15 and G21, exhibited long spike lengths.

Genetic diversity analysis

Diversity analysis of black pepper genotypes based on morphological characters is displayed as a dendrogram (Fig. 1). In this study, cluster analysis based on qualitative characters revealed that all the 21 genotypes could be grouped into 15 clusters at 73 % similarity. The similarity coefficient ranged between 0.47 and 0.81, indicating that

Table 2. Expressions and frequency of characters among the genotypes.

Sl. No.	Characters	Expression	Frequency (%)
1	Shoot tip color	Light Green	14.29
		Light Purple	76.19
		Dark Purple	9.52
2	Lateral branch pattern	Semi-Erect	9.52
		Horizontal	66.67
		Hanging	23.81
3	Lateral branch length	Short	23.81
		Medium	23.81
		Long	52.38
4	Number of nodes per lateral branch	Few	66.67
		Medium	33.33
5	Leaf lamina shape	Ovate	23.81
		Ovate- lanceolate	28.57
		Ovate- elliptic	19.05
		Cordate	28.57
6	Leaf base shape	Round	42.86
		Cordate	28.57
		Acute	28.57
7	Leaf margin	Even	90.48
		Wavy	9.52
8	Leaf petiole length	Short	19.04
		Medium	66.67
		Long	14.29
9	Leaf length	Short	23.81
		Medium	52.38
		Long	23.81
10	Leaf width	Narrow	28.57
		Medium	42.86
		Broad	28.57
11	Spike twisting	Absent	90.48
		Present	9.52
12	Spike setting	Loose	85.71
		Compact	14.29
13	Berry shape	Round	90.48
		Oval	9.52
14	Berry size	Small	19.05
		Medium	47.62
		Bold	33.33
15	Seed shape	Round	90.48
		Ovate	9.52
16	Spike peduncle length	Short	33.33
		Medium	61.91
		Long	4.76
17	Spike length	Short	42.86
		Medium	47.62
		Long	9.52
18	Number of berries per spike	Medium	71.43
		Many	28.57
19	Number of spikes per lateral branch	Few	9.52
		Medium	28.57
		Many	61.91
20	Bulk density	Low	38.10
		Medium	52.38
		High	9.52
21	Time of harvest maturity	Early	9.52
		Medium	85.72
		Late	4.76

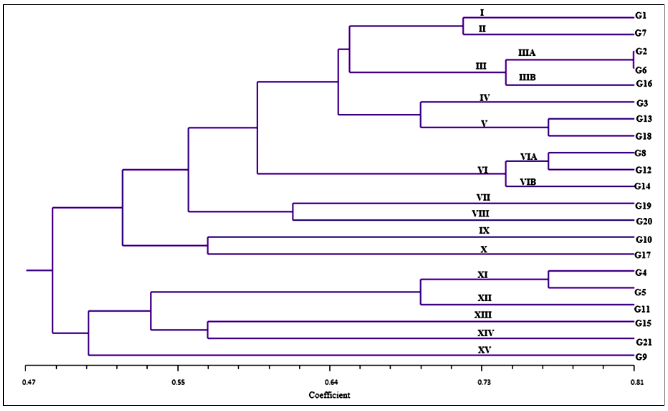

Fig. 1. Dendrogram showing NTSYS cluster analysis of 21 black pepper cultivars based on qualitative traits.

the black pepper genotypes used in the study have a low diversity or medium similarity. Cluster I comprised genotype G1 and Cluster II included genotype G7. Cluster III had 2 subclusters. Subcluster IIIA included genotypes G2 and G6 and subcluster III B included genotype G16 alone. The fourth group comprised G3. Cluster V contained G13 and G18, displaying their similar genetic backgrounds despite being selected from 2 locations. Cluster VI consisted of 2 subclusters, VIA and VIB. G8 and G12 belonged to subcluster VIA and G14 belonged to subcluster VIB. Cluster VII included G19, Cluster VIII contained G20, Cluster IX contained G10, Cluster X had G17, Cluster XI included G4 and G5, Cluster XII contained G11, Cluster XIII had G15, Cluster XIV included G21 and Cluster XV with G9. These results illustrated that clustered populations in the same group had similar genetic backgrounds. The genotype G9 in Cluster XV showed maximum diversity due to the dark purple colour of the runner shoot tip, semi-erect branching pattern, broad and cordate leaves, bold berries and low bulk density. Clusters III and VI had only three genotypes, while Cluster V and XI had two. The other clusters had only single genotypes, showing their divergence in the morphological characters considered. The genotypes with the highest level of characteristic similarity, 81 %, were G2 and G6. The morphological difference between these 2 cultivars was the size and shape of the leaves. The close relationship between these 2 indicates that they might have originated from the same parent but are scattered to different places. Thus, geographical location does not play much role in the distribution of black pepper populations.

Morphological characterisation based on quantitative traits

The quantitative traits of the evaluated black pepper germplasm are shown in Table 3. Summarising these quantitative characters using descriptive statistics revealed a wide range of variability in lateral branch length, number of well-developed fruits per spike, number of spikes per 30 cm^2, number of spikes per vine and bulk density. The vine height of the selected genotypes ranged from 4.10 m in G9 to 6.10 m in G18 and the vine column diameter ranged from 0.53 cm to 0.94 cm. The lateral branch length of the selected genotypes varied from 27.25 cm to 65.20 cm with a standard deviation (SD) of 10.90. The number of nodes per lateral branch ranged from 5.2 to 24.2. The length of the petiole varied from 1.52 cm to 3.51 cm. The length of the leaves ranged from 8.5 cm to 17.25 cm, and the leaf width ranged from 5.2 cm to 15.5 cm. The spike peduncle length ranged from 0.58 cm to 2.08 cm. Spike length varied from 6.5 cm to 17.5 cm.

