Received: 22 Jan 2020 | Revised: 17 Nov 2020 | Accepted: 18 Nov 2020

Electronic Journal of Plant Breeding

Research Article

Assessment of variation in volatile oil content in cardamom [*Elettaria cardamomum* (L.) Maton]

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Abstract
The volatile oil of cardamom capsules is an expensive ingredient in food preparation, beverages, perfumery and traditional medicines. The capsules of 65 promising cardamom accessions were used to evaluate the volatile oil content estimation. The volatile of cured capsules were extracted by hydro-distillation method and it was ranged from 2.2 % - 9.6%. Five cardamom accessions were observed highest volatile oil content, which was HY-3 and HY-4 (9.6 %), S1 × GG (Type 2) and SAM-5 (9.3%) and PPK-2 (9.1%). This higher volatile oil content is due to the more number of seeds per capsule, capsule maturity and curing period. The number of seeds per capsules was ranged 6.1 in SAM-3 and 17.8 in HY-3 and HY-4. In this study, we have identified 5 high volatile oil content cardamom accessions that could be used for future breeding programs for developing varieties with high yielding as well as high volatile oil content.

Keywords
Cardamom, Diversity, Variability, Volatile oil, Moisture, Number of seeds per capsule

INTRODUCTION

Elettaria cardamomum (L.) Maton belongs to family zingiberaceae, commonly known as small cardamom or green cardamom or true cardamom or Indian cardamom, is commercially cultivated in India, Guatemala, Sri Lanka, Nepal, Indonesia, Costa Rica, Mexico and Tanzania (Garg *et al*., 2016). In India, the cardamom is cultivated inthe southern states like Kerala, Karnataka and Tamil Nadu,in the higher uplands, altitude ranging from 900-1400 m above the sea level. The distinctive aroma of cardamom capsules is due to the existence of high value antioxidants and gastro-protective bioactive metabolites which play the roles of functional food, nutraceutical and pharmaceuticals (Hamzaa and Osman, 2012). Across the world, cardamom is prestigiously called as queen of spices. The spice is distilled for essential oil and the peculiar odour and flavour of cardamom oil is decided by the relative proportion of various key components present in the volatile oil. The concentration of volatile oil generally grades the aroma of cardamom as sweet, spicy andcamphory. The bitterness compond present in cardamom oil which is α-terpinyl acetate which comprises atleast 20% of the total fraction. A study by Pillai *et al*. (1984) showed that the high level of α-terpinyl acetate in Guatemalan cardamom make it superior to Indian cardamom.The cardamom capsules contain 6-14 % oil (volatile oil), which is responsible for its characteristic pleasant aroma (Menon, 2000). Interestingly, many factors influence the concentration of volatile oil across growing regions.

Several insect pests attack cardamom capsules there by changing the texture and appearance of the capsules. Commonly, the infestation by thrips (*Sciothrips cardamomi*), one of the major pests of cardamom results in increased accumulation and concentration of the volatile oils and bioactive phytochemicals (Murugan *et al*., 2002; Murugan *et al.,* 2019). The essential oil of cardamom is an expensive ingredient in food preparation, beverages, perfumery and traditional medicines (Kumar *et al*., 2005). Both the exportable and marketable quality of cured cardamom capsules should have 10-11% moisture content. Lower levels of moisture in cured capsules will

have better keeping quality for longer period. Therefore cured capsules from varieties having high volatile oil content and less moisture content will be of premium type. Cardamom oil contains many bioactive components of which 1, 8-Cineole contributes 28.94 – 34.91%. The concentration of bioactive compounds varies with varieties. Other major major bioactive components such as α-Pinene, Sabinene, Linalool, α-Terpineol and Nerol were present in considerable quantities (Ashokkumar *et al*., 2020a). The Germplasm Repository of the Cardamom Research Station, Pampadumpara is maintaining 187 accessions of cardamom (*Malabar, Mysore* and *Vazhukka*) which are the major sources of this research study. The study was conducted to estimate the volatile oil content and moisture content of cured capsules using 65 promising cardamom accessions (in terms of yield) maintained at the germplasm site. The best accessions from the study can be used for further quality improvement through appropriate breeding programs.

