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Pericarp as a new berry trait to define dry recovery and quality in black pepper (*Piper nigrum* L.)

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ABSTRACT

Black pepper is one of the oldest known spices cultivated for its berries. Pericarp is considered as a significant determinant of colour intensity, texture and yield of black pepper. However, pericarp thickness has not yet received its due importance as a trait selection criterion in breeding black pepper for enhanced productivity and quality. In this study, we examined the hypothesis that thick pericarp is associated with high dry berry recovery without any reduction in primary (starch, protein and reducing sugars) and secondary metabolite contents (piperine, oleoresin and phenols) in the pericarp, which imparts odour, flavour and pungency for which black pepper is known for. Eighteen black pepper genotypes were characterised for pericarp thickness, dry recovery and biochemical constituents such as piperine, oleoresin, protein, phenols, reducing sugars and starch content in pericarp and grouped them into thin and thick pericarp groups. Pericarp thickness ranged from 1.22 to 2.04 mm and pericarp dry recovery from 26.30 to 43.24 %. The pericarp contained 0.38 to 0.66 % and 1.60–4.35% of piperine and oleoresin, respectively. Wide variation was also observed for phenols, protein, reducing sugars and starch content in pericarp. Pericarp fresh weight, dry recovery, piperine and starch content differed significantly between thin and thick pericarp group genotypes. Thin pericarp in black pepper is more advantageous than thick pericarp for realizing high dry recovery (%). For white pepper production thin pericarp genotypes may have advantage in terms of recovery and processing as there is hardly any difference between thin and thick pericarp for primary and secondary metabolite contents.

1. Introduction

Black pepper (*Piper nigrum* L.) is a perennial climbing vine cultivated for its berries. It is one of the oldest known spices of the family Piperaceae. Though native to humid tropical evergreen forest of Western Ghats of South India, it is now cultivated in other countries like Indonesia, Malaysia, Brazil, Sri Lanka, Vietnam, China etc. Fruits of black pepper are botanically known as drupes but generally called berries. Whole berry consists of two parts viz. the pericarp and endocarp (Mangalakumari et al., 1983; Attokaran, 2017; Khew et al., 2020). Different types, viz., black, green, red and white peppers are produced from matured berries depending on the processing procedure followed after harvesting. Matured berries are freeze-dried to produce red pepper. Unripe but fully developed berries are dried or preserved in brine citric acid and acetic acid to produce green pepper. White pepper is obtained by removing the pericarp from the ripe berries, while black pepper is produced by drying the matured berries with the pericarp to a moisture level of 10–12 %. Besides indicating/defining the maturity bulk density and dry berry recovery, pericarp thickness also determines colour intensity and texture of the black pepper (Purseglove et al., 1981). Dry berry is the marketable product in black pepper. Colour intensity and texture influence market price and consumer acceptability of black pepper. Secondary metabolites such as piperine oleoresin and phenols impart a characteristic flavour, odour and pungency to black pepper (Singh et al., 2004; Hu et al., 2019). Plethora of information is available on biochemical composition and various quality attributes of whole black pepper and white pepper (Gopalakrishnan et al., 1993; Menon et al., 2003; Menon and Padmakumari, 2005; Friedman et al., 2008; Liu et al., 2013). Lee et al., 2020 observed difference in volume of oleoresin and piperine in black pepper before and after removing the outer skin.

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Table 1
Genotypes used in the study.