Table 3. Variation of quantitative characters observed among genotypes.

Sl. No.	Characters	Mean	Standard deviation	Minimum	Maximum
1	Vine column height (m)	5.05	0.52	4.20	6.10
2	Vine column diameter (cm)	0.69	0.11	0.53	0.94
3	Lateral branch length (cm)	40.83	10.90	27.25	65.20
4	Number of nodes per lateral branch	13.89	6.35	5.20	24.20
5	Leaf petiole length	2.45	0.52	1.52	3.51
6	Leaf length	13.24	3.03	8.10	17.41
7	Leaf width	8.88	3.01	5.20	15.50
8	Spike peduncle length (cm)	1.17	0.43	0.58	2.08
9	Spike length (cm)	10.89	2.89	6.5	17.5
10	No. of well-developed fruits per spike	46.35	15.53	30.31	88.25
11	No. of spikes per lateral branch	8.19	3.09	3.24	15.7
12	No. of spikes per 30 cm^2	37.13	10.34	18.69	57.12
13	No. of spikes per vine	419.14	64.82	303	542
14	Fresh spike yield per vine (kg)	3.35	0.41	2.3	3.85
15	Fresh berry yield per vine (kg)	2.69	0.43	1.45	3.15
16	Hundred fresh berry weight (g)	13.77	3.84	10.03	20.1
17	Hundred fresh berry volume (mL)	12.81	3.67	9.2	18.74
18	Berry diameter (mm)	4.1	0.83	2.8	5.11
19	Dry recovery (%)	33.33	1.88	30	37
20	Dry yield (kg)	0.96	0.17	0.57	1.23
21	Bulk density (g)	520.2	49.14	481	640

The number of well-developed berries per spike varied from 30.12 to 88.25, with a 15.53 SD indicating moderate variability. The number of spikes per lateral branch ranged from 3.24 to 15.7. The number of spikes per 30 cm^2 showed moderate variability, ranging from 18.69 to 57.12. There was a wide range of variability (SD = 64.82) for the number of spikes per vine, which ranged from 303 to 542. The number of spikes per lateral branch ranged from 3.24 to 15.7, the number of spikes per vine ranged from 303 to 542, fresh spike yield per vine varied from 2.3 kg to 3.85 kg, fresh berry yield per vine varied from 1.45 kg to 3.15, hundred berry weight ranged from 10.03 g to 20.10 g, hundred fruit volume ranged from 9.2 ml to 18.74 mL, bulk density ranged from 481.6 g to 640 g (SD=49.14), berry diameter ranged from 2.8 mm to 5.11 mm. In the 21 genotypes considered in this study, dry recovery ranged from 30 to 37 %, and dry yield varied from 0.57 to 1.23 kg.

Biochemical traits

All the selected black pepper genotypes were analysed for their biochemical constituents, such as piperine, oleoresin and essential oil (Table 4, Fig. 2). Considerable variations were found among the genotypes for piperine, oleoresin and essential oil content. In the present study, genotypes G16, G21, G7 and G19 had piperine content ranging from 5.6 % to 4.5 %, indicating high piperine content. The lowest piperine content was observed in G2 (3 %). The mean piperine content among the selected genotypes was 3.89 %, with a standard deviation of 0.66. In the present study, oleoresin content ranged from 6.3 % in G3 to 13.2 % in G16. G15, G19 and G17 cultivars had oleoresin contents of 10.9, 10.5 and 10.3 respectively. The essential oil content of selected genotypes ranged from 3.0 % in G3 and G8 to 4.5 % in G15, with a standard deviation of 0.39.

Table 4. Biochemical parameters of selected black pepper genotypes.

Sl. No.	Genotypes	Piperine (%)	Oleoresin (%)	Essential oil (%)
1	G1	3.7	7.50	3.20
2	G2	3.00	6.80	3.80
3	G3	3.9	6.30	3.00
4	G4	3.06	9.10	3.90
5	G5	3.9	8.50	3.10
6	G6	4.20	9.30	3.10
7	G7	4.50	7.90	3.30
8	G8	3.75	6.40	3.00
9	G9	3.40	8.40	3.90
10	G10	3.20	7.80	3.10
11	G11	3.85	6.70	3.60
12	G12	3.90	7.40	3.80
13	G13	3.40	7.80	3.80
14	G14	3.11	6.50	3.60
15	G15	4.90	10.90	4.50
16	G16	5.60	13.20	4.00
17	G17	4.21	10.30	3.70
18	G18	3.90	7.10	3.80
19	G19	4.50	10.50	3.70
20	G20	3.60	8.90	3.20
21	G21	5.20	10.30	3.40
SE (m)		0.15	0.40	0.09
SD		0.66	1.81	0.39
CV		0.17	0.21	0.11

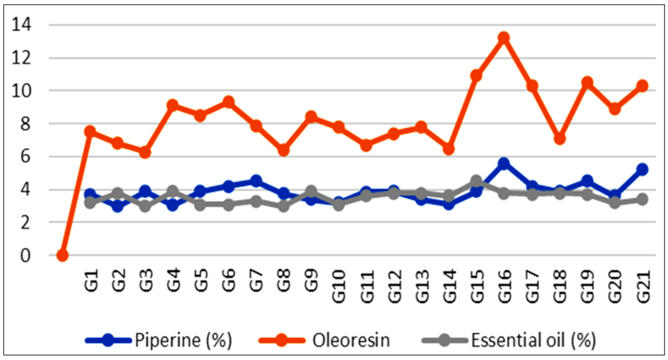

Fig. 2. Biochemical variation among black pepper cultivars.

