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Research Note

Genetic variation in black pepper (*Piper nigrum* L) accessions for piperine, essential oil and oleoresin, the biochemical principles of quality

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Abstract
Twenty elite high yielding blackpepper accessons with field tolerance to pollubeetle and phytophthora footrot were evaluated for biochemical principles of quality namely, piperine, essential oil and oleoresin. Two accessions, 7293 and 7252 recorded more than 6.5 per cent piperine which was greater than the maximum recorded value in the black pepper. Accession 7211 had high (5.87%) content of essential oil, while, accessions 7289,7276, 7229,7232 and 7252 211 had high amount of oleoresin . The clustering based on biochemical principles showed that the accessions can be grouped to five major clusters. Majority of the accessions belonged to the cluster V with high piperine, medium oil content and low oleoresin content. Correlation studies indicated that there is no correlation between yield and biochemical principles.

Keywords : Black pepper, piperine, essential oil, oleoresin

Black pepper, *Piper nigrum* L., is the most important spice crop in the world and is often described as the 'King of spices' (Purseglove *et al.,*1981). Indian pepper is preferred across the globe due to its intrinsic qualities. Intra-cultivar or inter varietal variability has been observed for morphological and qualitative characters (Ratnambal *et al.,* 1985). To ensure industrial and global demand for quality, varieties with more intrinsic properties are to be identified utilizing conserved germplasm. Essential oils, piperine and oleoresin content of berries are the quality parameters in black pepper that are important commercially (Mohammed *et al.,* 2016)

Sruthi *et al.* (2013) reported that there was profound variability in essential oil, oleoresin, piperine, total phenol, crude fibre, starch, total fat and bulk density in dried berries of black pepper. Gopalam and Ravindran (1987) performed quality indexing of all important cultivars of black pepper by categorizing them into three classes *viz*., low, medium and high.

Identification of black pepper accessions having high quality parameters is very important for its utilization in cultivation as well as in breeding .Conservation of genetic resources and pursuing research on black pepper in India is done by ICAR -Indian Institute of Spices Research (IISR). Calicut, Kerala. The existing germplasm collection of IISR consists of around 3,000 accessions of black pepper which may contain favorable traits like high yield, quality and resistance to biotic and abiotic stresses. In the present study, the accessions were assessed for various quality traits.

The present study was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara and ICAR-Indian Institute of Spices

Research, Calicut during the period 2016-2019. Berries collected from standing vines in the field germplasm of ICAR-IISR at Calicut was used for biochemical evaluation at biochemistry lab of IISR, Calicut using dried berries.

Twenty black pepper accessions selected based on yield (>400g dried berries), field tolerance to pollu beetle and phytophthora foot rot (Prakash *et al*., 2019 , 2020) were used as the mareials for the study and are detailed in **Table 1.** Spikes were harvested from vines at maturity and quality dried black pepper was made following hot water treatment (dipping berries for one minute in boiling water before drying). Dried and cleaned samples of 150 g were taken from each accession for analysis of the qualitative traits. Experiment was done with three replications. Piperine, essential oil and oleoresin content were estimated as per procedure described by ASTA (1997). Data was analysed using R software as completely randomized design. Data on biochemical parameters were compared by DMRT analysis. The accessions were subjected to multivariate analysis of clustering based on data on biochemical components using the soft ware R' SOFT. Correation coefficient were estimated between quality prameters and yield.

The selected accessions were subjected to evaluation for commercially important biochemical constituents in dried berries. Content of piperine, essential oil and oleoresin were analysed following standard procedures. The data were analysed statistically using online software RSOFT and the results are presented in **Table 2.** The results of biochemical analysis showed that there was significant variability among the accessions for all the characters of commercial value like piperine, oil and oleoresin content.

Piperine has numerous medicinal properties such as antioxidant, anti-platelet, anti-inflammatory, anti-hypertensive, hepato-protective, anti-thyroid, anti-tumor, anti-asthmatic activity and also have significant role as fertility enhancer (Chopra *et al.,* 2019). Accessions showed variability with respect to piperine content. Maximum piperine content of 6.96 per cent was observed for the accession 7293 followed by 6.71 per cent for the accession 7252. The accessions 7259, 7241, 7211 and 7249 also had high piperine content which was on par with each other. The minimum piperine content was observed for the accession 7229 (3.61%). The mean value of all the accessions was 5 per cent . Accessions 7285, 7286 and 7232 were placed in low piperine group. In popular black pepper cultivars, piperine concentration varied from 2.8 per cent to 3.8 per cent (Zachariah *et al.,* 2005). Piperine content of 6.6 per cent was recorded in Panniyur 2 (Zachariah 2008). The piperine content in 7293 and 7252 accessions recorded in the present experiment was greater than the maximum recorded value in the commercial variety Panniyur 2. Based on the study the accessions 7293, 7252, 7241, 7211 and 7249 had piperine content of more than 5.5 per cent.