MATERIALS AND METHODS

Cardamom capsules of sixty five promising accessions (in terms of yield) from the germplasm repository of the CRS farm were collected and used for the estimation of moisture, volatile oil content and the number of seeds per capsule **(Table 1 and Fig 1)**. These capsules were harvested from these accessions at brown to black seed stage and cured.The harvested wet capsules were thoroughly washed with good quality of water to remove any adhering soil or unwanted materials. Since the optimum duration for cardamom curing is 22-26 hours for getting better oil content and other quality traits it was followed in our study also. The cleaned capsules were spread and cured using special curing chambers under controlled temperature to retain good flavour and green colour. The curing chamber was initially heated to 45-50ºC for the first four hours and subsequently reduced to 40-45ºC by opening the ventilators and exhaust fans. Finally, the temperature was raised to 60ºC for one hour just before the end of curing time. The process of drying takes about 22-26 hours. Dried capsules were rubbed with coir mat/gunny cloth/steel mesh and sieved to remove the other plant debris. Only the good quality whole capsules were used for the estimation of volatile oil and moisture and the estimation was perfomed based on the method given by AOAC (2000).

The seeds as well as husk of cured capsules were separated and the powdered seeds were subjected to hydro-distillation using Clevenger apparatus for four to five hours. Fifteen gram of finely powdered cardamom sample was quantitatively transferred into 500 ml round bottom flask without much lapse of time and approximately 250 ml of distilled water was added into the flask, so as to immerse the sample completely in water. Refluxing was adjusted in such a way that one drop/second was maintained. The refluxing was continued until two to three consecutive readings were same. The apparatus was then allowed to stand to normal room temperature (25-27ºC) and the final reading was recorded. The quantity of volatile oil present in each sample was calculated by the following formula (AOAC, 2000).

$$\text{Volatile oil (\% v/w)} = \frac{\text{Volume of oil (ml)}}{\text{Weight of sample (g)}} \times 100$$

Twenty gram cured cardamom capsule was weighed and powdered and quantitatively transferred to a round bottom flask and approximately 125 ml of moisture free toluene was added and refluxed for about two hours. The refluxing was maintained at the rate of 4 drops/second and continued until there was no change in two to three consecutive reading for 15 minutes. The final reading was noted from the collecting tube. The moisture content was calculated using the following formula (AOAC, 2000).

$$\text{Moisture content (\% v/w)} = \frac{\text{Volume of water (ml)}}{\text{Weight of sample (g)}} \times 100$$

The data on biometric traits were subjected to basic statistical analysis like mean, standard error and standard deviation by Microsoft Excel and multivariate hierarchical cluster analysis was performed using NTSYSpcv2.02i software (Rohlf, 1998). The mean values were exposed to hierarchical cluster analysis accomplished by un-weighted pair-group arithmetic average (UPGMA) method (Sneath and Sokal, 1973) with sequential agglomerative hierarchical nested cluster analysis (SHAN) programme. A phenetic tree was constructed using the TREEPLOT programme of NTSYS pc (Rohlf, 1998).

RESULTS AND DISCUSSION

The results of the analysis showed that volatile oil content of 65 cardamom accessions ranged from 2.2 % to 9.6%. Among accessions, the lowest value of volatile oil was reported in BRINTH-4 (2.2%) followed by SAM-6 (3.6%), AEP-1 (4.3%), HY-5 and HY-11 (4.5%). Highest oil content was recorded in HY3 and HY4 (9.6 %), S1 × GG (Type 2) and SAM-5 (9.3%) and PPK-2 (9.1%), which was greater than that reported by Padmakumari *et al*. (2010) in four different cardamom types *viz*., *Malalabar, Mysore, Vazhukka* and *Guatemala* (7.9-8.8%). Ashokkumar *et al*. (2020b) stated that cardamom volatile oil content varied by based on the various extraction methods, varieties and plant parts. More number of seeds per capsule could have contributed to their increased oil levels. Since as much as 90% of the oil is present in the seeds they act as the main sources of volatile oil in cardamom. Accessions such as BRINTH-4, SAM-12, AEP-1, HY-5 and HY-11 were found to below oil yielding types due to lesser number of seeds present in each capsule of these cardamom accessions (**Table 1**). This finding is in confirmation with earlier reports (Miniraj *et al*., 2000). They reported high level of volatile oil in S1 compared to PS5 and PS12. Study by

