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Traditional knowledge and field marker development for essential oil content using peltate gland trichome and leaf colour in basil (*Ocimum basilicum* L.)

Parmeshwar Lal Saran*, Ram Prasanna Meena & Kuldeepsingh A

Kalariya ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi 387 310, Anand (Gujarat), India
E-mail: plsdehradun@gmail.com

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Twenty-six accessions of sweet basil collected from different parts of the country were observed for morphological and quantitative parameters. These accessions were recorded for oil gland type, size of peltate gland (PG), sepal, flower colour, stem girth, branching pattern, growth habits and leaf colour. In addition to that, accessions were observed for herbage parameters viz., maximum plant height, plant spread, stem girth, fresh leaf, dry leaf, herbage and oil yield, and DOB-8W was found superior as compared to checks. Maximum number of PGs on abaxial leaf as compare to adaxial leaf surface were recorded at all the three stages (young, mature and old based on leaf position on axis). Though, formation of PGs takes place in younger leaves only. Number of PGs on abaxial surface of young leaf, green leaf colour and oil content in leaf has positive relationship. The leaf colour intensity (purple) is to be marker character for evaluation of large number of accessions for oil content.

Keywords: Essential oil, Field marker, Leaf colour, *O. basilicum*, PGs

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Basil (*Ocimum basilicum*) is an aromatic perennial herb. It is used as a condiment and traditional medicament for several human ailments from prehistoric period in India. The genus *Ocimum* comprises about 150 species, of which *O. basilicum* L. is most widely grown throughout the globe for its green and aromatic herbage and also used for extraction of essential oil[1,2]. Sweet basil is cultivated in different climatic and ecological conditions with annual temperature between 6 to 24°C and receiving 500 to 8000 mm annual precipitation, the most favourable conditions are found in countries with a warm climate[3]. A characteristic chemical feature of the plant family *Lamiaceae* is lipophilic flavonoid aglycones secretion on leaf surface. These secretions protect the plant against harmful UV radiation, grown in wild, arid and semi-arid regions[4,5]. Another feature of this plant family is the possession of specialized glands, known as leaf trichomes (glandular), succulent stem, sepal, floral surfaces etc. These carpal and vegetative parts have several types of structures that include hairy trichomes, peltate and capitates glands[6] and filled largely with mono-terpenoid containing essential oil[7]. The capitate glands are numerous and small in size than peltate glands (PGs) on leaf surface, therefore, can't store methyl-chavicol compounds[8]. Among them PG takes major role for essential oil and natural compounds production from essential oil. The crop is commercially grown for production of fresh herbage (leaf, flower, tender branches) and dry herbage, which are natural source of essential oils[9]. The essential oil yield varies from 0.17 to 0.70%, based on ecological and agronomical conditions[2]. The peltate glands contain the enzymes catalyzing the first and last steps in the synthesis of the methylated phenyl propene methyl chavicol at much higher levels than elsewhere in the leaf[8]. The essential oil from different plant parts performs as protectant against herbivores and pathogens[10]. The secondary metabolites in secreted product are of the attention for flavouring, pharmaceutical, pesticides and fragrance industries[11]. Variation for morphological and chemical compositions of oil were also reported in basils[12-14], but no comprehensive studies have been carried out for herbage yield, oil yield number of PGs on leaf surface, disease susceptibility and field marker for essential oil content. This study was undertaken using traditional

*Corresponding author

knowledge in selection of superior accessions for high herbage, essential oil yield and quick screening through counting the PGs and leaf colour under field condition for oil content in fresh herbage.

Materials and Methods

Experimental site

The experiment was conducted at ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat during 2018. The research trial located at 22°35'N and 72°55'E at 45.1 m above mean sea level. The soils of experiment were sandy loam. The crop was managed as per standard horticultural practices. About six irrigations and three hand weeding were done.

Plant materials

Seeds of twenty-four sweet basil accessions were collected from Gujarat, Rajasthan and Haryana, India, were conserved and compared with two checks "GAB-1" (variety) and "DOB-1" (registered germplasm) in the present study. Pure seeds have been collected from different isolations of previous years. The nursery was raised and after forty-five days after seed sowing the seedlings were transferred in to the field at 54 cm × 45 cm. Standard agronomical practices were followed for raising of basil crop. Harvesting was done during first week of October, 2018. Green herbage was procured replication wise for essential oil extraction at full flowering stage. Field observation for growth parameters contributing in herbage yield and oil yield were recorded. Thirty-five plants in each replication were harvested for the same. The replication wise mean of each accession or parameters were carried out. The growth parameters like plant height, plant spread, plant weight, number of branches, number of leaves per plant, weight of leaves, stem diameter, root length, root weight, stem weight, dry leaf weight, dry stem weight, dry root weight and leaf area were observed at three months after planting at full bloom stage. The fresh leaf yield, dry leaf yield, fresh herbage yield and oil yield were measured using standard methods. The tagged plants were harvested for essential oil extraction during November-December months of year 2017 and 2018. The distinct morphological characters (PGs size, branching, growth habit/canopy type and calyx with flower colour) were observed at onset of flowering (50%) and stem girth at 90 days after planting. The trichome density was measured from branches (5 cm below the top) at onset of flowering (50%). The value of different parameters was obtained from three plants and averaged out.

