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Characterization of black pepper (*Piper nigrum* L.) varieties and landraces/farmers selections for spike and berry traits

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Abstract

Statistical tools such as analysis of variance, correlation, path coefficient analysis, Scott-Knott test and principal component analysis were used in the present study to characterize black pepper verities/hybrids for spike and berry traits. ANOVA indicated that fifteen traits under study were statistically significant. Traits like fresh pericarp weight and dry pericarp weight showed high positive correlation (>0.95) with spike weight. Path coefficient analysis revealed that berry weight and seed size are contributing directly to spike weight. Scott-Knott test identified Panniyur-1 and Nedumchola as the most contrasting genotypes for most number of traits studied. Based on Principal Component Analysis (PCA), first three principal components had an eigen value above unity and explained 88 per cent of cumulative variation. Principal component PC-1 accounted for maximum variation of about 42.4 percent which discriminated the genotypes based on fresh berry weight, dry seed weight and fresh pericarp weight. These traits serve as the selection criteria for improvement of yield in black pepper.

Keywords: berry components, landraces, correlation, principal components, Scott-Knott test.

Introduction

Black pepper (*Piper nigrum* L.) is an important spice cultivated for its berries. Cultivar diversity is very rich in black pepper. More than 100 cultivars widely differing in yield and quality are reported in black pepper (Ravindran *et al*. 2000). Released varieties, landraces, seedling progenies and in few cases the natural mutants are the major constituents of primary gene pool (Sasikumar *et al*. 2007). Whole berry of black pepper is divided into majorly two parts, pericarp and endocarp (Mangalakumari *et al*. 1983; Attokaran 2011). There are many variability studies in various genotypes for whole berry components such as matured and immature berries spike^{-1}, 100 berry weight, berry size and berry weight etc. (Ibrahim *et al*.

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1985; Shivakumar & Saji 2019). However, there are no reports on the contribution of pericarp/ seed and its association with yield and dry recovery in black pepper. This aspect assumes significance as two commodities *viz*., black and white pepper are obtained from the vine depending on the processing procedure of the matured harvested berries. Understanding the variability among the cultivars is very useful in conservation of cultivars and utilizing them in breeding programme. The present study was undertaken to estimate the variability among the black pepper genotypes for berry components especially the pericarp thickness/ weight and seed size/weight.

Materials and methods

The experimental material studied included 18 genotypes comprising of 8 improved varieties/hybrids (PLD-2, IISR-Thevam, Subhakara, Sreekara, Panniyur-1, IISR-Malabar Excel, IISR-Girimunda and IISR-Shakthi) and 10 land races/farmer's selections (Kalluvally, Arakulamunda, Thekkan, Mundi, Karuvilanchi, Chumala, Jeerakamundi, Narayakodi, Agali, Nedumchola). Details of the genotypes used for the study is described by Shivakumar *et al.* (2021). All the genotypes were grown under the same condition at the ICAR-Indian Institute of Spices Research (IISR), Experimental farm, Peruvannamuzhi (11°36′34″N 75°49′12″E), Kozhikode, Kerala as per the recommended package of practices.

Experimental procedure

The experiment was carried out in Randomized Block Design (RBD) with two replications. Samples of 20 matured spikes per genotype were selected randomly for experimentation. Data were recorded on 15 quantitative traits *viz*., peduncle length (cm), spike length (cm), no. of mature berries spike^{-1}, no. of immature berries spike^{-1}, berry weight (g), spike rind weight (g), berry size (mm), seed size (mm), pericarp thickness (pericarp thickness (mm) =berry size – seed size), pericarp weight (g), seed weight (g), dry rachis weight (g), dry pericarp weight (g), dry seed weight (g) and spike weight (g). Pericarp of each berry was carefully removed and observations were recorded.

Data analysis: Analysis of variance (ANOVA), descriptive statistics namely treatment mean, standard deviation (SD), coefficient of variation (CV) and critical difference (CD) for each of the 15 traits were calculated. Scott-Knot test, correlation coefficients and path coefficient analysis were also carried out. Principal component analysis (PCA) was performed to determine the relationships among genotypes and among variables. Statistical analyses were carried out using the INDOSTAT software developed by INDOSTAT Services, Hyderabad, India.

