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RESEARCH ARTICLE

Morphological Characterization of Black Pepper (*Piper nigrum* L) Accessions from Kerala

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Fifty accessions of black pepper maintained at the National Active Germplasm Site of ICAR-IISR, Kozhikode, Kerala were characterized to assess variability among the accessions for qualitative and quantitative characters. Majority of the accessions had dimorphic branching habit, light purple shoot tip colour, many runner shoots, weak holding capacity, few adventitious roots, horizontal lateral branching habit, ovate leaf lamina, round leaf base shape and round fruit shape. Five accessions produced more than 1.5 kg dry pepper. Clustering based on qualitative characters grouped the accessions into 4 clusters. Multivariate hierarchial cluster analysis based on quantitative traits grouped the accessions into five major clusters. Accession IC598883 remained as a single cluster with very high yield and tall vine. This accession was proposed to be used for further evaluation to release as a commercial variety.

Key Words: Black pepper, Morphology, Qualitative traits, Quantitative traits, Genetic diversity

Introduction

Black pepper (*Piper nigrum* L.), the most important spice crop in the world originated in the humid tropical forests of Western ghats of South India and is being grown in more than 25 countries. Indian pepper is preferred across the globe due to its intrinsic qualities. Selection over the years from the wild has resulted in cultivars with difference in morphology and yield (Ibrahim *et al*., 1985). Landraces, natural mutants, improved varieties and even true seedlings constitute the primary gene pool of black pepper (Sasikumar *et al*., 2007). Different methods of breeding like clonal selection, open pollination and hybridization have been utilized for development of improved pepper cultivars. (Krishnamoorthy and Parthasarathy, 2010). Intra-cultivar or inter varietal variability has been observed for morphological and qualitative characters (Ratnambal *et al*., 1985). Amenability of the species for sexual reproduction in combination with vegetative propagation has resulted in conservation of variability to a considerable extent (Dewaard and Zeven, 1969). Polyploidy observed in a few collections is also a reason for wide variability noted in the populations.

Indian Institute of Spices Research (ICAR-IISR), Calicut is the nodal agency in India for conserving genetic resources and persuing research on black pepper. The germplasm conservatory of IISR consists of 1075 wild, 1282 cultivar accessions and nine exotic species, besides 1375 hybrids (www.spices.res.in). Characterization of germplasm of high-value crops like spices is important for protection of bio wealth. Hence, morphological characterization of black pepper accessions was taken up with the objective of identifying superior black pepper germplasm for further breeding programmes.

Materials and Methods

Fifty accessions of three year old black pepper in bearing stage, recently collected from farmer's field from various parts of Kerala, formed the material for the study (Table 1). Accessions collected from farmers field were named landraces and those collected from wild were not named. Plants were maintained by contour planting as single plant accessions. They are maintained in the fields of IISR at a spacing of 3m x 3m under recommended management (Fig. 1). Observations were recorded on 15 qualitative and 17 quantitative traits of vine, leaf, spike and fruit (Table 2) as per standard descriptors

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Table 1. Passport details of Kerala accessions of black pepper used for the study