Physiological parameters

Physiological parameters include leaf thickness, relative water content, epicuticular wax, specific leaf area and stomatal density (Table 5). Leaf thickness of the selected black pepper genotypes ranged from 0.21 mm in G8 and G10 to 0.35 mm in G16, with a standard deviation of 0.04. Genotypes G16, G13 and G18 had high leaf thicknesses of 0.35 mm, 0.34 mm and 0.33 mm respectively. Relative water content ranged from 91.54 to 98.41 %, with a standard deviation of 1.92. Relative water content was highest in G16 and lowest in G2. The relative water content of G13 was 97.7 %, followed by G6, which was 97.11 %. The epicuticular wax of the selected genotypes ranged from 1.50 mg cm^{-2} in G4 to 1.82 mg cm^{-2} in G16. The mean epicuticular wax content was 1.63 mg $cm^{-2,}$ and the standard deviation was 0.10. The epicuticular wax recorded in G13 was 1.79 mg cm^{-2}, G20 was 1.78 mg cm^{-2} and G6 was 1.75 mg cm^{-2}. The specific leaf area of the 21 genotypes selected ranged from 160.25 cm^2 g^{-1} in G16 to 243.12 cm^2 g^{-1} in G14. The standard deviation for the specific leaf area was 28.24, indicating a wide variability among the selected genotypes for the specific leaf area. The stomatal density of the selected genotypes ranged from 8.01 to 13.5 per cm^2 area, with a mean of 11.24 and a standard deviation of 1.49. The mean stomatal density of the leaf was the lowest in G16 (8.01), followed by G13 (8.20) and G6 (9.50).

Principal component analysis

Approaches using multivariate analysis help identify genetic diversity and group genotypes. Among these techniques, PCA is a statistical approach for categorising multiple variables into primary uncorrelated variables. The present study conducted a multivariate analysis to summarise the variation among the selected genotypes for morphological quantitative traits, biochemical contents and physiological parameters. The results of PCA explained the genetic variation among the genotypes for all the characters under study. PCA based on 29 traits revealed that the first nine PCs accounted for an eigenvalue more significant than one (86.59 %) of the total variant amongst cultivars of black pepper (Table 6).

Table 5. Physiological parameters of selected black pepper genotypes.

Sl. No.	Genotypes	Leaf thickness (mm)	Relative Water Content (%)	Epicuticular wax (mg/ cm²)	Specific Leaf Area (cm²/ g)	Stomatal density (No/ cm²)
1	G1	0.28	92.23	1.52	210.45	11.2
2	G2	0.26	91.54	1.51	198.52	12.4
3	G3	0.24	92.10	1.61	200.3	13.5
4	G4	0.29	93.5	1.50	242.14	10.58
5	G5	0.28	92.2	1.70	213.25	11.6
6	G6	0.31	97.11	1.75	167.56	9.5
7	G7	0.30	94.0	1.68	190.14	10.2
8	G8	0.21	93.52	1.64	216.89	11.6
9	G9	0.26	92.79	1.61	240.13	12.5
10	G10	0.21	94.7	1.54	227.3	11.6
11	G11	0.26	93.8	1.57	218.6	11.4
12	G12	0.27	92.6	1.60	230.78	12.4
13	G13	0.34	97.7	1.79	165.78	8.20
14	G14	0.25	94.8	1.51	243.12	13.1
15	G15	0.32	93.69	1.57	231.12	12.4
16	G16	0.35	98.41	1.82	160.25	8.01
17	G17	0.25	92.8	1.72	200.12	12.4
18	G18	0.33	97.4	1.70	168.4	10.05
19	G19	0.29	94.6	1.52	230.2	12.4
20	G20	0.31	95.4	1.78	190.56	10.1
21	G21	0.24	93.2	1.67	230.41	10.80

Out of the 8 principal components (PCs) exhibiting more than 1.00 eigenvalue, PC1 had the highest variability (20.10 %), followed by PC2 (17.36 %). The first principal component, PC1, was mainly contributed by 6 yield and yield-attributing traits including leaf petiole length (0.30), leaf length (0.33), leaf width (0.30), fresh spike yield (0.34), fresh berry yield (0.35) and dry yield (0.30). This indicates that increased leaf size and leaf petiole length contribute to black pepper's high spike and berry yield. Thus, improving a given yield trait will direct the improvement of other yield-attributing characteristics collected in the same PC. The second principal component, PC2, was primarily contributed by 3 physiological traits: leaf thickness (0.30), relative water content (0.30) and epicuticular wax (0.31). However, specific leaf areas showed a more negative contribution (-0.25) in PC2. This suggests an increase in drought tolerance attributable to traits such as leaf thickness, relative water content and epicuticular wax due to the decrease in specific leaf area of black pepper. The PC3 contributed to 15.66 % of the total variability and was mainly attributed to spike length, number of spikes per laterals, number of spikes per 30 cm^2 and number of stomata. Principal component 4 contributed 10.35 % to the total variability and was mainly attributed to well-developed berries per spike, oleoresin and essential oil with their negative loadings. The PC5 described a contribution of 7.75 % to the total variability, illustrated primarily by the spike peduncle length with its favourable loading and negative loadings with lateral length, number of laterals, hundred berry weight and hundred berry volume. Principal component 6 contributed 6.25 % to the total variability, mainly ascribed to vine height. The PC7 contributed 4.81 % to the total variability and was depicted primarily to the number of spikes per vine. Principal component 8 contributed 4.32 % to the total variability. The variation in principal component 8 was mainly attributed to vine diameter with favourable loading and spike peduncle length with negative loading. When using these characters in a breeding program, it is possible to consider that crucial characters that tend to stick together in different PCs prefer to do so, resulting in a rapid improvement in yield and other associated qualities. The first 4 PCs exhibited high variation for the yield-attributing traits, physiological parameters and biochemical traits under study and therefore, black pepper improvement could be accomplished by considering these traits. The biplot gathered variables as vectors, with the relative length of the vector representing the degree of variability in each variable and the genotypes as scores. The genotypes farthest from their source had more diversity and less similarity to other types. The PC1 and PC2 determined that the cultivars are scattered each quarter and showed the highest genetic diversity in evaluating cultivars (Fig. 3).

