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Leaf biochemical constituents and its correlation with leaf oil percentage among sweet basil genotypes

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Abstract
Ocimum basilicum L. genotypes collected from NBPGR, New Delhi were analysed for its leaf biochemical constituents and correlation between them with percent oil from leaves on dry weight basis were studied and presented in this article. The maximum ascorbic acid content in leaf (91.95 mg 100 g^{-1}) was recorded in genotype IC326732 which was on par with IC110267 (90.60 mg 100 g^{-1}) whereas, IC344681 exhibited minimum (29.26 mg 100 g^{-1}). The highest β carotene in leaf (5.34 mg 100 g^{-1}) was recorded in IC110267 followed by IC326771 (3.58 mg 100 g^{-1}) while, genotype IC344681 exhibited lowest (0.76 mg 100 g^{-1}). Total phenols in leaf was recorded maximum in genotype IC395778 and minimum by IC344681 (260.81 and 84.25 mg 100 g^{-1}). IC395778 recorded the highest (4.44%) percent oil from leaves on dry weight basis followed by IC338959 (3.72%) whereas, the lowest (1.33%) was noticed in IC381552. The percent oil from leaf exhibited significant and positive correlation leaf chlorophyll content (0.668) whereas, phenols (0.775) and aroma score of leaves (0.775) showed highly significant positive association with leaf oil percentage on dry weight basis.

Keywords: Sweet basil, leaf biochemical traits, percent oil, correlation

Introduction
The use of plants as sources of medicines or human substance has been in vogue since ancient times. Large numbers of plants are utilized in various systems of medicine practiced in India and local health traditions for the treatment of human diseases since time immemorial. *Ocimum basilicum* Linn. is used for thousands of years in Ayurveda for its diverse healing properties [31]. *Ocimum* is a large genus belonging to the Lamiaceae family. The genus *Ocimum* includes 35-150 species of annual and perennial herbs and shrubs native in Asia, Africa, South and Central America, but widely distributed around the world. *O. basilicum* (sweet basil) is native to Asia [19]. It is to be found that the various *Ocimum* species are very much distinguished from each other. All the species are possessing different pharmacological activities since the huge variation in the chemical composition is there [32]. The volatile oil of sweet basil contains d-linalool and methyl chavicol as the major components. Other constituents in sweet basil leaf include protein (14%), carbohydrates (61%) and relatively high concentration of vitamin A and C. The herb, leaves and seeds are used medicinally in indigenous systems of medicine and homeopathy [9].
Although considerable research on the phytochemical analysis of sweet basil oil had been done but leaf biochemical constituent analysis is fewer therefore analysis of basil leaf biochemical composition has been the subject of considerable studies. There is extensive diversity in the constituents of the sweet basil leaf and several chemo-types have been established from various nutritional and phytochemicals investigations. The biochemical composition of sweet basil is highly complex, containing many nutrients and other biological active compounds in their leaves *viz.*, ascorbic acid, beta carotene, carbohydrates, proteins, phenols etc., thus nutritional properties of the whole herb in natural form gave the sweet basil importance as food and indicates its potential as a source of drugs and also perfumeries as it is rich in aromatic volatile oil. It is well known that the varieties, environmental condition and agricultural practices may significant influence on productivity, oil content, and bio chemical composition of basil leaf. The present study was aimed to evaluate the bio-chemical constituents in sweet basil leaves among different genotypes as preliminary investigation

under Godavari zone of Andhra Pradesh, India. Rather, character association study between leaf biochemical constituents with leaf oil percentage was also constituted with the data recorded to know the significant correlations and presented in this paper which may help the researchers in further investigations.