Sl. No.	Genebank Accession No.	Place of collection	District	Latitude/longitude
1	IC598866	Meenmutty	Wayanad	11.43.57/75.52.46
2	IC598869	Mayyil-Velam	Kannur	11.99.79/75.44.92
3	IC598872	Mayyil-Velam	Kannur	11.99.79/75.44.91
4	IC598873	Mayyil-Velam	Kannur	11.99.79/75.44.94
5	IC598874	Chapparakkunnu	Kannur	11.86.07/75.41.40
6	IC598875	Chapparakkunnu	Kannur	11.86.07/75.41.40
7	IC598877	Chapparakkunnu	Kannur	12.12.25/75.49.42
8	IC598880	Vellora	Kannur	12.12.25/75.49.42
9	IC598881	Vellora	Kannur	12.07.35/75.30.16
10	IC598883	Vellora	Kannur	12.07.35/75.30.11
11	IC598887	Kakkara	Kannur	12.07.35/75.30.16
12	IC598888	Kakkara	Kannur	12.07.35/75.30.16
13	IC598889	Kayapoil	Kannur	11.86.07/75.41.40
14	IC598890	Kayapoil	Kannur	12.92.25/79.13.90
15	IC598891	Kayapoil	Kannur	11.99.36/75.45.09
16	IC598893	Kakkara	Kannur	11.72.56/76.11.04
17	IC598899	Ammankad	Kannur	12.51.03/75.20.14
18	IC598901	Ammankad	Kannur	12.51.03/75.20.14
19	IC598902	Oduvally	Kannur	12.51.03/75.20.13
20	IC598903	Naduvil	Kannur	11.40.70/75.98.76
21	IC598904	Naduvil	Kannur	11.40.70/75.98.76
22	IC598905	Mandalam	Kannur	11.39.93/75.95.60
23	IC598906	Mandalam	Kannur	11.39.93/75.95.55
24	IC598907	Mandalam	Kannur	11.99.36/75.45.09
25	IC598909	Mandalam	Kannur	11.86.07/75.41.40
26	IC598911	Mandalam	Kannur	11.86.07/75.41.40
27	IC598920	Kattippara	Calicut	12.92.25/79.13.90
28	IC598921	Kattippara	Calicut	11.86.07/75.41.40
29	IC598927	Kattippara	Calicut	12.07.35/75.30.16
30	IC598929	Kodencherry	Calicut	11.86.07/75.41.40
31	IC598930	Kodencherry	Calicut	12.92.25/79.13.90
32	IC598932	Koodathai	Calicut	11.86.07/75.41.40
33	IC598933	Koodathai	Calicut	11.99.36/75.45.09
34	IC598936	Koodathai	Calicut	11.39.93/75.95.60
35	IC598938	Kodencherry	Calicut	11.40.70/75.98.76
36	IC598939	Kodencherry	Calicut	11.40.70/75.98.76
37	IC598940	Norramthodu	Calicut	11.23.41/75.79.55
38	IC598941	Norramthodu	Calicut	11.23.41/75.79.55
39	IC598945	Pallickal	Malappuram	9.66.43/77.09.74
40	IC598948	Pallickal	Malappuram	11.04.16/76.07.97
41	IC598949	Pallickal	Malappuram	11.14.29/75.92.20
42	IC598952	Anangapara-Pallickal	Malappuram	11.14.29/75.92.20
43	IC598967	Vettom- Tirur	Malappuram	11.14.29/75.92.20
44	IC598979	Nariampara- Kattappana	Idukki	9.84.75/76.98.09
45	IC598984	Nariampara- Kattappana	Idukki	10.90.07/75.89.71
46	IC598992	Kanjiyar- Udumbanchola	Idukki	9.66.43/77.09.74
47	IC598997	Konginikadavu- Kattappana	Idukki	9.84.75/77.11.68
48	IC599024	Santhigram-Irattayar	Idukki	9.84.75/76.98.09
49	IC599025	Santhigram-Irattayar	Idukki	9.84.75/76.98.09
50	IC599026	Santhigram-Irattayar	Idukki	9.84.75/76.98.09

Fig. 1. Field view of black pepper germplasm

(IPGRI 1995).Characterization was carried out at the field genebank of ICAR- IISR, Calicut, during the period from 2016 to 2019

Holding capacity of the vines was determined by counting number of sticky roots on young orthotropic stems. Orthotropic stems with less than five sticky roots were considered as weak and those with more than 5 sticky roots as having strong with respect to holding capacity. Vine length was measured as the vine column length from ground level to the top of orthotropic shoot using a measuring tape. The length of lateral was measured as average of 50 randomly selected laterals from the plant for every accession. The petiole length, leaf length, leaf width and spike length were measured as the average of 50 randomly selected leaf or spike. Petiole length was measured from leaves of lateral branches from base to the point of attachment with leaf blade. Leaf length was measured on mature leaves from lateral branches from base of lamina to tip. Leaf width was measured on mature leaves from laterals. The spike length was recorded on mature spikes collected randomly at the harvest and measured from tip to base using a measuring scale. Hierarchical cluster analysis was done separately for qualitative and quantitative characters using statistical package R.