Sl. No.	Name of genotype	Pedigree/Collection number	Remark
1	Sreekara	Clonal selection from Karimunda	Suitable for all pepper growing regions of India.
2	PLD-2	Clonal selection from Kottanadan	Late maturing, high quality cultivar, recommended for Trivandrum and Quilon districts of Kerala, India.
3	IISR Thevam	Clonal selection of Thevanmundi	Vigorously growing stable yielder, field tolerant to *Phytophthora capsci.* Suitable for all pepper growing regions of India.
4	Kalluvally	Coll. No.7229	A promising North Kerala cultivar, good yielder, medium in quality with high dry recovery, drought tolerant.
5	Arakulamunda	Coll. No. 7220	Moderate and regular bearer, medium in quality and suited to all pepper tracts of India.
6	Subhakara	Clonal selection from Karimunda	High quality variety (12.4 % oleoresin), with wider adaptability to all pepper growing tracts of India.
7	Thekkan	Farmer variety	Multi branched spike with varying berry size and maturity.
8	Mundi	Coll. No.7221	Spikes short to medium, moderate fruit set and medium sized berries.
9	Karuvilanchi	Coll. No.7216	Predominantly female, oblong berries medium in quality, poor yielder.
10	Chumala	Coll. No.7211	Spikes short to medium; good fruit set, medium yield and quality.
11	Jeerakamundi	Coll. No.7241	Cultivar with small leaves and short spikes with alternate bearing nature, small berries.
12	Narayakodi	Coll. No.7258	Popular in South Kerala, moderate yielder with medium quality. Not easily affected by foot rot.
13	Panniyur-1	F_1 of Uthirankotta x Cheriyakaniyakadan	High yielding hybrid, high oleoresin (11.8 %) long spikes & bold berries. Adapted to wide geographical locations.
14	Agali	Farmer variety	Bold berries with high (45−48%) dry recovery and high white pepper (37%) out turn.
15	Nedumchola	Coll. No.1058	A cultivar with small leaves and short spikes, moderate yielder.
16	IISR Malabar Excel	F_1 of Cholamundi x Panniyur-1	Recommended for all pepper tracts of India including high elevations.
17	IISR Girimunda	F_1 of Narayakodi x Neelamundi	Suitable for high elevation and plains of India.
18	IISR Shakthi	Open pollinated progeny of Perambramundi.	Tolerant to *Phytophthora capsici,* moderate yielder. Suitable for all pepper growing regions of India.

Despite being a significant determinant of colour intensity, texture and yield of black pepper, pericarp thickness has not yet received its due importance as a trait selection criterion in breeding black pepper for enhanced productivity and quality. However, in pursuit for targeting pericarp thickness, the contents of secondary metabolites which impart characteristic odour, flavour and pungency, for which black pepper is known for, should not be compromised.

The objectives of the present investigation are to examine the hypothesis that thick pericarp is associated with high dry berry recovery without any reduction in primary (starch, protein and reducing sugars) and secondary metabolite contents (piperine, oleoresin and phenols) in the pericarp.

2. Material and methods

2.1. Plant material

The plant material included 18 genotypes comprising of eight improved varieties/hybrids and 10 landraces/farmer's selections (Table 1). All these genotypes are being maintained at the ICAR-Indian Institute of Spices Research (IISR), Experimental farm, Peruvannamuzhi (11°36′34″N 75°49′12″E), Kozhikode, Kerala and all the recommended package of practices for cultivation of the crop are followed.

2.2. Methods

The experiment was set up in a Randomized Block Design with two replications. Samples of 20 matured spikes per genotype were harvested randomly for the study. Berries were harvested at maturity stage (to maintain uniformity spikes in which one or two berries turned into red colour were chosen). Pericarp was separated from the seed manually using a scalpel. Berry size and seed size were measured with digital calliper. Data were recorded on pericarp thickness (pericarp thickness (mm) =berry size – seed size), pericarp fresh weight (g) and pericarp dry weight (g). Dry recovery of pericarp was calculated as

$$Dry\ recovery(\%) = \frac{Drypericarpweight}{Freshpericarpweight} \times 100$$

The crude protein (Lowry's et al., 1951), reducing sugars, phenols and starch (Sadasivam and Manickam, 1992), and piperine and oleoresin contents (American Spice Trade Association (ASTA, 1968) in pericarp of all the 18 genotypes were estimated.