Traits such as leaf petiole length, leaf length, leaf width, fresh spike yield, fresh berry yield, dry yield, leaf thickness, epicuticular wax, spike length, number of spikes per vine, hundred berry weight, hundred berry volume and berry diameter were well represented with a high amount of variability compared to others, while the number of spikes per laterals, spike peduncle length and essential oil showed the lowest variability. Among these, leaf length,

Table 6. Component loadings of studied traits on eight PCs.

Variables	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Vine height	-0.04	0.191	-0.168	0.047	0.031	0.45	-0.331	0.218
Vine diameter	0.11	-0.162	-0.151	0.172	0.181	0.051	0.14	0.512
Lateral length	-0.027	0.144	0.223	-0.017	-0.417	-0.097	0.283	-0.05
No of nodes	0.004	0.238	0.173	0.011	-0.407	0.177	0.111	0.154
Petiole length	0.30	-0.04	0.021	-0.075	-0.156	0.273	-0.18	0.156
Leaf length	0.334	-0.069	-0.043	-0.106	-0.097	-0.105	0.143	0.094
Leaf width	0.304	-0.161	0.03	-0.105	0.19	0.092	0.113	0.062
Spike peduncle length	-0.032	-0.053	0.179	0.123	0.316	0.072	-0.069	-0.533
Spike length	0.21	-0.028	0.31	-0.299	-0.068	0.206	-0.057	-0.139
Well developed berries per spikes	0.21	-0.071	0.076	-0.336	0.212	0.096	0.022	-0.203
No. of spikes per lateral branch	0.037	0.131	0.364	0.243	0.032	-0.058	-0.01	-0.008
No. of spikes per vine	0.132	0.213	0.2	0.17	0.18	-0.037	0.406	0.184
No. of spikes per 30 cm^2	0.035	0.167	0.336	0.196	0.203	0.04	-0.074	0.101
Fresh spike yield	0.34	0.078	0.178	0.083	0.078	-0.068	-0.165	0.027
Fresh berry yield	0.35	0.139	0.135	0.07	0.073	-0.094	-0.116	-0.019
100 berry weight	0.231	-0.194	-0.084	0.212	-0.302	0.14	-0.027	-0.183
100 berry volume	0.235	-0.178	-0.067	0.224	-0.306	0.143	-0.012	-0.196
Bulk density	-0.155	0.248	0.119	-0.185	0	0.134	-0.359	0.11
Berry diameter	0.142	-0.221	-0.233	0.033	-0.03	-0.126	-0.005	0.102
Dry yield	0.30	0.156	0.122	0.135	0.006	-0.255	-0.233	-0.013
Dry recovery	-0.007	0.25	0.124	-0.3	-0.128	0.236	0.214	-0.024
Leaf thickness	0.145	0.30	-0.202	-0.01	-0.058	-0.336	-0.092	0.035
Relative water content	-0.019	0.30	-0.232	0.081	-0.079	-0.177	0.144	-0.092
Epicuticular wax	0.064	0.31	-0.194	0.097	0.012	0.226	0.082	-0.276
Specific leaf area	-0.001	-0.25	0.277	-0.246	-0.084	-0.061	0.181	0.036
Stomatal density	-0.125	-0.19	0.333	-0.13	0.023	0.038	-0.051	0.177
Piperine	0.169	0.19	-0.155	-0.076	0.193	0.204	0.17	0.111
Oleoresin	0.088	0.18	-0.185	-0.333	0.211	-0.067	0.231	-0.084
Essential oil	0.05	0.07	-0.009	-0.368	-0.14	-0.38	-0.331	0.069

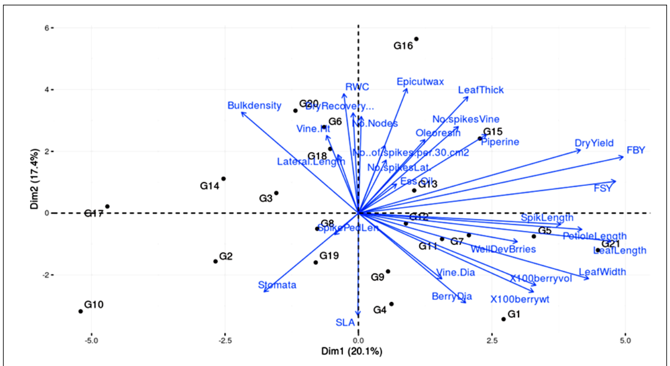

Fig. 3. Biplot distribution of 21 black pepper cultivars and studied traits depending on principal component axes PC1 and PC2.