Results and Discussion

Characterization Based on Qualitative Characters

Moderate variability was noticed among the accessions for 10 out of 15 qualitative characters (Table 3). The characters *viz.,* leaf margin, type of venation, spike orientation, spike shape and type of hermaphroditism were uniform in all accessions. All accessions were having pendant, light yellow, filiform spike with predominantly and bisexual flowers. Hence, these five characters were not included in further analysis. Similarity of various characters among the accessions could be due to chance crossing of accessions over generations of co-existence in the wild, sharing common support trees in the natural habitat and fixation due to successful vegetative propagation as proposed by Ravindran (1991). Selection for characters like yield and number of berries per spike of the vine could be the reason for retention of predominantly hermaphrodite nature of the accessions observed. The oval fruit shape of accession IC598890 resemble the fruit shape of *Piper attenuatum*, a wild relative of black pepper. Velayudan *et al.,* (2006) observed similar oval shaped berries among seven accessions they have studied.

The branching pattern of main shoot of majority of accessions was dimorphic. However, a few of them exhibited polymorphism also (Table 3). In majority of accessions shoot tip was light purple in colour (66%) (Fig. 2A). The shoot tip colour in black pepper might be due to the complementary factor interaction between

Table 2. Details of characters selected for morphological characterization of black pepper

Sl. No.	Vine characters	Leaf characters	Spike characters	Fruit characters
1	Vine length (m)	Petiole length (cm)	Orientation	Setting%
2	Branching habit	Length	Shape	Fruit shape
3	Shoot tip colour	Width	Colour	100 fruit weight (g)
4	Runner shoot production	Lamina shape	Length(cm)	100 fruit volume (cc^3)
5	Holding capacity	Base shape	Type of hermaphroditism	Green berry yield/plant (g)
6	Adventitious root production	Margin type	Peduncle length (cm)	Dry berry yield/plant (g)
7	Lateral branch production	Type of venation	No. of spikes/lateral branch	Driage (%)
8	Lateral branch length (cm)	–	Green spike yield/plant (g)	–
9	Number of nodes/lateral branch	–	No. of developed fruits/spike	–

Table 3. Variation among qualitative characters of 50 black pepper accessions

Character	Description	Percentage of accessions
Branching habit	Dimorphic	84
	Polymorphic	16
Shoot tip colour	Light purple	66
	Dark purple	32
	Light green	2
Runner shoot production	Many	26
	Few	56
	Absent	18
Holding capacity	Strong	38
	Weak	62
Adventitious root production	Many	40
	Few	60
Lateral branch habit	Horizontal	42
	Hanging	38
	Erect	20
Leaf lamina shape	Ovate	42
	Ovate-elliptic	28
	Ovate lanceolate	14
	Elliptic- lanceolate	4
	Cordate	12
Leaf base shape	Round	28
	Cordate	4
	Acute	68
Spike colour	Light yellow	60
	Green	38
	Greenish yellow	2
Fruit shape	Round	98
	Ovate	2

two pairs of genes as reported by Ravindran *et al*. (1992). Accordingly the light purple coloured accessions may have a genotypic constitution of $A_1a_1A_2a_2$, deep purple accessions with $A_1A_1A_2a_2$ and light green with $A_1a_1a_2a_2$ or $A_1A_1a_2a_2$.