Disease severity on basil accessions were observed considering whole plant as unit. The variable measures of disease were observed on 10 plants of each accession of accessions. The categorization of accessions was determined as per disease severity according to infested area and value assigned 0-4 as described (Table 1). The disease severity (DS) was calculated as per below given formula:

$$DS: \frac{\text{Some of individual ratings} \times 100}{\text{Number of leaves observed} \times \text{Maximum disease grade}}$$

Counting of PGs and correlation

Leaves were collected from experimental field during 2018-19 from main crop (first week of October) and from ratoon crop (first week of December 2018-19) at three stages viz., young (fully expanded), mature (dark colored) and old (before yellowing) leaves and from three different positions from tip of the axis to 2nd; 3th to 4th and 5th to 6th internodes, respectively for counting the PGs. These leaves were used for counting number of PGs in 0.5 mm^2 area from middle portion of leaves under light microscope at 10X visualization for both the surface (abaxial and adaxial). The correlation coefficient was worked out using SPSS program between number of PGs in young leaves and oil content. The genotype by trait biplot and parameter relationship analyzed by using statical software PAST (ver.2.17c).

For development of color scale on leaf colour intensity, a panel of five scientists were constituted. The observations were taken from experimental field

Table 1 — Description of disease infection type, scoring scale and host reaction.

Host reaction	Scoring scale	Description
Highly resistant (HR)	0	Leaves free from infection or 0 to 5% leaf area with lesions
Resistant (R)	1	Small irregular dark spots covering 6-10% leaf area
Moderately Resistant (MR)	2	Lesions enlarging, irregular dark brown spots covering 11-25% leaf area
Moderately Susceptible (MS)	3	Lesions coalesce and appears necrotic blackish spot covering 26-50% leaf area
Susceptible (S)	4	Lesions coalesce to form irregular and appears anthracnose symptom covering >50% leaf area

for leaf colour intensity[2]. The intensity of leaf color (purple) in mature leaves considered main features was experienced. The main aim was for field marker or morphological markers development. The degree of colour was apparent as critical factor for initial screening of accessions on the basis of oil content. The scoring procedure was excellent with the help of check list (0 to 3) put at proper stage for purple colour intensity of leaves. After emplacement the particular trait, selected accessions were analyzed for essential oil content in fresh herbage. The particular ranks were confirmed with given scores on majority basis.

Statistical analysis

The statistical data analysis was done by standard statistical procedures by using statistical software SAS 9.2 in randomized block design (RBD) for the different parameters of experiment. DMRT comparisons for the different growth attributing parameters, herbage yield as well as essential oil yield estimated from the accessions including checks. The results were presented at 5% level of significance (p=0.05). The critical difference (*CD*) values between different accessions were calculated to compare the various treatment means.

Results and Discussion

Distinct morphological characters

Selected sweet basil accessions were observed through naked eyes by traditional knowledge and validated by microscope for morphological variability with respect to crop improvement (Table 2 and Fig. 1). Morphological variations for PGs size, sepal and flower colour, stem girth, branching pattern and growth habit were reported (Table 2). The accessions DOB-13 for having larger size of PGs, Karam-1 for maximum number of branches, Sant-1 for erect and closed type canopy and DOB-8w for maximum plant height, spread, stem girth and white flower with green calyx colour as compared to checks were observed. The variation for morphological characters including flower colour (white, pink and violet) were reported in basil accessions[2,9]. All the accessions also have been observed for trichome / hairs variations on branch surface and AOB-3S(O) observed for glandular hairs and DOB-1 for glabrous (smooth) branch (Fig. 2). The diversity for secretory trichomes in *Lamiaceae*, was also reported[15].

Different sweet basil accessions were observed for yield and growth parameters (Table 3). The plant height, plant spread and plant weight were found maximum in DOB-8W (108.80 cm, 0.58 m^2 and 1000 g), whereas a minimum in B-6 (49.50 cm, 0.13 cm^2

Table 2 — Sweet basil accession with distinct morphological characters.