Results and discussion

The analysis of variance (ANOVA) and univariate statistics are listed in Table 1. ANOVA for means indicated that the differences among the black pepper genotypes were significant for all fifteen characters studied. Coefficient of variation varied from 1.30 for the trait berry size to 12.31 for number of immature berries. Coefficient of variation less than 20% suggested the absence of any skewed distribution (Bekele *et al*. 2017; Preethy *et al*. 2018). The trait pericarp thickness (mm) had a range of 1.18 to 2.04, indicating the genotypes varied significantly for this trait.

Correlation and path analysis

Correlation among the traits may be the result of genetic linkage. From breeders view point understanding the positive and negative association between traits is of paramount importance. High positive correlation was observed between spike length and berry weight (0.99) (Table 2). Traits like fresh pericarp weight and dry pericarp weight showed high positive correlation (>0.95) with spike weight. Whereas the number of immature berries/spikes showed a significant negative

Table 1. Analysis of variance for quantitative characters in black pepper genotypes.

SV	DF	PL	SL	NB/S	NIMB/S	BW	SRW	BS	SS	PT	FPW	FSW	DRW	DPW	DSW	SW
Replication	1	0.331	0.181	0.257	1.871	0.006	0.001	0.037	0.001	0.033	0.001	0.001	0.001	0.002	0.001	0.003
Treatment	17	0.113**	9.540**	327.9**	63.364**	11.235**	0.107**	0.445**	0.312**	0.129**	1.922**	1.593**	0.005**	0.111**	0.810**	12.51**
Error	17	0.005	0.1518	4.554	3.245	0.046	0.002	0.006	0.005	0.002	0.012	0.008	0.001	0.002	0.004	0.045
Mean		1.179	7.243	40.138	14.632	5.807	0.557	5.865	4.434	1.431	2.085	2.331	0.133	0.665	1.481	6.365
Minimum		0.690	3.749	19.900	6.500	2.205	0.178	4.839	3.589	1.189	0.678	0.956	0.060	0.292	0.613	2.383
Maximum		1.764	13.321	80.143	23.250	13.664	1.080	6.663	5.132	2.047	5.112	5.229	0.243	1.355	3.583	14.500
CV (%)		6.117	5.380	5.317	12.313	3.700	7.906	1.309	4.543	3.194	5.284	3.863	7.210	6.845	4.697	3.317

PL= Peduncle length; SL=Spike length; NB/S=No. of berries/spike; No. of immatures/spike: BW=Berry weight; SRW=Spike rind weight; BS=Berry size; SS=Seed size; PT=Pericarp thickness; FPW= Fresh pericarp weight; FSW= Fresh seed weight; DRW=Dry rind weight; DPW=Dry pericarp weight; DSW=Dry seed weight; SW=Spike weight. *, **, significant at P = 0.05, 0.01; values without asterisks are not significant

correlation with the seed size implying that as the number of immature berries increases seed size decreases. Ibrahim *et al*. (1985); Bekele *et al*. (2017) & Pradeep kumar *et al*. (2003) also observed high correlation between spike length and berry yield in black pepper.

Path coefficient analysis considering spike weight as the dependent trait revealed that most of the traits such as berry weight showed high positive direct effect while berry size showed negative direct effect on spike weight (Figure 1) confirming that berry weight and seed size directly influence spike weight. Hence berry weight and seed size could be targeted more confidently as selection criteria for yield improvement in black pepper (Ravindran *et al*. 1997). The residual effects estimated were only 0.001 indicating that the characters under study are sufficient to account for variability in spike yield. Fresh seed weight and dry pericarp weight showed high indirect effect through berry weight. Ibrahim *et al*. (1985); Thanuja & Rajendran (2003) reported high direct effect of spike weight and fresh berry weight on dry berry weight in black pepper. Thus, the traits like berry weight and seed size can be safely included in the selection criteria.

Grouping of genotypes

Scott-Knott test is a hierarchical clustering test used for grouping of genotypes. SK clustering is used where the comparison of genotypes mean is an important step to find out distinct homozygous groups of those means wherever the situation leads to a significant F-test (Scott & Knott 1974 & Jelihovschi *et al*., 2014). Among fifteen traits studied, Panniyur-1 and Nedumchola were contrasting for 9 traits *viz*., peduncle length, spike length, no. of berries spike^{-1}, berry weight, pericarp weight, seed weight, dry pericarp weight, dry seed weight and spike weight. For other traits also, Scott-Knott test grouped the contrasting genotypes. Nedumchola has the smallest spike among all the genotypes studies (4-5 cm). The vine is characteristically small statured. Similar

Table 2. Correlation coefficient of quantitative characters in black pepper genotypes/size.