Based on growth habits, morphological characters and biological functions, five distinct types of stem portions can be identified in the shoot system of a pepper vine (Krishnamurthy *et al.* 2000). The main orthotropic shoot has indefinite growth and produces lateral fruiting branches from the leaf axils. The accessions differed in runner shoot production from absent (0 per vine), few (<5per vine) to many (>10 per vine). More than half of the accessions had few runner shoots. and 18% of accessions did not have any runner shoots. The holding capacity of vine to the standard ranged from weak to strong. In 62% of accessions holding capacity was weak. Adventitious roots cling to the standards and help the plant to climb over the support trees. These clinging roots can develop into normal underground roots when they come in contact with soil or when stem cuttings are planted (Parthasarathy *et al*., 2007). Adventitious root production varied from few to many. In 40% of accessions many adventitious roots were found, while in 60 per cent of accessions it was less. The accessions differed in lateral branch habit as, horizontal, erect and hanging. The leaf shape in black pepper was reported to be of three basic shapes *viz.,* cordate, ovate and oblong-elliptical controlled by multiple alleles (Sasikumar *et al.,*1992). Slight variations in these basic classes are noted in the present study. In majority of accessions leaf lamina shape was oval (42%) followed by ovate-elliptical (28%), ovate-lanceolate (14%), cordate (12%) and elliptic-lanceolate (4%). The leaf base shapes recorded include acute, round and cordate (Fig. 2B). Krishnamurthy *et al.* (2000) reported that leaf characters form a major feature for cultivar identification in black pepper. The leaf size and shape on the emerging orthotropic shoots and runners differ from normal leaves found in lateral fruiting branches. The oblique shape reported in the descriptor was not observed in any of the accessions. Majority of accessions had horizontal branching habit (42%). Erect type of lateral branch habit which ensures better light penetration was observed in only 20% of the accessions. There were three types of spike colours observed among the accessions *viz.,*light yellow, green, and greenish yellow (Fig. 2C). The majority of accessions (60%) possessed light yellow on complete emergence.

The accessions were grouped based on morphological similarity for qualitative characters into four clusters (Table 4). The hierarchical cluster analysis showed that 26 out of 50 black pepper accessions are clustered in one group, which indicates the absence of significant diversity for qualitative characters.

Characterization Based on Quantitative Traits

Results of the study are presented in Table 5. The vine length ranged from 1.6 m (IC598952) to 3.95m (IC598883) with a mean value of 2.48m and CV of 25.77 indicating moderate variability for the trait.

Length of lateral had moderate variability with CV of 30.99 and ranged from 19.1cm (IC598883) to70.64cm (IC598984) with overall mean of 43.64cm.

Fig. 2. Variability in a. shoot tip colour, b. leaf traits and c. spike traits in black pepper germplasm

Number of nodes per lateral showed wide variation among the accessions with a CV of 51.05 and range of 7.2 (IC598902) to 52.8 (IC598932) and mean of 20.33.

Petiole length had mean value of 1.79cm with the values ranging from 1cm of IC598872 to 3.5cm of IC598901. It showed CV of 40.10 indicating wide variability among the accessions. The mean petiole length of leaves on lateral branches in cultivars ranged between 1.2cm in Sreekara and Subhakara to 1.9cm in Girimunda (Krishnamurthy *et al.,* 2000). They also reported that petiole length is higher in runner shoots compared to lateral shoots. To harvest maximum light, varieties with lower leaf petiole length at the top and increased petiole length at the bottom both in runner as well as lateral branches is ideal. However, none of the accessions in the present study had such varying petiole length.

Leaf characters form a major feature for cultivar identification in black pepper. The leaf size and shape on the emerging orthotropic shoots and runners differ from normal leaves on lateral fruiting branches. Accessions showed less variation for leaf length with CV of 12.81. It ranged from 11.14cm (IC598880) to 17.66cm (IC598979) with a mean of 14.05 cm. Similar values for leaf lamina length has been reported in black pepper cultivars by Ravindran and Johny (2000). Leaf width had CV of 17.62 and the values ranged from 5.32cm (IC598872) to 9.66cm (IC598940) with mean value of 7.83cm. According to Ravindran *et al*., 2000 leaf length and width were lowest in Sreekara (10.0 and 6.7 cm) and highest in HP 1411 (12.0 and 9.7 cm).

Similar to leaf length the spike length is also a key character for identification of a cultivars of black pepper (Plate 2C). It ranged from 4.98cm (IC599025) to 12.04cm in (IC598880) with a mean value of 8.63cm and CV of 19.65. Sujatha and Namboothiri (1995) reported positive and significant influence of spike length on yield.