Sr. No.	Accession	Distinct characters
1	DOB-8w	Green calyx with white flower colour, maximum stem girth with plant spread, high herbage yield.
2	DOB-13	Larger size of PGs in leaf (≈0.1 mm)
3	Karam-1	Leaf-green colour (Emerald), profuse branching (at base of leaf).
4	Sant-1	Growth habit-erect and closed type canopy.
5.	AOB-3S (O)	Glandular hair on branches

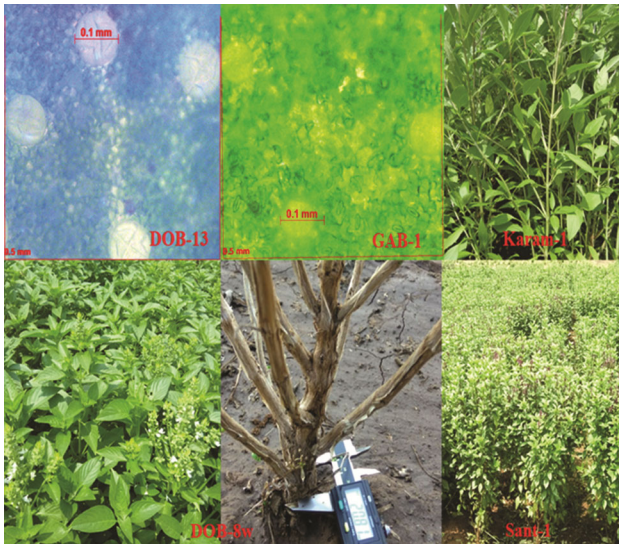

Fig. 1 — Sweet basil accessions with distinct morphological characters

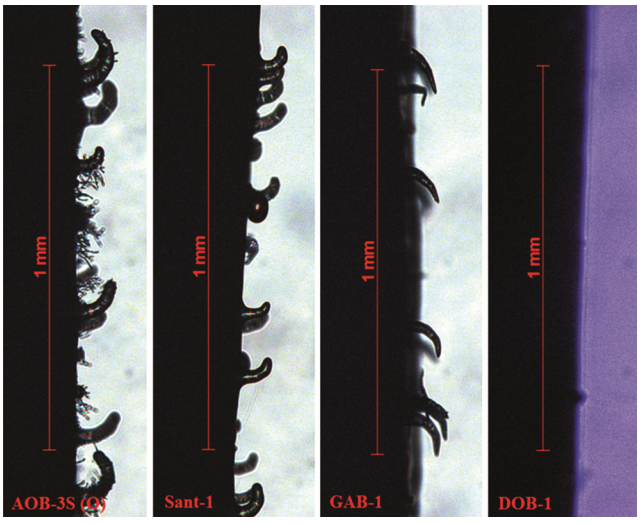

Fig. 2 — Sweet basil accessions with distinct trichome type and density on branches

Table 3 — Morphological variation for yield contribution traits in twenty-six accessions of sweet basil