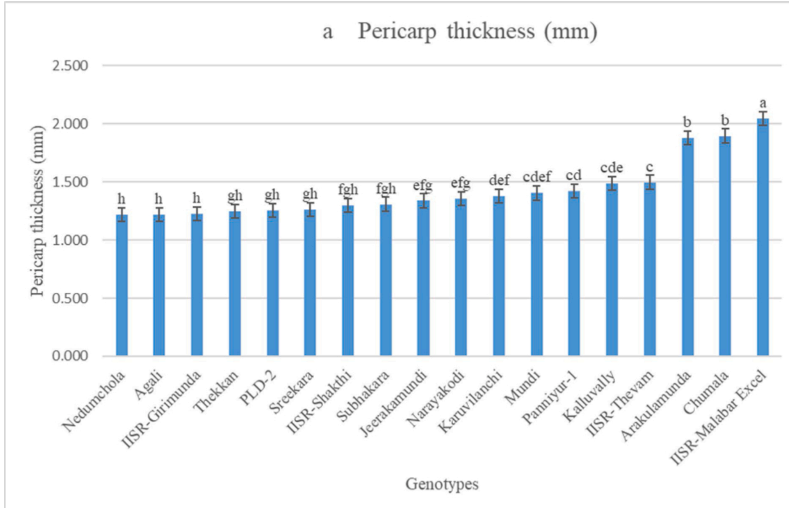
2.3. Statistical analysis

Descriptive statistics such as mean, range, standardized range, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) for each trait were calculated using statistical analysis option MS Excel (Burton and Devane, 1953). Genotypes were grouped into two categories as thin and thick pericarp group based on mean thickness – (0.5) standard deviation (SD). The significance of differences in pericarp fresh weight, pericarp dry weight, dry berry recovery, crude protein, reducing sugars, phenols, starch, piperine and oleoresisn contents in pericarp between thin and thick pericarp group of the genotypes were examined using two-sample 't' test with unequal variances. Box plots were plotted using Past software (version 4.02) to study the dispersion of the data and outliers in thin and thick pericarp groups of genotypes.

3. Results and discussion

3.1. Variability for pericarp thickness and dry berry recovery

Genotypes differed substantially for both pericarp thickness and dry recovery (%) (Fig. 1). Substantial variation for pericarp thickness among the 18 genotypes was evident from the estimates of absolute range, standardized range and GCV and PCV (Table 2). Berries of IISR Malabar Excel had the thickest pericarp, while those borne by Agali and Nedumchola had the thinnest pericarp. Pericarp also called as exocarp is one of the important components of the berry. It is not only a physiological maturity indicator but also serves as a protective coat to the developing endosperm and embryo and determines the texture and



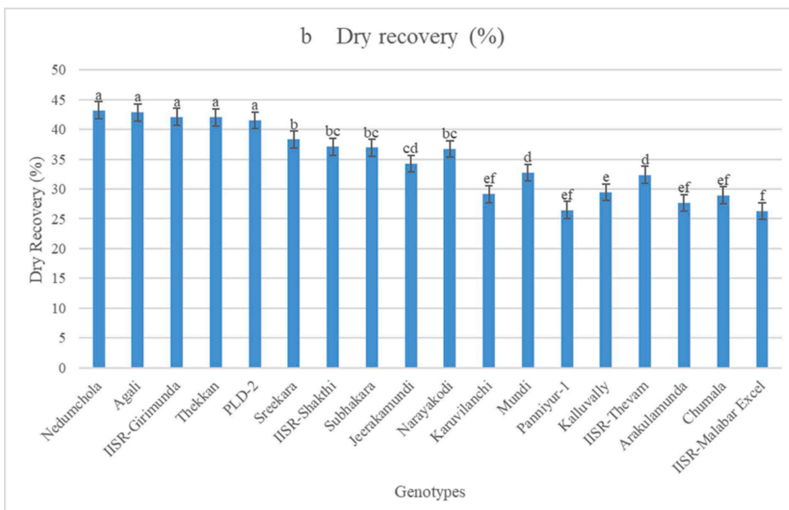


Fig. 1. Comparision of pericarp thickness (a) and dry recovery (%) (b) among 18 black pepper genotypes. Means followed by the same letter within each variety are not significant different by Tukey's test at $p < 0.05$.