leaf width, leaf petiole length and spike length, fresh spike yield per vine, fresh berry yield per vine and dry yield, oleoresin and piperine, hundred berry weight and hundred berry volume and number of spikes per vine and number of spikes per 30 cm^2 were strongly correlated with each other. Quality traits like piperine and oleoresin had less variability than yield and yield-related traits and physiological parameters. The number of stomata and specific leaf area were in the negative direction, indicating these 2 as the least desirable characters. From the biplot, it can be concluded that the genotypes G16, G15, G21, G1, G5 and G4 were promising and genetically divergent. Genotypes such as G10, G17, G14, G2 and G20 were genetically divergent, but these were in negative quadrants, indicating less desirability in the breeding program. Variability of cultivar 16 was contributed by epicuticular wax, relative water content, leaf thickness, oleoresin and number of spikes per vine, revealing it as a drought-tolerant, high-yielding cultivar with high oleoresin content. Cultivar 15 was influenced by piperine, dry yield, number of spikes per vine and oleoresin, indicating its high dry recovery combined with high oleoresin content, making it the most desirable cultivar. Variability in cultivar 21 was mainly contributed by leaf petiole length, leaf length, leaf width and spike length. Thus, this cultivar is vigorous, with large leaves and long spikes. The most important characteristics contributing to variability in cultivar 1 were berry weight, volume and diameter, indicating its bold berry traits. Variability in cultivar 5 was attributed to well-developed berries per spike, a desirable attribute in crop improvement. The high variability of cultivar 4 was due to specific leaf area and berry diameter. Drought tolerance combined with bold berries fetches more importance in black pepper improvement.

Discussion

Based on morphological differences, leaf, inflorescence and fruit are essential for differentiating black pepper cultivars (17). Significant variability among the black pepper landraces based on plant morphological characters was reported (18), granting them the status of different plant types, each with distinctive traits. The morphological variations exhibited by the landraces were stable and determined genetically. This study has revealed pertinent comparisons among black pepper cultivars in Kerala concerning vegetative and reproductive characteristics. The cultivars used in this study exhibited moderate variability. The similarity across the black pepper populations could be due to the chance crossing of cultivars through generations of co-existence in the wild, sharing common support trees in the natural habitat, and fixation due to successful vegetative propagation. The runner shoot tip colour of black pepper is an essential flag characteristic of black pepper cultivars and it was discovered to be regulated by 2 sets of genes having complementary actions. In the present study, the shoot tip colour of runner shoots was observed as light green, light purple and dark purple; the majority had light purple (76.19 %). Similar results (19) with 50 black pepper accessions were reported, where most accessions (66 %) exhibited light purple shoot tips. The lateral branch pattern refers to the orientation of the lateral branch for the horizontal axis. The erect branching type is considered the most efficient for trapping solar energy by leaves. However, in the present study, most cultivars had horizontal branch habits. The lateral or plagiotropic branch grows in a sympodial pattern, the apical bud develops into an inflorescence and the growth is perpetuated by the activity of the axillary bud (20). In this study, most cultivars produced long lateral branches with few nodes.

Usually, leaf characters are identified as a significant feature for cultivar identification in black pepper. The shape and size of the leaf lamina vary significantly among the cultivars (20). The genetics of leaf shape in black pepper are of 3 fundamental shapes: cordate, ovate and oblong elliptical, all governed by multiple alleles. There was some variation in these basic classes in the present study, for example, lamina shapes such as cordate, ovate, ovate-lanceolate and ovate-elliptic. Leaf base shapes were acute, round and cordate. It was reported oval, ovate-elliptical, ovate-lanceolate, cordate and elliptic-lanceolate leaf shapes and acute, round and cordate leaf bases in black pepper (19). The leaf margin had 2 variations, namely even and wavy. Spike variations such as spike length, floral composition, fruit number and size and spike twisting (20) are reported in black pepper. The arrangement of berries on the spike was compact in most of the cultivars studied. The high-yielding improved varieties such as "Panniyur-6" (clonal selection of landrace "Karimunda") and "Panniyur-7" (an open-pollinated progeny of landrace "Kalluvally") were also described as having compact spike setting (21).

Almost all the cultivars of black pepper have a round fruit shape, except for 2, which have an ovate fruit shape. Oval-shaped fruit only in one accession (22), similar to *P. attenuatum*, a wild relative of *P. nigrum*. Round and bold berries fetch more in international markets. Fruit size is a practical selection criterion in black pepper (10). Even though cell expansion rather than cell division is more important for fruit growth from the ovary to the mature fruit, fruit size differences among the cultivars are primarily caused by cell number. Fruit shape and size, although strongly associated, are less proper in cultivar delimitation, except in the case of cultivars with clearly defined characteristics, such as the oblong fruit shape of "Karivilanchy". The maturation period of pepper varies from 5 to 6 months in Indonesia and 7 to 8 months in India. The recent advances in product diversification have necessitated harvesting the berries at different maturity stages to meet the end product's specific needs (22). Most of the cultivars in this study were medium in terms of days to harvest maturity. The observed morphological variations were likely due to genetic and growing environmental effects.