Accession	Plant height (cm)	Plant spread (m^2)	Plant weight (g)	No. of branches/ plant	No. of leaves/ plant	Weight of leaves (g/plant)	Root weight(g/plant)	Root length (cm)	Stem diameter (mm)	Stem weight (g/plant)	Dry leaves weight (g/plant)	Dry stem weight (g/plant)	Dry root weight (g/plant)
DOB-1	75.00^{j}	0.32^{abcd}	470^{efghi}	13.60^{fghi}	1154.20^{klm}	180^{bc}	40.20^{efg}	7.54^{ab}	15.12^{bcdef}	220^{cdef}	31.40^{c}	57.00^{i}	11.80^{efgh}
DOB-2	98.60^{b}	0.44^{ab}	830^{ab}	16.20^{def}	2855.80^{cd}	260^{abc}	44.40^{def}	7.20 ^{ab}	16.30^{bcde}	490^{ab}	53.20^{a}	121.20^{b}	16.00^{d}
DOB-3	97.20^{b}	0.46^{ab}	770^{abc}	20.00^{bc}	3969.40^{a}	260^{abc}	52.40^{c}	8.36^{a}	15.33^{bcdef}	400^{abcde}	43.00^{b}	89.20^{e}	17.00^{cd}
DOB-4	71.00^{k}	0.29^{bcd}	490^{defgh}	12.00^{ghij}	2070.00^{f}	170^{bc}	33.50^{ghij}	7.25^{ab}	15.33^{bcdef}	270^{bcdef}	27.50^{cd}	72.50^{g}	11.50^{efgh}
DOB-5	91.20^{c}	0.39^{abcd}	550^{cdef}	14.80^{fghi}	1654.20^{ghi}	130^{bc}	31.20^{hijk}	7.54^{ab}	14.39^{bcdef}	300^{bcdef}	26.40^{cd}	71.60^{g}	11.00^{efgh}
DOB-8W	108.80^{a}	0.58^{a}	1000^{a}	15.80^{defg}	1912.40^{fg}	280^{a}	83.20^{a}	7.94^{ab}	20.74^{a}	580^{a}	51.60^{a}	146.00^{a}	27.80^{a}
B-1	81.20^{ghi}	0.40^{abc}	760^{abcd}	20.00^{bc}	2850.80^{cd}	250^{bc}	76.20^{b}	7.52^{ab}	17.52^{bc}	410^{abcde}	42.00^{b}	98.00^{d}	22.00^{b}
B-2	64.50^{m}	0.23^{bcd}	380^{efghi}	11.50^{ijk}	1399.00^{ijk}	140^{bc}	46.00^{cde}	7.35^{ab}	12.95^{fgh}	180^{def}	27.50^{cd}	44.50^{j}	19.00^{c}
B-3	67.50^{lm}	0.29^{bcd}	400^{efghi}	13.50^{fghi}	933.00^{mn}	140^{bc}	32.50^{hij}	6.25^{abc}	15.38^{bcdef}	200^{def}	26.50^{cd}	45.00^{j}	6.50^{kl}
B-4	55.00^{o}	0.17^{cd}	220^{hi}	12.00^{ghij}	632.00^{o}	070^{c}	28.50^{ijkl}	5.25^{bc}	11.10^{ghi}	110^{f}	14.50^{f}	29.00^{lm}	11.50^{efgh}
B-6	49.50^{p}	0.13^{d}	190^{i}	13.50^{fghi}	670.50^{no}	70^{c}	15.50^{m}	8.55^{a}	8.51^{i}	100^{f}	12.00^{f}	21.50^{n}	5.00^{l}
B-7	70.00^{kl}	0.26^{bcd}	330^{efghi}	16.00^{def}	1608.00^{hi}	140^{bc}	23.50^{l}	7.40^{ab}	10.94^{hi}	160^{ef}	24.00^{cde}	37.50^{k}	7.50^{jk}
B-8	65.50^{m}	0.31^{bcd}	260^{ghi}	15.50^{efgh}	1512.00^{ij}	90^{bc}	25.50^{kl}	6.00^{abc}	10.98^{hi}	140^{ef}	17.50^{ef}	33.00^{kl}	7.00^{kl}
B-9	54.50^{o}	0.20^{bcd}	270^{fghi}	8.00^{k}	644.00^{o}	100^{bc}	45.50^{cde}	6.10^{abc}	13.19^{defg}	110^{f}	15.50^{f}	26.00^{mn}	10.00^{ghij}
B-13	59.00^{n}	0.21^{bcd}	330^{efghi}	9.50^{ijk}	766.50^{no}	100^{bc}	33.00^{hij}	4.00^{c}	13.06^{defg}	170^{def}	19.50^{def}	43.50^{j}	13.50^{e}
B-14	78.00^{ij}	0.46^{ab}	460^{efghi}	15.00^{efghi}	1279.00^{ijkl}	160^{bc}	30.50^{ijkl}	7.10^{ab}	13.26^{defg}	260^{bcdef}	29.50^{c}	66.00^{h}	10.00^{ghij}
Badrinath-1	75.50^{j}	0.28^{bcd}	430^{efghi}	14.00^{fghi}	1210.50^{klm}	150^{bc}	33.00^{hij}	6.75^{ab}	14.26^{cdefg}	220^{cdef}	30.50^{c}	56.50^{i}	12.50^{efg}
Dedia-1	87.00^{de}	0.42^{abc}	770^{abc}	20.50^{bc}	2682.50^{d}	270^{ab}	28.00^{jkl}	6.15^{abc}	15.06^{bcdef}	440^{abcd}	46.50^{ab}	107.50^{c}	10.50^{fghi}
OB-10	89.00^{cd}	0.38^{abcd}	530^{cdefg}	19.50^{cd}	2355.00^{e}	160^{bc}	52.00^{c}	7.00^{ab}	16.66^{bcd}	260^{bcdef}	29.00^{c}	56.50^{i}	18.00^{cd}
JHU-1	98.40^{b}	0.39^{abcd}	840^{ab}	20.60^{bc}	2597.33^{de}	250^{bc}	47.60^{cd}	6.84^{ab}	17.62^{b}	480^{abc}	46.40^{ab}	124.00^{b}	16.20^{d}
Sant-1	86.20^{def}	0.29^{bcd}	440^{efghi}	15.40^{efghi}	1815.40^{fgh}	110^{bc}	24.40^{kl}	6.46^{abc}	13.66^{defgh}	270^{bcdef}	23.20^{cde}	80.80^{f}	8.20^{ijk}
Srinagar-1	80.40^{hi}	0.44^{abc}	490^{defgh}	18.80^{cde}	2649.20^{d}	150^{bc}	24.20^{kl}	5.72^{abc}	12.96^{fgh}	260^{bcdef}	27.60^{cd}	58.40^{i}	8.40^{ijk}
Karam-1	83.00^{fgh}	0.27^{bcd}	540^{cdefg}	28.60^{a}	3080.40^{bc}	150^{bc}	38.20^{fgh}	7.56^{ab}	13.13^{defg}	300^{bcdef}	29.60^{c}	75.20^{g}	13.00^{ef}
SEL-3C	88.80^{cd}	0.19^{bcd}	470^{efghi}	11.80^{hij}	1077.60^{lm}	150^{bc}	36.40^{ghi}	7.32^{ab}	16.92^{bcd}	240^{bcdef}	27.20^{cd}	56.40^{i}	9.80^{hij}
GAB-1	84.20^{efg}	0.38^{abcd}	860^{ab}	23.40^{b}	3292.20^{b}	250^{bc}	51.80^{c}	7.16^{ab}	15.25^{bcdef}	490^{ab}	43.60^{b}	120.60^{b}	19.40^{c}
AOB-3S (O)	92.00^{c}	0.46^{ab}	600^{bcde}	23.40^{b}	3111.60^{bc}	170^{bc}	36.80^{ghi}	7.20^{ab}	16.00^{bcdef}	350^{abcdef}	39.60^{b}	92.80^{de}	12.60^{efg}