Peduncle length of accessions ranged from 0.76cm (598873) to 1.58cm (IC598936) with a mean value of 1.15cm and CV of 14.87 indicating less variability for the trait.

The number of spikes per lateral showed wide variation with CV of 80.91 and range of 2.20 (IC598945) to 40.4 (IC598904) with mean value of 9.22 indicating possibility of selection of accessions with more number of spikes.

Fruit setting percentage and number of developed fruits per spike showed less variability. Fruit setting percentage ranged from 10.25 (IC598888) to 88.49 (IC598902) with mean of 70.68 and CV of 16.96. The number of developed fruits per spike ranged from 34.21 (IC598938) to 81.2 (IC598891) with a mean of 48.86 and CV of 17.22.

Hundred fruit weight had CV of 15.20 with a mean value of 11.16g. It ranged from 9.58g (IC598939) to 17.77g (IC598903). Hundred fruit volume which indicates the boldness of berries had CV of 17.32 and range of 9.00cc to 16.00cc.

High variation with CV more than 155.00 per cent was observed in green spike yield, green berry yield and dry berry yield per plant indicating scope of selection from the accessions for yield. Green spike yield ranged from 0.31kg (IC598984) to 15.60kg (IC598883) with mean yield of 2.08kg. Berry yield per vine ranged from 0.23kg (IC598984) to 14.00kg (IC598883) with mean value of 1.75kg. Berries/ spike also varied among cultivars. In general, cultivars with increased spike length will have more berries/ spike. Krishnamurthy *et al.* (2000) reported that pollination, water and nutrient availability and pest and disease attack during initial berry development period also influence berry number/ spike. Panniyur 1 grown under irrigated condition has an average 75-80 berries/ spike compared to 30-40 berries/ spike when grown under rainfed condition.

Table 4. Cluster groups and characteristics of black pepper accessions in groups based on qualitative traits

Group	Accessions	No. of accessions	Characteristics
1	IC598875 and IC598909	2	Polymorphic branching with deep purple shoot tip colour, more runner shoots, bearing horizontal lateral branches and light yellow spikes.
2	IC598948, IC599025, IC598939, IC598938, IC598945, IC598940, IC598984, IC598887, IC598949, IC598979, IC598877, IC598881, IC598929, IC598911, IC598927, IC598890, IC598902 and IC598893	18	Dimorphic branching with deep purple shoot tip, few runner shoots, erect lateral branches, ovate - lanceolate leaves with acuminate leaf base and greenish yellow spike
3	IC598941, IC598874, IC598997, IC598932, IC598907, IC598901, IC598880, IC599026, IC598936, IC598921, IC598872, IC598920, IC599024, IC598866, IC598933, IC598883, IC598869, IC598888, IC598904, IC598906, IC598904, IC598891, IC598902, IC598903, IC598889 and IC598899.	26	Dimorphic branching with few runner shoots, hanging lateral branches, oval leaf with acute leaf base, light yellow spikes and round berries
4	IC598873, IC598952, IC598967 and IC598930.	4	Dimorphic branching with few runner shoots, weak holding capacity, horizontal lateral branches and ovate - lanceolate leaf with acute leaf base and round fruits

Dry berry yield/vine ranged from 0.07kg (IC598911) to 4.52kg (IC598883) with a mean value of 0.55kg. Variability among the accessions for driage was less. The driage of accessions ranged from 24.84% (IC598930) to 37.04% (IC598869) with mean value of 31.05 per cent and CV of 7.89.

Ibrahim *et al.* (1985) reported that spike yield and spike number in black pepper are important traits contributing for yield for which straight selection can be practiced for improvement. The quantitative traits, green berry yield per vine, spike number, spike length, and angle of insertion of the fruiting branch directly affect yield (Sujatha and Namboodiri, 1995).