Diversity analysis is essential in determining the genetic associations within available genetic resources. As a result, estimating the genetic diversity among genotypes has become necessary for locating superior genetically diverse parents who also possess desirable traits. Morphological traits have long been used to investigate interactions between plant genotypes and estimate genetic diversity (23). The application of cluster analysis in taxonomic studies has previously been reported in *Piper* species. In the present study, cultivars were distributed randomly into different clusters based on morphological traits. Among the 15 clusters formed at a similarity coefficient of 0.73, two (III and VI) are relatively large, holding 3 members. The distribution of various characters among the cultivars shows that a considerable degree of solidarity exists in each cluster. This grouping pattern could be caused by differences in genetic makeup and the continuous influence of environmental conditions. Also, cultivars belonging to different geographic origins were included in the same clusters. This might have been due to the free exchange of propagating materials from one place to another. Two cultivars, *viz*., G2 and G6, showed 81 % similarity, suggesting their common genetic origin or similar genetic makeup. Cultivar G9 showed maximum diversity (55 % similarity) due to the dark purple colour of the runner shoot tip, semi-erect branching pattern, broad and cordate leaves, bold berries, low bulk density and late maturity. Genetic divergence among genotypes has practical applications in breeding programs, as choosing relatively divergent parents will maximise the frequency of progeny with superior characteristics. In a study on cluster analysis of 44 cultivars and 7 wild black pepper accessions using 22 morphological characters, 11 clusters were identified. “Karimunda”, “Panniyur 1”, “Vadakkan” and “Kuthiravally” were the cultivars that were unique and did not cluster with any other cultivars (24). The presence of 28 cultivars in one category demonstrates that most common cultivars closely mimic one another and most likely have a common origin. In a study, 50 accessions were grouped based on morphological similarity. The hierarchical cluster analysis showed that 35 out of 50 black pepper accessions clustered in a single group, which indicated the absence of significant morphological divergence among them (19).

Black pepper can reach a height of 10 m or more. The mature vine has a columnar appearance and a height of around 4 m when limited. In the present study, the vine length ranged from 4.20 m to 6.10 m. A variation in the vine length of 3 years old black pepper accessions, ranging from 1.6 m to 3.95 m was reported (19). Variations in vegetative characters such as vine column height, leaf weight, leaf width and internodal length among the 10 black pepper accessions were studied (25), of which “Karimunda” had the shortest leaf length and width. Moderate variability in the lateral branch length and wide variation in number of nodes per lateral branch were also observed (19). The ideal characteristics of black pepper indicate leaf length ranges from 10 to 12 cm, leaf width ranges from 7 to 8 cm and petiole length is between 1.2 and 1.5 cm at the top (26). However, the leaf size may be varied from 8 to 20 cm in length and 4 to 12 cm in width or longer (24). The leaf characteristics of pepper cultivars evaluated in this study showed wide variations. Some leaves had petiole lengths longer than 3 cm were also noted. Studies also reported low intraspecific variability for leaf petiole length and significant intraspecific variability for leaf length and leaf breadth (18). Wide variability for leaf petiole length and less variability for leaf length and width were also reported (19).

The spike peduncle length of selected genotypes ranged from 0.58 cm in G13 to 2.08 cm in G9, with a mean value of 1.17 cm and a standard deviation of 0.43, indicating less variability for the trait. Low intraspecific variability for spike peduncle length was noted (18). a peduncle length variation from 0.76 cm to 1.58 cm with a CV value of 14.87, indicating less variability was reported (19). In this study, black pepper cultivars differed in their spike length, which ranged from 6.50 cm in G3 to 17.5 cm in G15. Cultivars with increased spike length generally will have more berries per spike. Spike length is a significant yield-contributing character in black pepper and is thus amenable to selection (26). In the present study, the number of well-developed berries per spike varied from 30.12 in G8 to 88.25 in G21. According to (24), the number of berries per spike in black pepper ranges typically from thirty to less than a hundred, depending on the variety or hybrid. Pollination, water and nutrient availability and pest and disease attacks during the initial berry development period influence berry number per spike. Failure of berry set or undeveloped ovules on pepper varieties has been associated with insufficient pollination, unfertilised flowers or imperfect fertilisation and loss of stigma receptivity before pollination either singly or as combination of these factors (27). Significant variations in spike length, berry size and berries among all genotypes were reported (1). “Karimunda” was found to have a higher number of laterals with more spread and a higher number of spikes (28). High fresh berry weight in “Karimunda” and “Panniyur 1” were recorded (25). Similarly, a significant variation in hundred fruit weight and a wide variation in hundred fruit volume were also observed (19). Bulk density ranged from 481.6 g in G1 to 640 g in G17, with a mean of 520.20 in the present study, indicating a highly variable trait with a standard deviation of 49.40. An increase in bulk density with an increase in berry size was noted in similar studies (29). It was found to decrease when the berry size was above 4.8 mm. Bulk densities of dried berries of “Panniyur 1” collected from different locations ranged from 460.60 g to 608.70 g (30). Significant variation in berry size was reported (1). The variation in vegetative, inflorescence and yield characteristics among black pepper types grown in Kerala may be attributed to several factors, such as differences in genetic makeup, edaphic factors and the influence of environmental conditions. The productivity of black pepper depends on elevation, soil fertility, cultural practices, temperature, rainfall, age of the crop and climatic conditions during flowering, fruit set and development. Other findings (8, 31) also showed that yield in pepper is influenced by many factors, including soil fertility, cultural practices and the age of the crop. In this study, we focused on cultivars selected from the Southern Western Ghats, which share a similar tropical humid climate. This uniformity in climatic conditions suggests that the observed variability is likely due to genetic differences among the cultivars. However, the lack of soil analysis represents a limitation in the present study, as soil fertility is a significant factor affecting yield. Growing the germplasm collections at our institute to validate the genetic basis of the observed variability is highly recommended. This will minimize environmental influences, helping to confirm if the differences in vegetative, inflorescence, yield and biochemical characteristics are primarily due to genetic factors.

The quality of black pepper is as vital as yield and is attributed to the content of secondary metabolites such as piperine, essential oil and oleoresin. Piperine is primarily attributable to pungency (32) and oleoresin is the commercial spice flavour of black pepper. This spice is known for its aroma, which is generated by volatile oil. The production of these metabolites is controlled by genetics and fluctuations in ecosystem balance, such as seasonality and pest and disease activity (33, 34).