Means with the same letter (superscript) in the columns do not showing significantly different (p=0.05) – (Duncan Multiple Range Test).

and 190 g), respectively. Maximum number of branches per plant was found to be in Karam-1 (28.60) followed by GAB-1 and AOB-3S(O) (23.40), while the minimum was observed in B-9 (8), whereas highest number of leaves per plant was reported in DOB-3 (3969.40), while the minimum was observed in B-4 (632). The fresh leaf weight, root weight, stem diameter, fresh stem weight, dry stem weight and dry root weight per plant were found maximum in DOB-8W (280 g, 83.20 g 20.74 mm,580 g, 146 g and 27.80 g), while minimum in B-6 (70 g, 15.50 g, 8.51 mm, 100 g, 21.50 g and 5 g), respectively. The root length was found maximum in B-6 (8.55 cm), while the minimum in B-13 (4 cm). The dry leaves weight per plant was found maximum in DOB-2 (53.20 g) followed by DOB-8W and Dedia-1 (51.60 g and 46.50 g, respectively), while the minimum dry leaves weight was found in B-6 (12 g). The basil accession was categorized in dwarf, semi dwarf and vigorous based on plant growth parameters under Gujarat conditions[14].

The fresh leaf yield and green herbage yield were found maximum in DOB-8W (122.40 q $ha^{-1}$and 250.50 q ha^{-1}), while the minimum in B-6 (28.80 q ha^{-1} and 49.80 q ha^{-1}), respectively. Similarly, oil yield was observed maximum in DOB-8W (94.20 kg ha^{-1}), while the minimum in B-4 (11.36 kg ha^{-1}) from green herbage (Table 4). The diversity for morphological parameters has been reported earlier under Indian conditions[2]. Similarly, the variation was also reported growth parameters, herbage and oil yield. The Egyptian variety gave the higher herbage yield, while the French variety gave the higher essential oil yield[16].

The accessions were also evaluated for incidence of anthracnose disease incidence (Table 4) and categorized in different classes according to the host reaction to the pathogen. The accession OB-10 shows highly resistance reaction, while Jhu-1 observed most susceptible for basil anthracnose incidence.

Table 4 — Variation for different leaf, herbage and essential oil yield parameters in twenty-six accessions of sweet basil

Accession	Fresh leaf yield (q ha^{-1})	Green herbage yield (q ha^{-1})	Essential oil yield (kg ha^{-1})	Anthracnose severity	Host reaction
DOB-1	78.67^{d}	126.50^{cd}	78.38^{c}	15	R
DOB-2	114.75^{b}	222.80^{b}	20.94^{p}	45	MR
DOB-3	114.40^{b}	202.50^{b}	30.99^{m}	45	MR
DOB-4	74.36^{ef}	133.70^{cd}	28.60^{mn}	50	MR
DOB-5	56.58^{m}	122.20^{cde}	44.72^{j}	50	MR
DOB-8W	122.41^{a}	250.50^{a}	94.20^{a}	25	R
B-1	108.42^{c}	197.70^{b}	86.97^{b}	15	R
B-2	62.92^{k}	103.50^{def}	31.36^{m}	40	MR
B-3	63.58^{jk}	107.00^{def}	30.40^{m}	15	R
B-4	29.92^{r}	53.70^{hi}	14.71^{q}	55	MS
B-6	28.82^{r}	49.80^{i}	20.53p	15	R
B-7	59.62^{l}	95.30^{efg}	55.06^{f}	15	R
B-8	37.84^{q}	68.30^{ghi}	24.46^{o}	60	MS
B-9	45.54^{o}	69.50^{ghi}	27.46^{n}	55	MS
B-13	42.68^{p}	80.70^{fgh}	36.25^{l}	70	MS
B-14	69.74^{gh}	126.80^{cd}	79.65^{c}	15	R
Badrinath-1	66.88^{i}	115.70^{de}	54.39^{fg}	50	MR
Dedia-1	120.12^{a}	216.40^{b}	77.68^{c}	55	MS
OB-10	72.16^{fg}	130.20^{cd}	41.94^{k}	0	HR
JHU-1	109.38^{c}	215.20^{b}	92.95^{a}	85	S
Sant-1	48.66^{n}	108.90^{def}	52.29^{gh}	55	MS
Srinagar-1	67.76^{hi}	123.90^{cde}	49.30^{i}	35	MR
Karam-1	66.264^{ij}	131.90^{cd}	51.17^{hi}	35	MR
SEL-3C	66.18^{ij}	119.40^{de}	62.93^{e}	50	MR
GAB-1	110.62^{c}	218.60^{b}	74.74^{d}	60	MS
AOB-3S (O)	76.30^{de}	152.20^{c}	78.10^{c}	40	MR

Means with the same letter (superscript) in the columns do not showing significantly different (p=0.05) – (Duncan Multiple Range Test).