Table 2
Descriptive statistics for pericarp related traits in black pepper genotypes.

Statistics	Pericarp thickness (mm)	Pericarp fresh weight (g)	Pericarp dry weight (g)	Dry recovery (%)	Piperine (%)	Oleoresin (%)	Protein (%)	Phenols (%)	Reducing sugars (%)	Starch (%)
Mean	1.43	2.09	0.69	34.91	0.66	2.77	13.9	3.00	5.44	17.48
Lowest	1.22	0.68	0.29	26.30	0.38	1.60	5.50	1.15	2.74	11.00
Highest	2.05	5.11	1.36	43.24	0.96	4.35	18.3	6.22	9.90	28.51
Observed range	0.83	4.43	1.06	16.94	0.58	2.75	12.8	5.07	7.16	17.51
Standardized range	0.58	2.13	1.53	0.49	0.88	0.99	0.92	1.69	1.32	1.00
GCV	17.42	46.86	34.30	16.87	31.31	33.25	25.13	42.78	30.73	26.25
PCV	17.81	47.16	34.76	17.36	31.58	33.67	25.89	42.94	31.25	26.85

appearance of dry black pepper. The fleshy and red coloured pericarp serves as attractant to birds in seed dispersal. Dry recovery per cent is the recovery of marketable dry berries from fresh berries. From producer point of view, dry recovery is an important determinant of black pepper yield. In the present study, dry recovery ranged from 26.30–43.24 per cent which is much higher than the reported whole berry dry recovery range (28–38 %) (Ravindran et al., 2000; Shivakumar and Saji, 2019).

3.2. *Variability for primary and secondary metabolite contents in pericarp*

Genotypes differed substantially for primary and secondary metabolite contents in pericarp (Fig. 2). Piperine, which is one of the secondary metabolites imparts characteristic aroma and flavour to black pepper (Purseglove et al., 1981). Gaikar and Raman (2002) and Mangalakumari et al. (1983) reported that 7.8 % of total piperine of black pepper is derived from the pericarp. Raman spectroscopy mapping of green and black peppers too indicated the presence of pungent principles in whole perisperm (Schulz et al., 2005). In the present study,