Table 1. Genetic variability of volatile oil and moisture content of 65 cardamom accessions

Sl.No.	Name of the entry	Accession No.	Volatile oil (%)	Moisture (%)	No. of seeds per capsule
1	CHETTI 1	CRSP 117	6.5	10.0	16.2
2	HY1	CRSP 87	6.5	8.0	12.1
3	HY2	CRSP 88	6.3	8.1	12.4
4	HY5	CRSP 91	4.5	8.0	10.1
5	HY6	CRSP 92	5.5	10.0	11.6
6	HY11	CRSP 97	4.5	8.0	10.4
7	HY14	CRSP 100	5.7	10.0	13.2
8	HY16	CRSP 102	5.0	6.6	10.2
9	HY18	CRSP 104	5.5	7.3	11.1
10	GG × PV1 TYPE-2	CRSP 78	7.5	8.6	16.6
11	GG × PV1 TYPE-3	CRSP 79	5.0	8.0	12.7
12	PS1	CRSP 112	4.0	7.2	9.8
13	PS2	CRSP 113	5.0	6.0	15.2
14	PS3	CRSP 114	6.0	8.0	16.1
15	PS5	CRSP 116	5.5	6.5	6.2
16	PS6	CRSP 117	5.5	7.0	12.1
17	PS10	CRSP 121	6.2	8.0	11.8
18	PS12	CRSP 123	5.0	8.0	16.2
19	PS21	CRSP 132	5.7	8.5	12.8
20	PS29	CRSP 140	6.1	8.5	12.4
21	PALAKUDDI	CRSP 183	7.0	10.0	13.2
22	ICRI-2	CRSP 184	7.0	11.0	13.6
23	NS18	CRSP 149	5.5	11.3	15.2
24	ELARAJAN-2	CRSP 187	6.7	8.6	12.2
25	PRO107	CRSP 145	5.0	7.3	16.2
26	CLONE SELECTION	CRSP 124	4.4	9.0	10.0
27	MYLADI3	CRSP 185	6.7	8.5	13.2
28	AEP1	CRSP 4	4.3	8.0	10.4
29	BABU-2	CRSP 128	5.0	6.5	10.2
30	PS9 × GG TYPE2	CRSP 86	5.0	10.5	10.2
31	PS9 × GG TYPE3	CRSP 85	6.0	10.7	12.1
32	ASK2	CRSP 20	5.0	11.0	10.6
33	BRINTH2	CRSP 139	5.6	8.0	11.4
34	BRINTH4	CRSP 133	2.2	8.2	6.2
35	BRINTH9	CRSP 137	7.5	8.5	14.1
36	MBP	CRSP 110	5.7	8.5	12.7
37	PV 35	CRSP 186	5.0	8.0	12.4
38	SAM 4	CRSP 30	6.0	7.5	13.6
39	SAM 5	CRSP 31	9.3	7.4	17.4
40	SAM 6	CRSP 32	3.6	7.0	10.2
41	SAM 12	CRSP 106	3.0	8.2	7.6
42	SAM13	CRSP 107	6.7	8.0	12.5
43	SAM16	CRSP 109	5.0	7.5	10.6
44	COMPOUND PANICLE	CRSP 126	7.4	10.0	15.9
45	HY-17	CRSP 103	8.5	8.7	11.2
46	BRINTH-5	CRSP 182	8.5	8.7	11.6
47	S1 × GG (Type 2)	CRSP 76	9.3	8.0	17.4
48	PS 23	CRSP 134	7.8	8.0	14.1
49	PS 21 × S1	CRSP 74	8.5	8.0	15.1
50	HY-4	CRSP 90	9.6	10.0	17.8
51	HY-3	CRSP 89	9.6	10.0	17.8
52	PS 9 × GG (Type 2)	CRSP 86	8.9	10.7	16.4
53	SAM-3	CRSP 181	5.3	7.5	6.1
54	SAM-8	CRSP 34	7.2	7.0	11.2
55	BABU-3	CRSP 129	8.1	6.0	10.6
56	ASK-1	CRSP 19	6.6	10.5	11.2
57	ASK-4	CRSP 22	8.9	6.0	11.6
58	ASK-5	CRSP 23	7.4	11.0	11.2
59	PV1 × GG Type 1	CRSP 77	8.6	11.0	10.8
60	NS 34	CRSP 154	8.1	8.0	12.1
61	BEP-2	CRSP 3	8.1	8.9	16.1
62	PPK-2	CRSP 5	9.1	7.5	14.2
63	BRINTH-8	CRSP 136	8.1	6.3	16.4
64	MCC-34	CRSP 120	8.6	6.6	16.6
65	PV-5	CRSP 156	7.5	8.0	14.4
	Mean	-	6.4	8.4	12.7
	Standard Error	-	0.12	0.25	0.42
	Standard Deviation	-	1.62	1.40	2.62