The properties of spices, such as flavour, colour and pungency, vary among cultivars and varieties. India has been exploring the black pepper cultivars available in the country, especially the main producing areas in the state of Kerala. Current research investigated all aspects with a particular focus on high productivity, quality and biotic and abiotic resistance. The present study investigated the extent of quality variation regarding piperine, essential oil and oleoresin content among the popular cultivars in Kerala's major black pepper growing areas. The genotypes G7, G15, G16, G19 and G21 had high piperine content ranging from 5.6 % to 4.5 %. Biochemical characterisation of black pepper accessions (35) identified 2 accessions with high piperine content, *viz*., 7293 with 6.96 % and 7252 with 6.71 %. Genotypes rich in bioactive constituents, such as piperine, could be explored in breeding programs and used in pharmaceutical industries for their various pharmacological properties.

The essential oil content of selected genotypes ranged from 3.0 in G3 and G8 to 4.5 % in G15, which was less than the oil content of “Sreekara” and “Subhakara” (> 6 %) (7). G7, G9, G15 and G16 had high essential oil contents. A volatile oil range of 3.0 % to 5.87 % among 20 black pepper accessions studied (35). Volatile oil and oleoresin content variability in 14 black pepper accessions, ranging from 2.7–5.1 % and 7.6–9.4 % respectively (36). Oleoresin is the commercial spice flavour of black pepper. In the present study, oleoresin content ranged from 6.3 % in G3 to 13.2 % in G16. Genotypes G15, G16, G17, G19 and G21 had a high level of oleoresin. However, all the genotypes in the experiment expressed values lower than the maximum recorded value of oleoresin (15.45 %) of the commercial variety PLD2. Indian cultivars “Kottanadan,” “Kumbakodi,” “Kuthiravally” and “Nilgiri” had high piperine and oleoresin content, whereas “Balankotta,” “Kaniyakadan” and “Kumbakody” had high essential oil content (7). The metabolic profiling of black pepper genotypes grown in Sikkim was analysed and variations of volatile oil ranging from 2.01 % to 0.02 % were reported (37). Also, the piperine ranged from 2.75 to 0.02 % 24 black pepper genotypes collected from south Sikkim. In the present study, it was evident that the genotypes G15 and G16 were better for quality traits.

Drought is the most adverse environmental factor regarding the growth and productivity of black pepper cultivars. Thus, a preliminary study was performed on naturally developed physiological characteristics that withstand adverse climatic conditions. All the genotypes evaluated in this study were found in natural ecosystems without much human care. Physiological parameters such as leaf thickness (38), specific leaf area (39), epicuticular wax (40), relative water content (11) and stomatal density (41) are associated with drought response. In this study, cultivars G6, G13, G16 and G18 showed reduced specific leaf area, indicating a strategy of acclimatisation to water stress with tremendous potential for maintaining crop productivity. The leaf area is extremely sensitive to soil water conditions, particularly in more susceptible varieties (42) and water stress affects the photosynthetic process and as a result, biomass production (43). Reducing specific leaf area in plants under water stress indicates higher leaf thickness, which may help maintain water in the leaf tissue, allowing a more favourable response to drought (39). Drought tolerance is associated with increased leaf thickness (44). The most significant leaf thickness was observed in G6, G13, G15, G16, G18 and G20. The cultivars differed in their relative water content. Leaf relative water content (RWC) indicates a plant's water status since it depicts the balance between the water supply to the leaf tissue and the transpiration rate (45). RWC has been used to evaluate black pepper cultivars (11, 46, 47). Three cultivars, *viz*., G6, G13 and G16, had RWC greater than 97 %. Landraces that grow in arid areas for years have a higher RWC throughout the drought stress. Among the cultivars studied, 3 cultivars, *viz*., G6, G13 and G16, are grown in areas that experience some degree of water deficit stress during summer. Epicuticular wax, a waxy coating, is an active interface between the plant and the external environment. It shields the plant against various abiotic and biotic stresses (47). The epicuticular wax of the selected genotypes ranged from 1.50 mg cm^{-2} in G4 to 1.82 mg cm^{-2} in G16. Accessions G6 (1.75), G13 (1.79), G16 (1.82) and G20 (1.78) showed the highest epicuticular wax content. Plant drought tolerance is often related to their morphological traits. Among the morphological characters, stomata are specialised epidermal structures that control water and carbon dioxide exchange between the plant and the atmosphere. Water use efficiency depends on stomata regulation. Stomata density and stomata opening modulation can control the transpiration rate. Water stress resistance could be obtained by reducing the frequency and size of stomata (48). In the present study, the number of stomata per cm^2 varied from 8.01 to 13.50. Low stomatal density genotypes were G6, G13 and G16. Genotypes with low stomatal density can conserve more water; thus, these can be considered drought tolerant. Evaluation of the physiological traits revealed 3 cultivars, *viz*., G6, G13 and G16, as the most drought-tolerant types, which could be further studied and preserved for breeding programs. Moreover, enhanced drought tolerance was observed in black pepper cultivars with less vigorous canopy, producing lower foliage biomass.