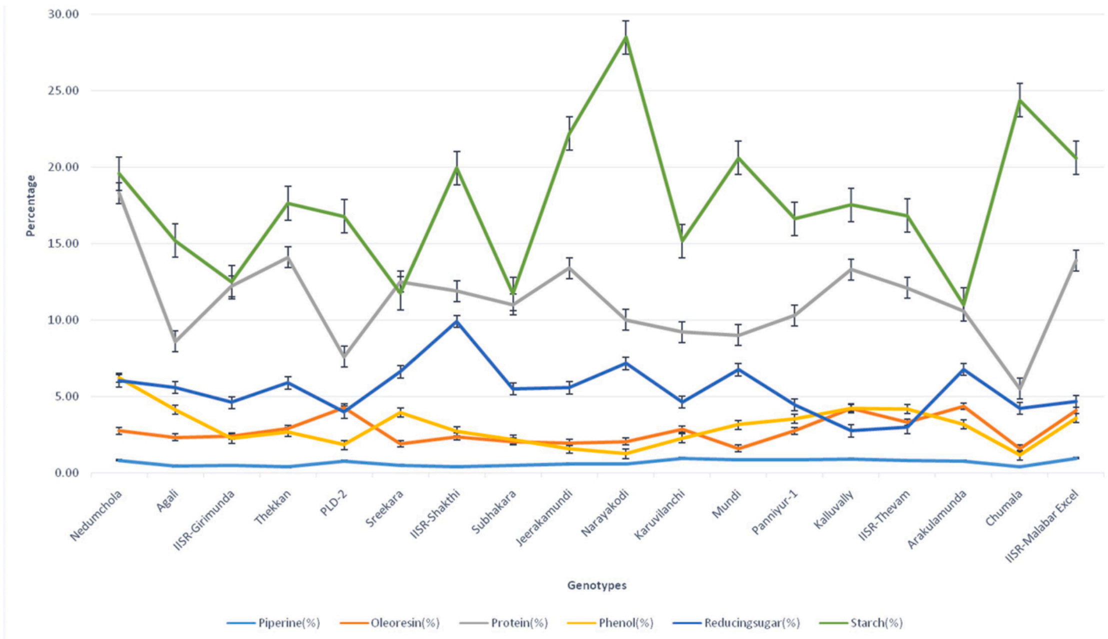


Fig. 2. Comparision of concentration of different biochemical compounds in pericarp of 18 black pepper genotypes.

Table 3
Comparison of means of pericarp traits and biochemical constituents using *t*-test for thin and thick group of black pepper genotypes.

Trait	Thin pericarp (n = 8)		Thick pericarp (n = 10)		Trait Difference	't' statistic	Probability
	Mean	Variance	Mean	Variance			
Pericarp thickness(mm)	1.255	0.001	1.570	0.07	0.316	−3.745	0.002
Pericarp fresh weight (g)	1.526	0.21	2.533	1.14	1.007	−2.676	0.010
Pericarp dry weight (g)	0.62	0.04	0.75	0.07	0.14	−1.24	0.12
Dry recovery (%)	40.52	6.87	30.41	12.10	−10.11	7.03	0.00
Piperine (%)	0.53	0.03	0.77	0.04	0.24	−2.93	0.01
Oleoresin (%)	2.62	0.55	2.88	1.15	0.26	−0.60	0.28
Protein (%)	12.0	0.11	10.8	0.06	−1.20	0.90	0.19
Phenols (%)	3.24	2.13	2.80	1.36	−0.44	0.69	0.25
Reducing sugars (%)	6.00	3.16	4.99	2.40	−1.02	1.28	0.11
Starch (%)	15.14	8.93	19.35	24.98	4.21	−2.21	0.02

pericarp contained 0.38 to 0.66 % and 1.60–4.35% of piperine and oleoresin, respectively. Piperine content in the pericarp was higher in the thick pericarp genotypes such as IISR Malabar Excel while oleoresin was high in Kalluvally (Fig. 2). Protein content in pericarp varied from 5.5 to 18.3%. Higher percentage of protein was found in Nedumchola and IISR Malabar Excel. Phenols in pericarp ranged from 1.15 to 6.22 %. Histochemical studies also reported the presence of phenols in pericarp (Mangalakumari et al., 1983)). Polyphenols are the ones which give dark brown/black colour during drying due to enzymatic reaction (Bandyopadhyay et al., 1990). Reducing sugars ranged from 2.74 to 9.90 %. IISR Shakthi recorded the maximum followed by Narayakodi. Zachariah et al. (2010) reported variation in phenols, starch and protein in berries of 26 black pepper genotypes. Starch content in black pepper is about 28–49 % (Murthy and Bhattacharya, 2008). Starch level in black pepper is directly related to maturity of the berries. Mature berries containing highest starch than green berries (Rathnawathie and Buckle, 1984). In pericarp, starch varied from 11.76 to 28.52 %. High starch in pericarp was found in Narayakodi and Chumala. Starch in berries and other seeds have relevance to seed germination and early seedling vigour (Yu et al., 2015)