Counting and correlation study of PGs

The accessions have been observed sufficient variation for number of PGs on leaves at different positions and surface at full flowering stage (Table 5). The average maximum sum from young, mature and old leaf for number of PGs per 0.5 mm^2 area was found in DOB-5 (19.2), whereas minimum was found in OB-10 (8.3). On an average of PGs were found (2.5 and 3.4) in 0.5 mm^2 area in adaxial and abaxial side of young leaf, respectively. In mature leaf, number of PGs was observed (2.2 and 2.9), whereas, in old leaf 1.7 and 2.2 number of PGs were observed in adaxial and abaxial side, respectively. The variation for PGs in Lamiaceae was reported[15]. Overall, maximum number of PGs was observed (3.4, 2.9 and 2.2) in abaxial side of young, mature and old leaves, respectively. There are approximately twice as many glands recorded on the abaxial surface than the adaxial surface of peppermint[7]. The young leaves are biogenetically more active as compare to mature and old leaves for the amount of the essential oil in lemongrass[17]. In Japanese mint, individual leaves show a progressive decrease in oil percentage with age of the leaf[15]. The rate of initiation and the developmental progress of peltate glands in the *Lamiaceae* vary considerably between different regions of a leaf and depend to a large extent on the maturity of tissues[10]. Similarly, lower number of PGs was reported in most mature leaves as compare to young leaves of peppermint[7]. It means the PG formations takes place in young leaves and remains constant with progression of leaf age due to only leaf area expansion with age of leaf.

Accessions were evaluated for number of PGs on abaxial surface of young leaves only and oil content in fresh herbage for the purpose of field marker development (Table 6 and Fig. 3). The number of PGs in 0.5 mm^2 area and essential oil content (%) in fresh herbage were observed same trend in both the years. The positive and significant correlation between PGs

Table 5 — Variation for number of PGs in young, mature and old leaf in twenty-six accessions of sweet basil

Accessions	Young leaf		Mature leaf		Old leaf	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
DOB-1	2.40^{de}	6.00^{a}	1.67 ^{def}	3.40^{bc}	2.00^{ab}	1.73^{d}
DOB-2	1.00^{h}	1.33^{h}	1.57^{ef}	2.23^{e}	1.00^{c}	2.00^{cd}
DOB-3	2.00^{efg}	2.33^{gh}	1.00^{f}	2.00^{e}	1.40^{bc}	1.40^{de}
DOB-4	2.00^{efg}	2.60^{efg}	1.73^{def}	2.00^{e}	1.40^{bc}	1.40^{de}
DOB-5	3.40^{bc}	4.73^{b}	3.00^{ab}	3.40^{bc}	2.00^{ab}	2.73^{b}
DOB-8W	2.67^{de}	3.67^{bcde}	2.40^{bcd}	3.00^{cd}	2.00^{ab}	2.67^{bc}
B-1	3.40^{bc}	4.50^{bc}	2.67^{bc}	3.67^{bc}	1.00^{c}	2.00^{cd}
B-2	1.67^{fgh}	2.67^{efg}	2.67^{bc}	2.67^{de}	2.73^{a}	3.00^{ab}
B-3	2.33^{def}	4.33^{bc}	1.67^{def}	2.67^{de}	1.00^{c}	2.00^{cd}
B-4	3.00^{cd}	3.00^{defg}	3.00^{ab}	4.00^{ab}	1.00^{c}	1.00^{e}
B-6	3.00^{cd}	4.00^{bcd}	3.00^{ab}	3.00^{cd}	1.50^{bc}	1.50^{de}
B-7	1.50^{gh}	3.00^{defg}	2.00^{cde}	3.00^{cd}	2.00^{ab}	3.50^{a}
B-8	2.00^{efg}	2.00^{gh}	3.00^{ab}	3.00^{cd}	2.00^{ab}	2.00^{cd}
B-9	3.00^{cd}	3.00^{defg}	3.00^{ab}	3.00^{cd}	2.00^{ab}	2.00^{cd}
B-13	1.50^{gh}	2.50^{fg}	2.00^{cde}	2.00^{e}	1.50^{bc}	2.50^{bc}
B-14	2.50^{de}	3.50^{cdef}	3.50^{a}	4.50^{a}	2.00^{ab}	3.00^{ab}
Badrinath-1	2.00^{efg}	2.50^{fg}	2.00^{cde}	2.50^{de}	1.83^{b}	2.83^{b}
Dedia-1	2.67^{de}	3.50^{cdef}	2.00^{cde}	3.50^{bc}	1.50^{bc}	3.00^{ab}
OB-10	2.00^{efg}	2.33^{gh}	1.00^{f}	1.00^{f}	1.00^{c}	1.00^{e}
JHU-1	4.00^{ab}	4.00^{bcd}	2.00^{cde}	4.00^{ab}	1.00^{c}	3.00^{ab}
Sant-1	3.00^{cd}	4.33^{bc}	1.50^{ef}	2.50^{de}	2.67^{a}	2.00^{cd}
Srinagar-1	2.50^{de}	3.50^{cdef}	2.00^{cde}	2.50^{de}	1.50^{bc}	2.00^{cd}
Karam-1	2.50^{de}	4.00^{bcd}	1.50^{ef}	2.50^{de}	1.50^{bc}	2.50^{bc}
SEL-3C	4.33^{a}	4.67^{b}	2.00^{cde}	2.50^{de}	2.00^{ab}	2.00^{cd}
GAB-1	2.50^{de}	3.00^{defg}	2.00^{cde}	3.00^{cd}	2.00^{ab}	2.50^{bc}
AOB-3S (O)	2.50^{de}	4.67^{b}	3.00^{ab}	3.50^{bc}	2.00^{ab}	3.00^{ab}
Average	2.5	3.4	2.2	2.9	1.7	2.2