Traits	PL	SL	NMB/S	NIMB/S	BW	FRW	BS	SS	PT	FPW	FSW	DRW	DPW	DSW	SW
PL	1	0.60* *	0.59 ** *	0.16	0.56 * **	0.44 * *	0 .07	-0.02	0.17	0.54* **	0.53* **	0 .48 * *	0 .53 * * *	0.54 ***	0.58***
SL		1	0.77 ** *	0.33 *	0.82 ** *	0.80 ** *	0.34 *	0.14	0.42 *	0.86 ** *	0.712** *	0.79* * *	0.87* * *	0.74 ***	0.85***
NB/S			1	0.08	0.87 ** *	0.44 * *	0 .14	0.07	0.15	0.81 ** *	0.82 * **	0.54 * **	0.85* **	0.85 ***	0.87***
NIMB/S				1	0.04	0.55 ***	-0.01	-0.43 * *	0 .56 ** *	0.13	-0.06	0.40 *	0.08	-0.16	0.09
BW					1	0.52 ** *	0.51 * *	0 .42 *	0.30	0.97 ** *	0.96* * *	0.53 ** *	0.95 * **	0.95 ***	0.99***
FRW						1	0.2311	-0.08	0.56 * **	0.60 ** *	0.41 *	0.91 * **	0.64 * * *	0.38 *	0.59***
BS							1	0.84 * * *	0.54 ** *	0.52* *	0 .53 * **	0.08	0.43 * *	0 44 **	0.51***
SS								1	0.01	0.35 *	0.51**	-0.08	0.32	0.47 **	0.38*
PT									1	0.42 * *	0 .20	0.28	0.30	0.09	0.34*
FPW										1	0.88 ** *	0.56** *	0.94* * *	0.89 ***	0.97***
FSW											1	0.45 * *	0 .88* * *	0.95 ***	0.95***
DRW												1	0.68* **	0.48 **	0.59***
DPW													1	0.91***	0.96***
DSW														1	0.93***
SW															1

PL= Peduncle lengh; SL=Spike lebgth; NB/S=No. of berries/spike; No. of immatures/spike: BW=Berry weight; SRW=Spike rind weight; BS=Berry size; SS=Seed size; PT=Pericarp thickness; FPW= Fresh Pericarp weight; FSW= Fresh Seed weight; DRW=Dry rind weight; DPW=Dry Pericarp weight; DSW=Dry Seed weight; SW=Spike weight. *, **, *** significant at P = 0.05, 0.01, 0.001; values without asterisks are not significant.