Studying genetic variation is especially crucial for properly maintaining, evaluating and using accessions since germplasm is the only source that can be utilised to investigate novel varieties during breeding projects. Conserving the genetic diversity of cultivars and their wild relatives is the goal of germplasm collection. This study revealed significant genetic diversity among the black pepper cultivars from Kerala, following the findings of similar study (18). The findings of the present study provide more comprehensive insights into genetic diversity than previous studies assessing morphological, biochemical and physiological traits. PCA is used to measure the influence of each trait on overall variation. PCA based on 29 traits extracted nine PCs with eigenvalues of more than 1. These components explained 86.59 % of the total variance. Investigations have revealed the relative contributions of various characters in cultivar differentiation and identified 8 PCs as the most important, accounting for 75 % of the total variation (24). They had leaf size index, leaf length and leaf breadth with high loading in PC1 and leaf thickness, lower epidermal thickness and upper epidermal thickness in PC2. Principle component analysis in black pepper using 27 morphological characteristics explained 95.79 % of the total variance with 7 PCs (17). In the present study, variability was mainly contributed by leaf petiole length, leaf length, leaf width, fresh spike yield, fresh berry yield, dry yield, leaf thickness, relative water content, epicuticular wax, specific leaf area, spike length, number of spikes per laterals, number of spikes per 30 cm^2, number of stomata, well-developed berries per spike, oleoresin, essential oil, spike peduncle length, lateral length, number of spikes per laterals, hundred berry weight and hundred berry volume, vine height, number of spikes per vine and vine diameter. A strong influence of leaf width, spike length and berry diameter on the grouping of black pepper cultivars were identified by researchers (17). High variation in yield has been reported in black pepper (25). Higher the value of coefficients for the direction (positive or negative), the more efficient they will be in distinguishing the genotypes (49). The traits such as leaf petiole length, leaf length, leaf width, fresh spike yield, fresh berry yield, dry yield, leaf thickness, epicuticular wax, spike length, number of spikes per vine, hundred berry weight, hundred berry volume and berry diameter had a high amount of variability. In contrast, number of spikes per laterals, spike peduncle length and essential oil content showed less variability, indicating that these 2 parameters are less important in cultivar selection. In this study, leaf size and spike length were highly correlated, indicating that the greater the leaf size, the longer the spikes. A positive correlation between leaf length and width was observed by researchers in their study (25). Increases in photosynthetic areas, such as those in leaves, have been reported to boost agricultural yield.

The present study found a strong correlation between fresh spike yield per vine, fresh berry yield per vine and dry yield, indicating that the higher the number of spikes, the more berries they produce, contributing to a high dry yield per plant. Black pepper's fresh and dried yields was reported to have a significant positive correlation (36). Oleoresin content was positively correlated with piperine, confirming that piperine is one of the components of oleoresin. This suggests that concurrently improving these characters by a simple selection program is the best tool for improving quality traits in black pepper. Hundred-berry weight and hundred-berry volume were positively correlated, as supported by other researchers (25). An increase in spikes per 30 cm^2 increased the total number per vine. Therefore, a selection program based on the number of spikes per 30 cm^2 would significantly improve the yield of black pepper. Quality traits like piperine and oleoresin had less variability than yield and yield-related traits. Similarly study on less variability in oleoresin was also reported (36). The number of stomata and specific leaf area were negative in this study. Low stomatal density and reduced leaf area (39) can conserve water, allowing the plants to survive better under drought-stress conditions. Genotypes G16, G15, G21, G1, G5 and G4 were considerably more isolated from the rest and thus genetically divergent. They were found promising as good candidates for black pepper improvement in their respective superior traits. Cultivar 16 was identified with high epicuticular wax, relative water content, leaf thickness, oleoresin and number of spikes per vine. Comparatively, higher RWC (11) and leaf thickness (38) are associated with plant drought tolerance. Thus, it can be concluded that cultivar 16 could provide a good source of genes for high yield, quality and drought tolerance. Cultivar 15 was influenced by piperine, dry yield, number of spikes per vine and oleoresin. This local cultivar may be a prime candidate since it produced many spikes per vine with high dry recovery and good quality attributes. The number of fresh pepper berries produced was strongly inversely related to the number of spikes, with no significant relationship between yield and the number of well-developed berries per spike. On the contrary, the number of berries per spike is the most important morphological trait with a direct and positive effect on black pepper productivity identified by researchers (8). Variability of cultivar 21 was contributed mainly by leaf petiole length, leaf length, leaf width and spike length and was found to be a high-yielding one. Since the leaf lamina is the major photosynthetic organ of the plant to intercept sunlight, the productivity of a plant depends on its leaf surface area. This reveals that greater leaf areas and spike length contribute to better cultivar yield. However, this does not corroborate with some findings (17), in which leaf areas did not contribute to better cultivar yield, even though flower intensity per spike, inflorescence length, and fruit spike has positive relationships with fruit sizes. The bold berry trait of Cultivar 1, denoted by its hundred-berry weight, hundred-berry volume and berry diameter, explains its high-yielding behaviour.

Conclusion

Due to their simplicity, agro-morphological traits related to yield are commonly used as a preliminary evaluation tool. A wide range of diversity was observed in black pepper cultivars in terms of lateral branch length, number of well-developed fruits per spike, the number of spikes per 30 cm^2, number of spikes per vine and bulk density. Significant variability exists in the germplasm and associated traits, creating scope for additional enhancement in crop improvement programs for black pepper. Genotypes G16, G15, G21, G1, G5 and G4 were identified as promising and genetically divergent. The results from this study indicate that many cultivars possess potential characteristics that can be utilized to improve black pepper production in the country. The study revealed that genotypes 1, 4, 5, 15, 16 and 21 were better in terms of yield parameters, 15 and 16 for quality traits and 6, 13, 16 and 18 for physiological aspects. This information from the present research can be translated for black pepper germplasm conservation, categorisation and future breeding as well as for developing regional-specific superior varieties. Furthermore, future research will focus on molecular analysis to gain a deeper understanding the genetic basis of these traits, enhancing the precision of breeding programs.

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Authors' contributions

RP- wrote the manuscript and carried out the experiments with the help of SGS, DSN and RS, SGS- planned the experiment

Compliance with ethical standards

Conflict of interest: The authors have no conflict of interest.

Ethical issues: None.

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