Table 1. List of black pepper accessions selected for evaluation of biochemical principles of quality

S. No	Accession Number	IC Number	Place of collection	District
1	7211	598866	Meenmutty	Waynadu
2	7215	598869	Mayyil-Velam	Kannur
3	7219	598872	Mayyil-Velam	Kannur
4	7221	598874	Chapparakkunnu	Kannur
5	7222	598875	Chapparakkunnu	Kannur
6	7229	598880	Vellora	Kannur
7	7232	598883	Vellora	Kannur
8	7240	598890	Kayapoil	Kannur
9	7241	598891	Kayapoil	Kannur
10	7243	598893	Kakkara	Kannur
11	7249	598899	Ammankad	Kannur
12	7252	598902	Oduvally	Kannur
13	7254	598903	Naduvil	Kannur
14	7255	598904	Naduvil	Kannur
15	7259	598906	Mandalam	Kannur
16	7276	598920	Kattipara	Kozhikkod
17	7285	598929	Kodenchery	Kozhikkode
18	7286	598930	Kodenchery	Kozhikkode
19	7289	598933	Koodathai	Kozhikkode

Table 2. Biochemical principles of quality in black pepper accessions

Accession Number	Piperine %	Essential oil %	Oleoresin %
7211	5.68^{BC}	5.87^{A}	9.64^{C}
7215	5.49^{CD}	3.60^{IJ}	8.38^{E}
7219	4.33^{GHIJ}	3.87^{GHI}	10.79^{AB}
7221	4.28^{HIJ}	4.50^{CDE}	9.09^{CD}
7222	5.47^{CD}	4.67^{CD}	9.46^{C}
7229	3.61^{K}	3.47^{J}	7.10^{F}
7232	4.65^{FGH}	3.60^{IJ}	10.34^{B}
7240	4.28^{HIJ}	4.00^{FG}	7.43^{F}
7241	5.93^{B}	4.80^{C}	9.67^{C}
7243	4.35^{GHI}	4.27^{EF}	8.32^{E}
7249	5.68^{BC}	4.47^{DE}	9.58^{C}
7252	6.71^{A}	4.67^{CD}	10.28^{B}
7254	4.09^{IJ}	3.00^{K}	8.59^{DE}
7255	3.92^{JK}	5.47^{B}	7.52^{F}
7259	6.01^{B}	3.60^{IJ}	7.10^{F}
7276	3.99^{IJK}	3.93^{GH}	10.85^{AB}
7285	4.85^{EF}	4.00^{FG}	9.67^{C}
7286	4.73^{FG}	3.07^{K}	8.32^{E}
7289	5.18^{DE}	3.67^{HIJ}	11.18^{A}
7293	6.96^{A}	4.13^{FG}	9.04^{CD}
Mean	5.00	4.13	9.12
SE	0.05	0.03	0.13
CV	4.52	4.29	4.02

Foot note . Values having the same alphabet as suprcript are on par

Black pepper essential oil showed antioxidant, carminative, larvicidal, antibacterial, and antifungal activities. Essential oil of blackpepper were reported to have strong *in vitro* and *in vivo* antioxidant and radical scavenging activities (Dosoky *et al.,* 2019). The Essential oil content of the accessions ranged widely among the accessions. Maximum oil content of 5.87 per cent was recorded for the accession 7211 followed by 5.47 per cent for the accession 7255. The mean value observed was 4.13 per cent . Accessions 7241, 7222 and 7221 were on par for essential oil content. The minimum oil content of 3 per cent was observed for the accession 7254. The poor oil containing accessions were 7229, 7232, 7289 and 7215. The maximum value of oil content observed for the accession 7211 in the experiment (5.87%) was less than the maximum recorded value of 6 per cent, as reported by Krishnamurthy *et al.* (2010) .

Black pepper oleoresins impart taste and also have antioxidant properties, so they are used in foods and health products. They are also used as a colourant and a food preservative because of their antimicrobial properties. The oleoresin content of dry fruits of accessions showed wide variability and ranged from 7.10 per cent (7229) to 11.18 per cent (7289) among the accessions with a mean value of 9.12 per cent. There was high oleoresin content in accessions *viz.*,7289,7276, 7229,7232 and 7252 compared to rest of the accessions. The oleoresin content was low for accessions *viz.*7229 (7.10%), 7259 (7.15%), 7240 (7.43%), 7255 (7.52%), 7243 (8.32%) and 7286 (8.32%). However, all the accessions in the experiment expressed values lower than the maximum recorded value of oleoresin (15.45%) of commercial variety PLD2.