Material and Methods

An investigation entitled "Performance of sweet basil (*Ocimum basilicum* L.) genotypes in Godavari zone of Andhra Pradesh" was carried out during *kharif* season, 2018-2019 at COH, Venkataramannagudem, West Godavari district. The location falls under Agro-climatic zone-10, humid, East Coast Plain and Hills (Krishna-Godavari zone) with an average annual rainfall of 900 mm at an altitude of 34 m (112 feet) above mean sea level. The geographical situation is 16º 63' 120" N latitude and 81º 27' 568" E longitude. It experiences hot humid summer and mild winter. A total of thirteen sweet basil genotypes *viz*., IC110267, IC201233, IC281185, IC326732, IC326771, IC336833, IC338959, IC344681, IC369247, IC381552, IC395778, IC469923 and IC469938 sourced from NBPGR, New Delhi were taken for study. The experiment was laid out in RBD with three replications. The crop was raised at a plant spacing of 50 cm × 40 cm with plot size 7.2m^2. The seedlings were transplanted during August, 2018 and herb was harvested during 2nd fortnight of November. A basal fertilizer dose of 25 kg N, 15 kg P_2O_5 and 10 kg K_2O ha^{-1} was given at the time of soil preparation. One month old seedlings were transplanted and need-based plant protection measures were taken up to raise a healthy crop.

Essential oil percentage was recorded by using Clevenger apparatus [5] and expressed on dry weight basis by taking 100gram fresh sample for analysis. Chlorophyll content of randomly selected 20 leaves from each tagged plant was measured at harvest with the help of a SPAD Meter at full bloom stage and the average chlorophyll content of leaf was calculated. Carbohydrate, total phenol and protein content of leaves were estimated by using Anthrone reagent, Folin-Ciocalteau reagent and Lowry's method respectively [27]. Ascorbic acid and β carotene content of fresh leaf was estimated as suggested by Ranganna [26] and Srivastava and Kumar [30], respectively. Sensory evaluation was carried out on the fresh leaves using 10-man panel of judges from different departments of Horticulture. Aroma of leaves was rated separately on a scale of 1 to 5. Scores were defined as follows: 1 - dislike extremely, bad; 2 - like only slightly, tolerable; 3 - like, good; 4 – like very much, very good; 5 – like extremely, excellent. Numerical averages were then calculated for a composite test score.

The data obtained in respect to all the characters was subjected to the following statistical analysis. The data were analyzed by the methods outlined by Panse and Sukhatme [22] using the mean values of five random plants in each replication from all genotypes to find out the significance of genotypes effect. Correlations were worked out by using the formula suggested by Karl Pearson [16]. Significance of correlation coefficients was tested by comparing correlation coefficients with the table values [10] at n-2 degrees of freedom at 5% and 1% levels where 'n' denotes the total number of pairs of observations used in the calculation.

Results and Discussion

Leaf Chlorophyll (SPAD)

The leaf chlorophyll content varied significantly among the different genotypes (Table 1). Average chlorophyll content recorded by leaves was 34.22. The maximum total chlorophyll (41.55) content was recorded in IC110267 which was on par with IC326732, IC395778 and IC281185 (41.27, 37.62 and 36.90, respectively), but significantly superior to IC326771 (35.10). Whereas the lowest leaf chlorophyll (27.30) was noticed in IC381552.

β carotene in leaf (mg 100 g $^{-1}$)

The genotypes varied significantly in terms of leaf β carotene content (Table 1). Mean β carotene in leaves recorded was 2.88 mg 100 g^{-1}. The highest β carotene in leaf (5.34 mg 100 g^{-1}) was recorded in IC110267 followed by IC326771 (3.58 mg 100 g^{-1}). The genotype IC344681 exhibited minimum content of leaf β carotene (0.76 mg 100 g^{-1}) [6, 24].

Carbohydrate content in leaf (%)

Considerable variation was recorded for carbohydrate content among the genotypes (Table 1) with a mean 28.32% and the maximum carbohydrate content was observed in IC110267 (45.24%) which was on par with the genotype IC326732 and IC395778 (40.84% and 38.82%, respectively), but significantly superior to IC469923 (32.81%) while, the genotype IC469938was showing the lowest (17.38%) carbohydrate content [8, 13].

A perusal of the results in the contents of chlorophyll, carotene and carbohydrate revealed that those genotypes with higher leaf contents of chlorophyll and carotene pigments also had a higher content of carbohydrates which might be due to the reason that they had more quantum of photosynthetic apparatus in their leaves and *vice versa.* Similar results were obtained by [2, 20, 25, 31].