Means with the same letter (superscript) in the columns do not showing significantly different (p=0.05) – (Duncan Multiple Range Test).

Table 6 — Pearson's correlation matrix between essential oil in herbage and number of PGs in young leaf (abaxial) across the years

Year	Particulars	Essential oil content in herbage (%)
2017	PGs on young leaf (Abaxial)	0.642**
2018	PGs on young leaf (Abaxial)	0.622**

** Significant at 0.005 %

number in young leaf (abaxial) and oil content (%) in fresh herbage during 2017 and 2018 (0.642 and 0.622, respectively) were observed. The diversity among accessions were explained by GT biplot (Fig. 4). Variation for essential oil content in herbage may be due to change in weather parameters during crop season. The PCA analysis shows that two major group of traits namely oil content (%) and PGs on abaxial side of leave were very closely and positively associated. It is interesting to note that these two traits were plotted nearly at 90° with host reaction and disease severity which were negatively correlated and plotted nearly at 180°. Accessions B-3, DOB-8W, B-6, B-7, B-1, OB-10 and Srinagar-1 were plotted negatively with disease severity. The bar length of GT plot denotes their effect in deciding the dropping of an accession in PCA analysis. The essential oil content (%) and number of PGs in upper side of leaves showing positive relationship and accession SEL-3C, DOB-5, AOB-3S(O), B-14, Karam-1 and DOB-1 rich in essential oil or PGs content. Principal component analysis exposed that there were accessions DOB-1, OB-10, DOB-2 and Jhu-1 were most diverse accessions. Variations for number and type of glandes were reported in mint on the aerial epidermis of leaves, stems and floral organs[7]. Positive relationship between oil yield from leaves and number of PGs were reported in holy basil and sweet basil[9,18,19]. The