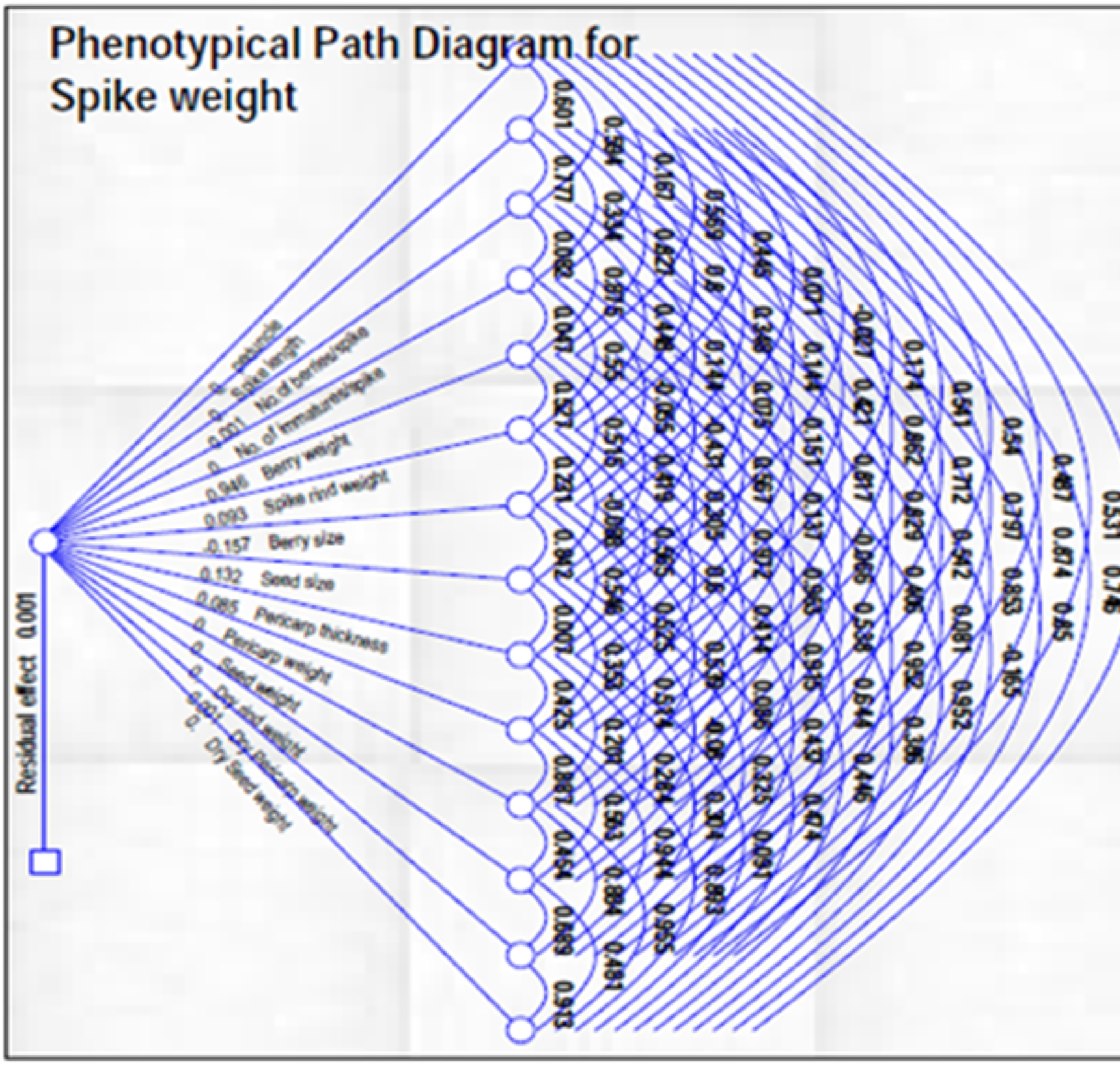

Fig. 1. Path diagram showing direct and indirect effects of quantitative traits on spike weight in black pepper.

observations were also recorded by Ravindran *et al*. (1997). Contrasting genotypes for some of the derived traits like pericarp to seed ratio, dry/fresh pericarp weight ratio and dry/fresh seed weight ratio is presented in Table 3. IISR Malabar Excel recorded maximum pericarp to seed size ratio and in contrast Agali genotype recorded the lowest pericarp to seed size ratio. Likewise, Nedumchola and IISR Malabar Excel, Jeerakamundi and IISR Thevam were contrasting for dry/fresh pericarp weight ratio and dry/fresh seed weight ratio respectively. This information is very useful in identifying the genotypes for developing single/multi trait mapping population.

Principal component analysis (PCA)

Principal component (PC) analysis reorients data sets containing many related variables into smaller sets of components of the original variables. Each set is uncorrelated with any other, but components within sets are related. The criterion established by Kaiser (1960) which is based on selection of those PC with a Eigen value above unity was used in the present study to prove the significance of Eigen values.

Table 3. Contrasting genotypes for seed and pericarp traits in black pepper genotypes

Trait	Contrasting genotype	
	High	Low
Berry size	Chumula, Panniyur-1, Agali and IISR Malabar Excel	Thekkan, Sreekara, Jeerakamundi and Karuvilanchi
Seed size	Agali, IISR Girimunda, Panniyur-1 and Naraykodi	Thekkan, Karuvilanchi, Sreekara and Jeerakamundi
Pericarp thickness	IISR Malabar Excel, Chumula, Arakulamunda and Thevam	Nedumchola, Agali, IISR Girimunda and Thekkan
Pericarp to seed size ratio	IISR Malabar Excel (47.93%), Arakulamunda (44.31%), Chumula (39.80%) and Thekkan (34.81%)	Agali (23.77%), Girimunda (24.64 %), Nedumchola (27.74%) and IISR Shakthi (28.22%)
Dry/fresh pericarp weight ratio	Nedumchola (43.24%), Thekkan (43.07%), PLD-2 (41.51%) and Subhakara (36.94%)	IISR Malabar Excel (26.30%), Panniyur-1 (26.49%), Arakulamunda (27.64%) and Karuvilanchi (29.13%)
Dry/fresh seed weight ratio	Jeerakamundi (71.92%), Agali (71.84%), IISR Malabar Excel (70.85%) and Mundi (69.86 %)	IISR Thevam (49.18), Chumula (49.87), IISR Girimunda (54.97%) and Arakulamunda (54.99%)