Total phenols in leaf (mg 100 g $^{-1}$)

Total phenols in leaf differed significantly among the genotypes and the mean recorded was 179.67 mg 100 g^{-1} (Table 1). The highest total phenols in leaf (260.81 mg 100 g^{-1}) was recorded in genotype IC395778 followed by IC326732 (250.87 mg 100 g^{-1}). The minimum phenolic content was recorded by IC344681 (84.25 mg 100 g^{-1}) [4, 14, 17, 21].

Ascorbic acid in leaf (mg 100 g $^{-1}$)

There were significant differences in ascorbic acid content of leaf among the different genotypes with mean 49.62 mg 100 g^{-1} (Table 1). The maximum ascorbic acid content (91.95 mg 100 g^{-1}) was recorded in IC326732 which was on par with IC110267 (90.60 mg 100 g^{-1}), but significantly superior to IC338959 (59.61 mg 100 g^{-1}). Genotype IC344681 exhibited minimum ascorbic acid content in leaf (29.26 mg 100 g^{-1}) [7, 15].

Aroma score for fresh leaves

The sensory evaluation of aroma in fresh leaves using 5-point hedonic scale revealed that there were significant differences among the various genotypes (Table 1) with a mean of 3.44. IC326732 genotype had the highest (4.89) aroma which was on par with IC338959 (4.78), but significantly superior to IC469938 (4.22). The least aroma was exhibited by the genotype IC344681 (1.56).

It is interesting to note from the results of the above parameters that those genotypes where the leaves were more aromatic had the higher contents of total phenols and ascorbic acid in their leaves. This is in support of the fact that the aromatic principles (methyl chavicol derivatives) are chemically phenolic in nature and the aroma is preserved by the presence of ascorbic acid and thus the availability of more phenols and ascorbic acid could have helped in maintenance of more aroma in the leaves of corresponding genotypes and *vice versa*. Similar significant differences in these parameters were reported in the earlier findings of [1, 8, 11, 23, 32].

Protein content in leaf (%)
The results on protein content revealed that there were significant differences among the various genotypes (Table 1) with a mean of 8.24%. IC395778 recorded maximum protein content in leaf (13.00%) followed by IC469938 (11.02%) whereas, IC369247 recorded minimum protein content in leaf (4.14%) [3, 18, 20, 28]. Also reported significant differences among the protein contents of genotypes which reveals that it might be a varietal character and governed by genotype.

Percent oil from leaves (Dry weight basis)
The variation was significant for percent oil from leaves (Table 1). The mean value recorded was 3.05%. IC395778 recorded the highest (4.44%) percent oil from leaves followed by IC338959 (3.72%), whereas the lowest percent oil from leaves (1.33%) was noticed in IC381552 [12] [29].

Character association among leaf biochemical constituents
Correlation coefficients among different biochemical constituents in leaves of sweet basil are presented in Table 2.

Leaf chlorophyll (SPAD)
Leaf chlorophyll content recorded significant positive association with carbohydrate content (0.577) and percent oil (0.668); highly significant positive association with β carotene (0.896), phenols (0.773), ascorbic acid (0.697) and aroma (0.687).

β carotene in leaf (mg 100 g $^{-1}$)
Carbohydrate (0.609), phenols (0.599) and ascorbic acid (0.566) recorded significant positive association with β carotene in leaf. Highly significant positive association was found with carbohydrates content in leaf (0.721).

Carbohydrate content in leaf (%)
This character exhibited significant and positive correlation with leaf chlorophyll (0.577), β carotene (0.609), phenols (0.571) and ascorbic acid (0.629).

Total phenols in leaf (mg 100 g $^{-1}$)
This trait had significant positive association with leaf β carotene content (0.599), carbohydrate content (0.571) and ascorbic acid content (0.556); highly significant positive association with leaf chlorophyll (0.773) aroma score of leaf (0.703) and percent oil from leaf (0.775).

Ascorbic acid in leaf (mg 100 g $^{-1}$)
Significant and positive association of this trait was observed with leaf β carotene content (0.566), carbohydrate (0.629), phenols (0.556), and aroma score of leaf (0.614) whereas leaf chlorophyll (0.697) showed highly significant positive association with ascorbic acid in leaves.