Correlation of yield with various biochemical principles *viz.,* piperine, essential oil and oleoresin content were estimated (**Table 3**). None of the biochemical principles exhibited correlation with yield in black pepper. However, Sruthi *et al.* (2013), observed positive correlation of essential oil, piperine and oleoresin with each other based on their study. Shivakumar *et al.* (2020) observed highly significant and positive correlation of dry berry yield per wine with rachis weight vine-1, fresh berry yield vine-1, spike length and lateral branch length . In chilli quality parameters *viz*., ascorbic acid, capsaicin, red and yellow carotenoids had positive direct effect on fruit yield

Table 3. Correlation between yield and biochemical priciples of quality in black pepper accessions

	Yield	Piperine	Essential oil	Oleoresin
Yield	1.00	-0.21	0.05	-0.03
Piperine		1.00	0.26	0.21
Essential oil			1.00	0.11
Oleoresin				1.00

per plant (Janaki *et al.,* 2018). Hence, the correlation of biochemical components, yield and other traits , may vary depending on the traits , accessions and species studied .

Cluster analysis of 20 accessions was done based on various biochemical parameters. The number of accessions falling under each cluster, based on piperine, oil and oleoresin content is presented in **Table 4.** The clustering showed that the accessions can be grouped to five major clusters (**Fig.1**.) on the basis of biochemical principles like piperine, essential oil and oleoresin content. Majority of the accessions belonged to the cluster V with high piperine, medium essential oil content and low oleoresin content. Cluster IV had five accessions namely,

Table 4. Distribution pattern of black pepper accessions based on biochemical principles

Cluster	Number accessions	Accession number	Characteristics
I	2	7215 and 7259	High piperine Low oil Low oleoresin
II	4	7286,7254,7221and 7243	Medium piperine Medium oil Low oleoresin
III	3	7255,7240 and 7229	Low piperine Low oil Low oleoresin
IV	5	7232,7285,7289,7276 and 7219	Medium piperine Medium oil Medium oleoresin
V	6	7293,7252,7211,7241,7249 and 7222	High piperine Medium oil Low oleoresin

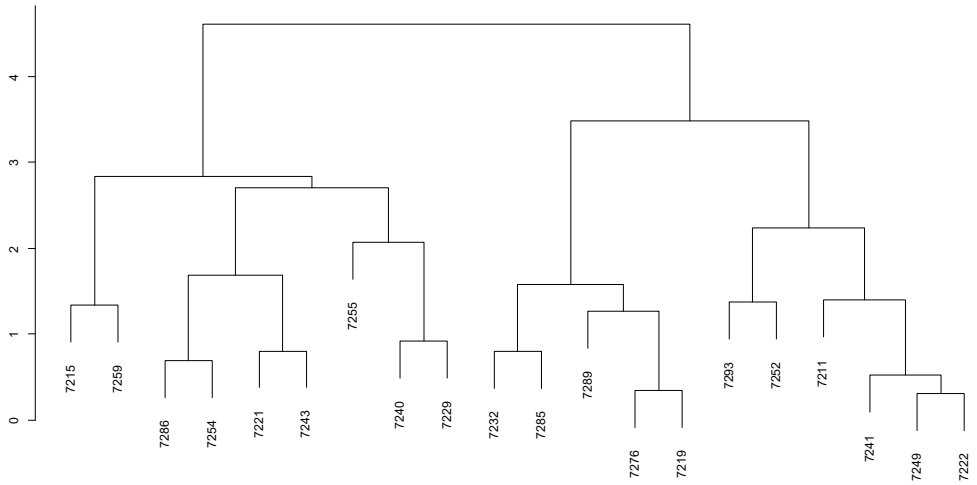

Fig.1. Dendrogram of black pepper accessions for biochemical principles of quality

7232,7285,7289,7276 and 7219. They had medium content of piperine, essential oil and oleoresin. While, cluster III comprised of three accessions with inferior biochemical status. In a similar study on divergence of 42 black pepper germplasm accessions, oleoresin exhibited more variability than piperinee (Pradeepkumar *et al*., 2003). Gopalam and Ravindran (1987) performed quality indexing of all important cultivars of black pepper by categorizing them into three classes namely, low, medium and high. The biometric observation and visual assessment of plants based on qualitative traits brought intra group, interclone and inter varietal variation of different characters among tissue cultured and conventional clones of four pepper varieties (Sujatha, 2001). The Indian cultivars Kottanadan, Kumbakodi, Kuthiravally and Nilgiri were rich in piperine and oleoresin, where as Balankotta, Kaniyakadan and Kumbakody were rich in essential oil (Krishnamoorthy and Parthasarathy, 2010). Profound variability in essential oil, oleoresin, piperinee, total phenol, crude fibre, starch, total fat and bulk density in dried berries of black pepper variety, Panniyur-1 was observed by Sruthi *et al*. (2013).

Biochemical characterization of twenty accessions for piperine, essential oil and oleoresin content showed that high piperine content of 6.96 per cent was observed for the accession 7293 followed by 6.71 per cent for the accession 7252. This was greater than the highest reported value of 6.6 per cent in the commercial variety Panniyur 2. Essential oil content of the accessions varied from 3 - 5.87 per cent with a mean value of 4.13 per cent .Oleoresin content of accessions ranged from 7.10 - 11.18 per cent with a mean value of 9.12 per cent .Correlation studies indicated that there is no correlation between yield and biochemical principles in pepper .

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