The importance of principal component is showcased by the proportion of total variation explained by this factor. In the present study the share of the first three components is 88% of total variation and all had Eigen value above 1 (Table 4). Higher the coefficient values (positive or negative signs), higher the efficiency in discriminating genotypes.

The first PC accounted for 42.4% variability. The positive contributors in first PC are the fresh berry weight, dry seed weight and fresh pericarp weight. As a result, the first PC differentiated those genotypes that had high berry weight, seed weight and less pericarp thickness. The second PC accounted for 26.50% of total variation. The major positive contributing traits are those which are correlated to spike characters like spike rachis weight, numbers of berries/spike and peduncle length. Most of the others traits like berry size, spike weight contributed negatively. (The second PC differentiated the black pepper genotypes based on spike characters). Third PC accounted for 12.35% of total variation. Seed weight is the major positive contributor whereas seed size recorded highest negative value (-0.43) indicating (third PC group differentiated the black pepper genotypes based on fresh seed weight and seed size). Chen *et al*. (2018) also reported fruit size and seed diameter were the important diagnostic key characteristics to differentiate black pepper cultivars.

A scatter plot was drawn using the first two principal components (Figure 2). The genotype Panniyur-1 followed by Agali and Narayakodi recorded very high values of first component and it is expected that these genotypes have high values for the traits