Sayed *et al.* (1979) revealed positive correlation between cardamom seeds and volatile oil and negative correlation between husk percentage and volatile oil. Seeds were main source of volatile oil whereas; the husk hardly contains 0.2% oil (John and Korikanthimath, 2002).In general, the accumulation and quantity of volatile oil in cardamom capsules is depending mainly on the number of seeds per capsule, capsule rind, size of the seeds, capsule maturity, harvest time and curing methods. Study of John and Korikanthimath (2002) indicated that the oil content of cardamom vary from 3-8% with varieties, grade, age of the sample, stage of harvest, distillation method and nature of capsule (bleached or not). He also noted that the geography of the land where the crop is grown also determine the level of constituents of volatile oil. *Mysore* and *Vazhukka* possessed the highest percentage of oil compared to Malabar type (Sayed *et al*., 1979). According to Vasanthakumar *et al.* (1989) the black seed stage of cardamom capsule yield more essential oil. This may be due to the accumulation of oil constituents with advancement of seed maturity as a result of cardamom source-sink relationship (Sheeba, 2004). Padmakumaria *et al.* (2010) showed that the major components of volatile oil that determine cardamom flavour and aroma were 1,8-cineole (20-60%) and α-terpinyl acetate (20-55%). Ratios of these components decide the flavour of the oil. Malabar type cardamom have camphor aroma due to high concentration of 1, 8 cineole. Gopalakrishnan *et al*. (1989) found that volatile oil in thrips affected capsule contain higher level of 1,8-cineole. Kumara *et al*. (1985) noticed that the appearance and green colour has no impact on volatile oil content, but oil loss is high from bleached cardamom due to loss of husk turgidity (Govindarajan *et al*., 1982). Higher oil content in HY-3, HY-4, S1 × GG and SAM 5 is due to more number of seeds per capsule, correct stage of seed maturity (brown to black seed stage) and slow curing process of the varieties evaluated. Higher volatile oil and longer duration of curing with gradual increase of temperature were well correlated and documented.