Table 5. Variability in the black pepper germplasm

Character	Range	Mean	SD	CV %
Vine length (m)	1.6 - 3.95	2.48	0.64	25.77
Length of lateral (cm)	19.1-70.64	43.64	13.55	30.99
No. of nodes/lateral	7.2 - 52.8	20.33	10.46	51.05
Leaf petiole length cm)	1.00- 3.50	1.79	0.68	40.10
Leaf length(cm)	11.14 - 17.66	14.05	1.80	12.81
Leaf width(cm)	5.32 - 9.66	7.83	1.38	17.62
Spike length (cm	4.98 - 12.04	8.63	1.69	19.65
Peduncle length (cm)	0.76 - 1.58	1.15	0.17	14.87
No. of spikes /lateral	2.20 - 40.4	9.22	7.75	80.91
Fruit setting (%)	10.25 -88.49	70.68	11.99	16.96
No. of fruits /spike	34.21 - 81.2	48.86	8.41	17.22
100 fruit weight (g)	9.58 - 17.77	11.16	1.70	15.20
100 fruit volume (ml)	9.00 -16.00	10.67	1.85	17.32
Green spike yield/ vine (kg)	0.31-15.60	2.08	3.23	155.23
Green berry yield/ vine (kg)	0.23 -14.00	1.75	2.78	158.98
Dry berry yield/ vine (kg)	0.07 - 4.52	0.55	0.87	159.26
Driage (%)	24.84 -37.04	31.05	2.45	7.89

Cluster Analysis of the Accessions for Quantitative Characters

The 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 (Table 6). The accession IC598883 remained as a single cluster. The accession exhibited very tall vine, very long laterals, medium number. of laterals, medium long leaf petiole, long leaf, medium leaf width, medium long spike, medium long peduncle, low spiking intensity, medium dense berries, medium sized berries, very high yield and medium driage. Accessions from clusters 4 and 5 were having high yield hence can be used for further evaluation to develop high yielding black pepper varieties.

Conclusion

Evaluation of fifty black pepper accessions maintained at ICAR–IISR Calicut showed no substanical morphological diversity. Some traits like green spike yield, green berry yield and dry berry yield showed high variability. Accession IC598883 (Fig. 3) had a very high yield of green and dry pepper berries and can be used for further evaluation to develop a commercial variety.

Table 6. Clustergroups and characteristics of black pepper accessions based on quantitative traits

Group	Accessions	No. of accessions	Characteristics
1	IC598904, IC598890, IC598902, IC598906, IC598872	5	Medium tall vine, medium long laterals, medium no.of nodes, short petiole, long leaf, medium broad leaves, short spikes, medium long peduncle, medium dense berries, large berry, high yield and low driage.
2	IC598869, IC598866, IC598903, IC598929, IC598930, IC598880	6	Medium tall vine, medium long lateral, medium petiole length, medium long leaf, narrow leaves, short spikes, medium long peduncle, medium no.of nodes per node, high density berries, large berry size, medium yielders with high driage.
3	IC598920, IC598984, IC598911, IC598921, IC598940, IC598901, IC598907, IC598877, IC598938, IC598941, IC598997, IC598992, IC598889, IC599024, IC598939, IC599025, IC598949, IC598952, IC598887, IC598881, IC598945, IC598948, IC598904, IC598909, IC598888, IC598927, IC598873, IC598967, IC598979, IC598932, IC599026, IC598936, IC598933, IC598891, IC598899	35	Medium tall vine,long laterals, medium no of nodes/lateral, medium leaf petiole length, long leaf, medium wide leaf, medium long spike, medium long peduncle, low spike intensity, low density berries, medium fruit size, low yield and high driage.
4	IC598893, IC598874, IC598875	3	Tall vine, medium long lateral, low no. of nodes/lateral, medium petiole length, medium long leaf, medium wide leaf, medium long spike, medium long peduncle, medium spike intensity, low density berries, high yield and low driage.
5	IC598883	1	Very tall vine, very long laterals, medium no. of laterals, medium leaf petiole length, long leaf, medium wide leaf, medium long spike, medium long peduncle, low spiking intensity, medium dense berries, medium sized berries, very high yield and medium driage.

Fig. 3. Accession IC598883 a. plant in the field, b. spike formation, and c. mature berries in spike

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