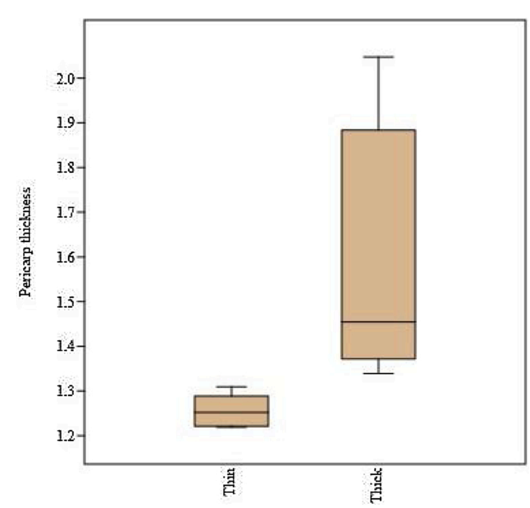


Fig. 3. Box plot for thin and thick pericarp group classified based on pericarp thickness.

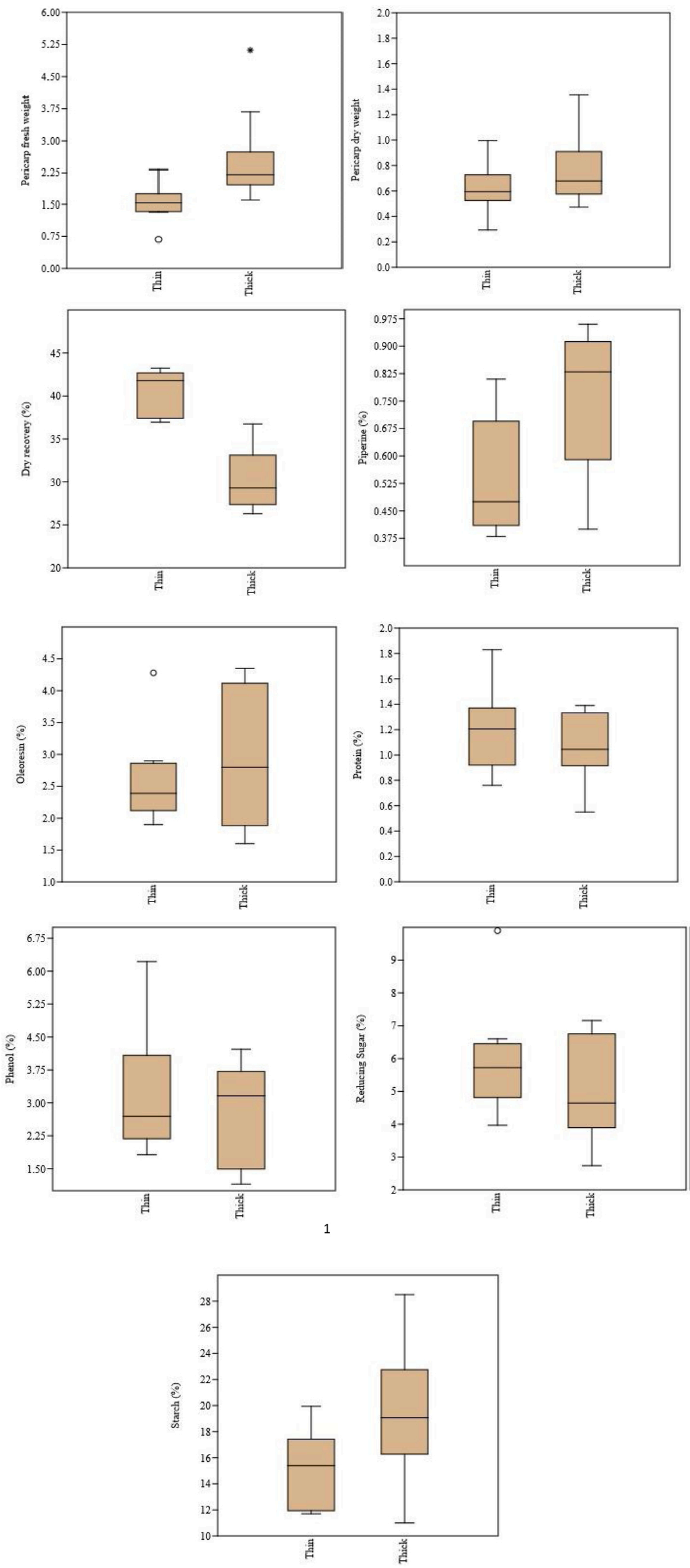


Fig. 4. Box plot for pericarp fresh weight, pericarp dry weight, dry recovery, piperine, oleoresin, protein, phenols, reducing sugars and starch for thin and thick pericarp group (ᵒ and * in box plot indicate the mild and extreme outliers, respectively).

3.3. Relationship of pericarp thickness with traits preferred by producers and consumers/industries

The genotypes were grouped into two distinct groups. The classification of 18 genotypes into thin and thick pericarp groups was justifiable as two groups differed significantly at $P < 0.05$ (Table 3). Thin pericarp group comprised of eight genotypes, while thick pericarp genotypes comprised of 10 genotypes. It was interesting to note the grouping was independent of the pedigree of the genotypes indicating the lack of selection pressure for this trait. We observed a greater variability for thick pericarp group with no outliers (Fig. 3).

The *t*-test revealed significant difference ($P < 0.05$) between thin and thick pericarp genotypes for traits like pericarp fresh weight, dry recovery, piperine and starch content. However, no significant difference between the groups was observed for pericarp dry weight, oleoresin, protein, phenols and reducing sugars (Table 3). Dry recovery percentage was significantly higher in thin pericarp group than thick pericarp group. Even though there was no statistical significance for many quality attributes, thick pericarp genotypes showed higher oleoresin and pericarp dry weight, whereas protein, phenols and reducing sugars were higher in thin pericarp genotypes. Higher variability was observed in the thick pericarp group than thin pericarp group except for reducing sugars for which both groups showed similar pattern. Outliers were noticed for pericarp fresh weight in thick pericarp group and for oleoresin and reducing sugars in thin pericarp group (Fig. 4).