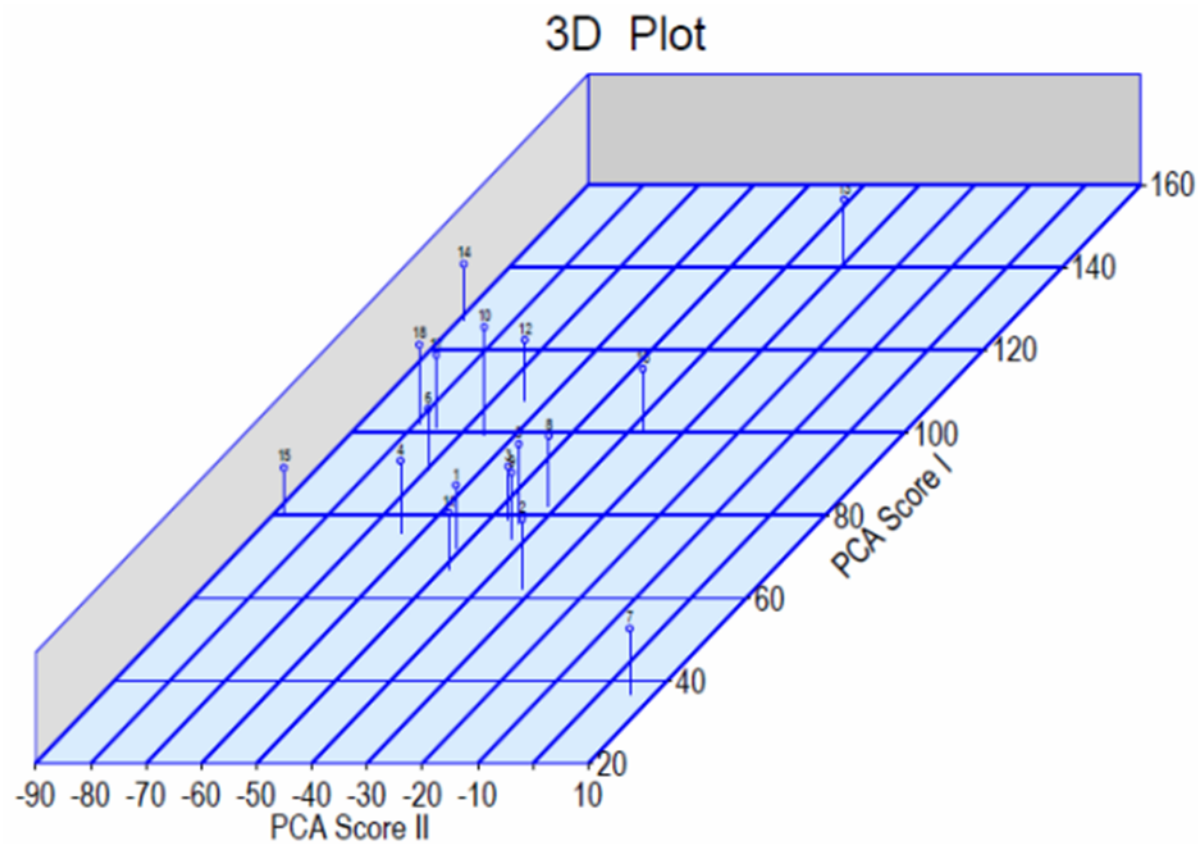

Fig. 2. Plot of principal components of 18 black pepper genotypes.

(Genotypes 1= Sreekara; 2= PLD-2; 3= IISR Thevam; 4=Kalluvally; 5=Arakulamunda; 6=Subhakara; 7=Thekkan; 8=Mundi; 9=Karuvilanchi; 10=Chumala; 11=Jeerakamundi; 12=Narayakodi; 13=Panniyur-1; 14=Agali; 15=Nedumchola; 16=IISR Malabar Excel; 17=IISR Girimunda; 18=IISR Shakthi)

which are highly correlated. Whereas, second component genotypes like Agali, Nedumchola and IISR Shakthi recorded high negative values. Thekken is the only accession which had positive values in both PC1 and PC2. Panniyur-1, Nedumchola and Thekken are two genotypes which clustered away from most of the other genotypes indicating their divergence from each other and from other genotypes, both first and second PC are important in separate these cultivars from others (Ravindran *et al*. 1997b; Mathew *et al*. 2001). Genotypes Agali and Nedumchola showed large negative PC2 values, thereby indicating that characters of second PC are important in differentiating them from other genotypes. Ravindran *et al*. (1997b) while characterizing the 50 black pepper germplasm accessions also reported Panniyur-1 and Nedumchola as independent entries during PCA analysis.

In conclusion, among all the traits studied berry weight, dry seed weight and fresh pericarp weight are the most important traits which recorded high positive correlation; high direct and indirect influence on spike weight. These three traits also played an important role in PC components and clustering of genotypes. These are the traits targeted for yield improvement in black pepper. Panniyur-1 and Nedumchola (Coll No.1058; IC 316610) are the most divergent genotypes, which can be used in exploitation of heterosis and for developing multi-trait mapping population. The study

Table 4. Eigen values, total variance, cumulative variance, and eigenvectors for 15 quantitative characters in black pepper genotypes

	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen value (Root	6.36708	3.97517	1.85252	0.99389	0.76241
% Var. Exp	42.44722	26.50114	12.35014	6.62595	5.08275
Cum. Var. Exp	42.44722	68.94836	81.29851	87.92445	93.00721
Peduncle length	0.09963	0.32818	0.33918		
Spike length	0.24346	0.25793	-0.21245		
No.of berries/spike	0.22969	0.37581	-0.1505		
No. of immatures/spike	-0.22514	0.20518	-0.09272		
Berry weight	0.3678	0.13229	0.02311		
Spike rind weight	0.06552	0.39219	0.21629		
Berry size	0.15067	-0.41657	0.21558		
Seed size	0.18996	0.16126	-0.43321		
Pericarp thickness	-0.35113	0.05347	-0.19545		
Fresh pericarp weight	0.33361	-0.02739	-0.22485		
Fresh seed weight	0.22062	-0.04378	0.54003		
Dry rind weight	0.24307	-0.24916	-0.28353		
Dry pericarp weight	-0.34164	-0.13152	-0.20821		
Dry seed weight	0.35968	-0.11302	-0.15138		
Spike weight	0.20141	-0.41704	-0.00873		

implies that there exists exploitable variability for berry components in black pepper. Hence, along with other berry components pericarp too must be accorded due weight in selecting high yielding and high quality genotypes.

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