Moisture contents of 65 cardamom accessions evaluated were found to be varying between 6-11.3% and the values fell within the range of 6-11% for the most of the cardamom accessions. The highest value of 11.3 % was reported in NS 18 followed by ICRI 2, ASK-5, PV1 × GG Type 1 and ASK 2 (11%). The lowest moisture content was 6 - 6.5 %, recorded in ASK-4, BABU-3, BABU-2 and BRINTH-8, PS-3 and PS-5. In our study, we maintained the curing temperature levels between 40-60°C. The reason for the slight variation in the moisture content of these accessions could be due to the variations in capsule size and thickness of the capsules' rind as well as initial moisture and wetness of the harvested produce. Smaller sized capsules have lower moisture content and *vice versa* (Sheeba, 2004). The moisture level in cardamom capsules at the time of harvest (70-80%) mainly depends on its stage of maturity as well as environmental condition. While curing, the moisture level should be brought down to 8-10% for better shelf life and maintenance of green colour (John and Korikanthimath, 2002). They also reported that the curing temperature requirement for good capsule colour is between 45-50°C which is achievable with uniform heat distribution. Alteration or break or abrupt increase in the curing temperature affects the colour and shelf life of the produce. Capsules cured at 45 and 60°C yield almost same quantity of volatile oil in GLC studies (Anonymous, 1991). The above results support our findings. Number of seeds per capsule were ranged between 6.1 – 17.8 **(Table 1)**. The cardamom accessions HY-3, HY-4, SAM-5 and S1 × GG Type-2 recorded the highest number of seeds, which also recorded higher volatile oil content. Hence, this study confirmed that the number of seeds per capsule was positively correlated with volatile oil content in cardamom. Furthermore, in this study we have observed that the lowest number of seeds per capsules contains lesser volatile oil content **(Table 1)**.

The hierarchical clustering was performed to know the relationship among the 65 cardamom accessions based on the biometrical traits using Euclidean distance measuring dissimilarity between the samples. Dendrogram constructed based on UPGMA cluster analysis with biometric data shown in **Fig 1**. The Jaccard's

Table 2.Cluster analysis of genetic similarities and distance among 65 cardamom accessions

Cluster group	No. of genotypes	Name of genotypes
I	17	CHETTI 1, HY 6, HY 14, PALAKUDI, COMPOUND PANICLE, HY 4, HY 3, HY 18, PS 5, PS 6, NS 18, HY 2, PS 29, PS 21, MBP, MYLADI 3 & BRINTH 9
II	30	HY 1, NS 34, HY 5, HY 11, AEP 1, PS 3, PS10, BRINTH 2, SAM 13, PS 23, PS 21 × S1, PV 5, SAM 5, S1 × GG TYPE 2, HY 16, BABU 2, PS 9 × GG TYPE 2, GG × PV1 TYPE 3, PS 12, PV 35, PRO 107, PS 2, ASK 2, SAM 16, PS 9 × GG TYPE 3, BABU 3, ASK 4, BEP 2 & BRINTH 8
III	14	SAM 6, SAM 8, HY 17, BRINTH 5, ASK 1, ASL 5, ICRI 2, SAM 3, SAM 4, PPK 2, GG x PV1 TYPE 2, ELARAJAN 2, PV 1 × GG TYPE1 & MCC 34
IV	2	PS 1 & CLONE SELECTION
V	2	BRINTH 4 & SAM 12

Fig.1. Cardamom accessions used for volatile oil estimation by hydro-distillation method