From farmers' point of view thin pericarp is advantageous as they contribute to higher dry recovery than thick pericarp and the berry endosperm stands a better chance as a sink in thin pericarp genotypes. Thin pericarp contributes to fine attractive berries without glaring crinkling/wrinkles, thus increasing the acceptance by consumers. For white pepper industry too thin pericarp genotypes may have advantage in terms of recovery and processing as there was hardly any difference between thin and thick pericarp groups for primary and secondary metabolites (Khew et al., 2020). Lower piperine and oleoresin contents in thin pericarp genotypes indicate little/negligible loss in these metabolites during retting for production of white pepper whereas Oil and oleoresin industry can target for genotypes with thick pericarp.

4. Conclusions

Genetic variability for quantitative and qualitative traits of pericarp was observed in black pepper primary gene pool. On the whole, the genotypes differed significantly for most of the pericarp traits. Grouping of 18 genotypes in to thick and thin pericarp types was done. The study for the first time underlined the significance of pericarp as a yield and quality attribute in black pepper. Thin pericarp genotypes were better in terms of dry yield while the thick pericarp genotypes exhibited better quality traits. A judicious selection of the parental genotypes from the contrasting group will be useful in evolving value added high yielding black pepper varieties. The study also has relevance for selecting suitable varieties with thin pericarp for white pepper production. Oil and oleoresin industries can target for genotypes with thick pericarp.

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CRediT authorship contribution statement

Shivakumar Mundagodu Somashekar: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, original draft, Writing - review & editing. **Krishnamurthy Kuntagod Subraya:** Formal analysis, Investigation, Writing - review & editing. **Saji Koryampalli Vijayan:** Resources; Supervision. **Sasikumar Bhaskaran Pillai:** Conceptualization, Project administration, Visualization, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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