Aroma score for fresh leaves
The character exhibited highly significant and positive correlation with leaf chlorophyll content (0.687), and percent oil from leaf (0.755).

Protein content in leaf (%)
Protein content in leaf had no significant association with any of the biochemical traits studied.

Percent oil from leaves (Dry weight basis)
Leaf chlorophyll content (0.668) exhibited significant and positive correlation with percent oil from leaves whereas, phenols (0.775) and aroma score of leaves (0.775) showed highly significant positive association.
A perusal of results on association among the biochemical traits and oil percentage in leaves brings a fact into light that oil percentage was positively and significantly associated with chlorophyll content, phenols and aroma scores. This might be because of the reason that the total phenolic compounds in the leaf would have maintained a dynamic equilibrium with fatty acids and essential oil principles which were phenolic in nature; thus, maintaining both metabolites at high level since they might be involved in biosynthetic pathway of the aromatic principles.

Table 1: Leaf biochemical characters in sweet basil (*Ocimum basilicum* L.) genotypes

Genotype	Leaf chlorophyll (SPAD)	β carotene in leaf (mg 100 g^{-1})	Carbohydrate content in leaf (%)	Total phenols in leaf (mg 100 g^{-1})	Ascorbic acid in leaf (mg 100 g^{-1})	Aroma score of fresh leaves	Protein content in leaf (%)	Leaf oil percent (DWB)
IC110267	41.55	5.34	45.24	241.02	90.60	4.11	4.90	3.47
IC201233	29.24	3.20	31.95	120.48	31.91	2.44	9.82	2.54
IC281185	36.90	2.78	24.78	140.46	52.89	3.56	10.35	3.32
IC326732	41.27	3.23	40.84	250.87	91.95	4.89	8.97	3.22
IC326771	35.10	3.58	23.38	195.82	48.66	2.67	8.35	2.84
IC336833	34.34	2.85	17.38	164.09	33.11	3.22	8.32	3.16
IC338959	32.38	2.29	22.17	180.14	59.61	4.78	7.52	3.72
IC344681	28.91	0.76	18.54	84.25	29.26	1.56	7.88	1.33
IC369247	34.31	3.05	26.78	152.38	31.58	3.89	4.14	3.52
IC381552	27.30	2.05	27.96	104.74	47.61	2.22	5.08	1.33
IC395778	37.62	3.07	38.82	260.81	36.82	3.56	13.00	4.44
IC469923	32.25	2.36	32.81	223.99	47.51	3.33	7.78	3.45
IC469938	33.71	2.89	17.78	216.67	43.61	4.22	11.02	3.27
Mean	34.22	2.88	28.32	179.67	49.62	3.44	8.24	3.05
S Em±	1.35	0.06	2.39	0.34	0.53	0.2	0.24	0.22
CD (0.05)	3.95	0.16	6.96	0.98	1.56	0.55	0.70	0.63

Table 2: Correlation matrix among different leaf biochemical characters of sweet basil (*Ocimum basilicum* L.) genotypes

	A	B	C	D	E	F	G	H
A	1.000							
B	0.721**	1.000						
C	0.577*	0.609*	1.000					
D	0.773**	0.599*	0.571*	1.000				
E	0.697**	0.566*	0.629*	0.556*	1.000			
F	0.687**	0.463	0.311	0.703**	0.614*	1.000		
G	0.142	-0.106	-0.063	0.299	-0.199	0.063	1.000	
H	0.668*	0.513	0.341	0.775**	0.226	0.755**	0.343	1.000

A	: Leaf chlorophyll content (SPAD)	D	: Total phenols in leaf (mg 100 g $^{-1}$)	G	: Protein content in leaf (%)
B	: β carotene in leaf (mg 100 g $^{-1}$)	E	: Ascorbic acid in leaf (mg 100 g $^{-1}$)	H	: Percent oil from leaves (DWD)
C	: Carbohydrate content in leaf (%)	F	: Aroma score for fresh leaves		
*	Significant at 5% level of significance (significant correlation)	**	Significant at 1% level of significance (highly significant correlation)		

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