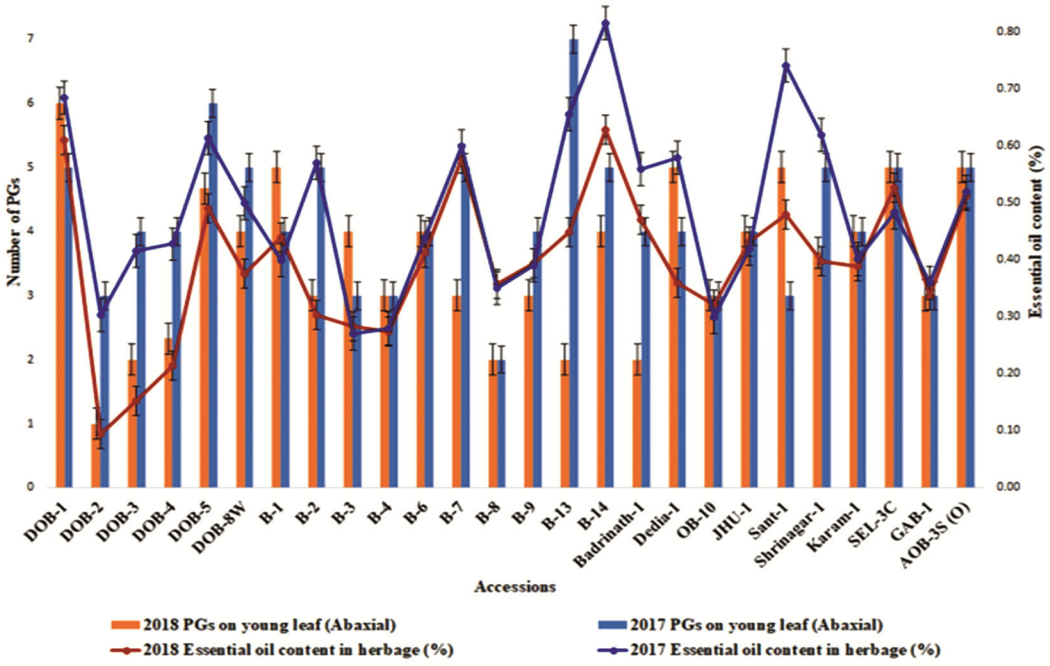

Fig. 3 — Relationship between number of PGs in abaxial side of young leaf and essential oil content in fresh herbage

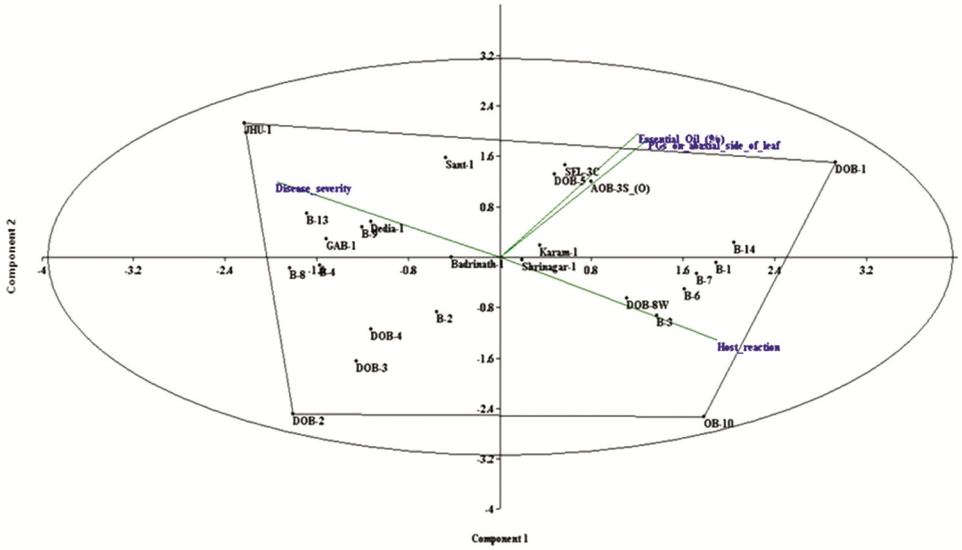

Fig. 4 — Genotype by trait biplots and trait relationship analysis

results revealed that the accession was easily screened for essential oil content under field condition by counting PGs on abaxial leaf surface through using compound microscope[19].

Sufficient diversity in basil was observed for leaf pigmentations; especially purple pigments are highly marketable for herbal and ornamental industries. The green leaf accessions were good source of essential oil, while greenish purple (DOB-3, DOB-4) and purple (DOB-2) leaf-colored accessions were comparatively low in essential oil (Table 7 and Fig. 5). Similarly, *Lamiaceae* viz., Rama (green colour) and Shyama (purple) tulsi were identified from long back for leaf colour variation[18-21]. Over all the correlation become field marker for essential oil production and screening of larger number of accessions.

Table 7 — Purple and green leaf colour intensity as a morphological trait for essential oil content in herbage.

Score	Accession	Leaf colour	Average oil content (%)
0	DOB-1, DOB-5, DOB-8W, B-1, B-2, B-3, B-4, B-6, B-7, B-8, B-9, B-13, B-14, Badrinath-1, Dedia-1, OB-10, JHU-1, Sant-1, Srinagar-1, Karam-1, SEL-3C, GAB-1, AOB-3S (O)	Fully developed leaves are green in colour on both sides	0.43
1	DOB-3, DOB-4	Adaxial and abaxial side is greenish purple	0.18
3	DOB-2	Adaxial side is purple and abaxial side is green with purple veins	0.09

Fig. 5 — Sweet basil accessions with distinct leaf colour traits

Conclusion

In conclusion the abaxial young leaf surface constitute maximum numbers of the peltate glands which are positively correlated with higher essential oil content. The number of PGs and oil content does not affect the anthracnose disease incidence. Therefore, harvesting of green herbage at premature stage for higher oil yield recovery by exploring the accessions of basil has more numbers of PGs on the leaf surface. The number of PGs and green leaf colour has positive relationship with essential oil content and vice versa with purple colour intensity of leaves.

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Conflict of Interests

The authors state that they have no any conflict of interest.

Authors' Contributions

PLS conceived, designed the experiments, collection of all accessions, multiplication, conservation, performed the experiments, data collection, interpreted the data, tables, figure's preparation and writing of manuscript. RPM executed the scale development and edit manuscript. KAK performed scale development for leaf colour and data analysis in this manuscript.

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