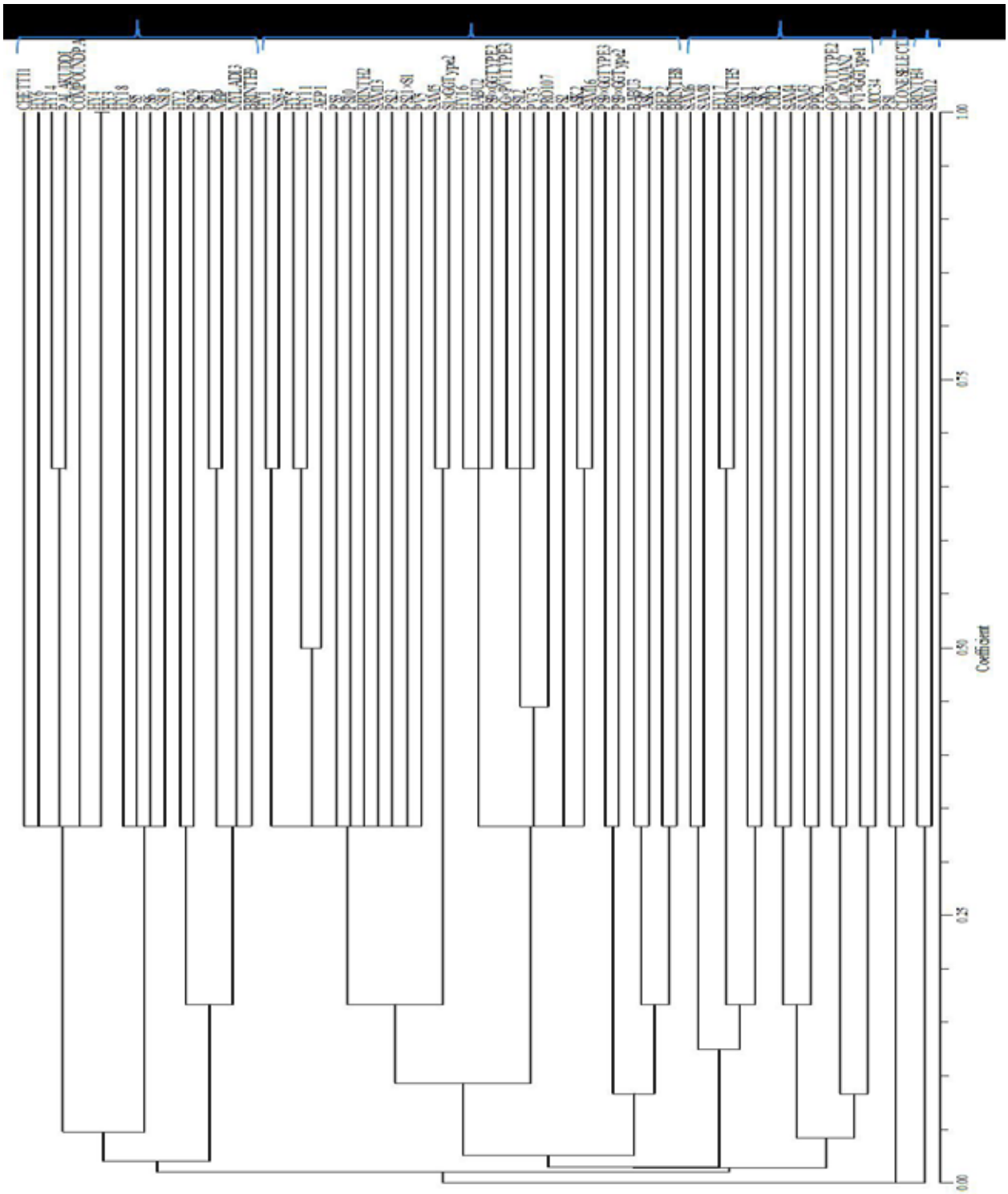

Fig 2. UPGMA dendrogram of genetic similarities and distance among cardamom accessions

similarity coefficient ranged from 0.00 to 1.00. At 50% variation, the dendrogram separated the 65 cardamom genotypes into 5 major clusters: Cluster I, II, III, IV and V consisted of 17, 30, 14, 2 & 2 accessions respectively **(Table 2)**. The lowest oil content and the lower number of seeds per capsule accessions namely, BRINTH 4 & SAM-12 and PS1 & CLONE SELECTION were grouped in two separate clusters IV and V, respectively. However, the highest oil content accessions SAM-5 and S1 × GG Type-2 were separately sub-grouped in a cluster II.

Conclusion of present study is the accessions HY-3, HY-4, SAM-5 & S1 x GG Type-2 have recorded the highest number of seeds per capsule and positively correlated with higher oil levels. These identified high volatile oil content cardamom accessions could be used as breeding material for developing varieties with high oil content as well as yield. From the study, HY-3 and HY-4 and S1 × GG were identified as superior clones in terms of higher yield, volatile oil content and optimum moisture content. Therefore, they can be successfully incorporated in future breeding for enhancing the cardamom oil content. UPGMA based cluster analysis had 50% variation and 65 genotypes were grouped in to 5 major clusters. Future studies are need to be focused on the investigation of phytochemical variation as well as profiling of volatile oil for identifying the better clones having biologically active and potential compounds that can be used in food and nutraceutical industries.

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