International Journal of Crop Science and Technology
IJCST
Volume 2, Issue 1, 2016, Page:32-44 ISSN: 2458-7540

# Population Polymorphism Study among Tulsi (*Ocimum* sp.) Ecotypes in Assam, India using Morphological and Biochemical Parameters

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**ABSTRACT**

The genus *Ocimum,* (family *Lamiaceae*), displays great variability. Prevalence of cross pollination, polyploidy and interspecific hybridization has complicated its taxonomy, making its systematic study difficult. This study investigated the morphological and biochemical variability among 18 ecotypes collected from different parts of the Assam state, India. Considerable diversity was found using all the approaches. The quantitative morphological parameters showed wide variability, not always co-relating with their geographical distances. The Euclidean distance ranged between 6.06 (between the closest accessions) and 59.00 (between the most distant accessions). Phylogenetic analysis divided the accessions into two major clusters sharing approx. 76% identity, and a minor cluster (with three accessions) sharing only approx. 65% identity with the other two. A great deal of diversity was found in the qualitative characters too; that divided the ecotypes into two to five groups. The biochemical characters also revealed wide polymorphism; the distance varied from 1.690 to 58.574. Similar to the morphological characters, the biochemical parameters too did not always correlate with geographical distances. The phylogenetic mapping divided the accessions into two major clusters, sharing approx. 80% similarity. Further work will be needed to correlate the distance based on the morphological and the biochemical characters.

**Key words:** Diversity, Morphological, Biochemical, Phylogeny, *Ocimum*, Tulsi

## INTRODUCTION

Tulsi is one of the most cherished medicinal herbs of India. Because of its popularity, tulsi (popularly called basil in English) is often referred to as ‘King of herbs’. Over 175 species of the genus *Ocimum* are reported world-wide. The genus is well represented in the warmer parts of both the hemispheres from sea level to 1800 meter with three centres of diversity (i) tropical and subtropical regions of Africa (ii) tropical Asia and (iii) tropical parts of America (Kewalanand 2007). The plant is distributed throughout India and Pakistan, as well as in Malayasia, Australia, the Philippines, Brazil, Western Asia and the Arab countries, Nepal and Egypt (Grieve 1992, Bhattacharjee 1998). There are three main varieties of Tulsi; namely, Rama Tulsi (*O. sanctum*), Krishna Tulsi (*O. tenuiflorum*) and Vana Tulsi (*O. gratissimum*) in India (Balaji *et al.* 2011).

A great deal of diversity exists in the habit, morphological and the biochemical characteristics among the tulsi ecotypes that have been shown by several groups of researchers. For example, Quereshi *et al.* (2011) extracted the essential oils from the leaves of three *Ocimum* species, *viz. O. gratissimum*, *O. sanctum* and *O. americanum*, and reported different oil content in them which was also subjected to GC and TLC profiling. Similar investigation had been reported the variations in the morphological characters to be useful in identification and evaluation of the plant (Choudhury *et al.* 2011, Gupta *et al.* 2011). Tewari *et al.* (2012) reported an *Ocimum* species, *O. kilimandscharicum* from western Himalayan region and evaluated certain nutritional characters among its population of *O. kilimandscharicum*. Analyses of diversity among tulsi ecotypes have also been done using molecular markers by several groups (Omidbaigi *et al.* 2010, Carovic-Stanko *et al.* 2011). However, there is little information available regarding the plant's diversity in India and in Asia; there is confusion regarding even its genome structure. The northeastern part of India, including the state of Assam, being a biodiversity hotspot, would be a good start point to study the diversity of the plant in the country as well as in the region. To our knowledge, this is the first report on the categorization of the morphological and biochemical parameters of the plant.

## MATERIALS AND METHODS

### Plant Materials and Growth Conditions

The plant materials were collected from 18 different districts of Assam and were named as accession (Acc) #1 to accession #18 (Table 1; Fig 1). Seeds were also collected for all the ecotypes, and were germinated in laboratory on moist filter paper in Assam Agricultural University (AAU), Jorhat. The seedlings were grown in the potting mixture prepared by mixing soil, sand and dried manure in the ratio of 2:1:1 v/v, and the plants were maintained in typical green-house conditions (16/8 hours of light/dark cycle and 22-25°C temperature) in the AAU facility. The morphological data were recorded at the vegetative (150 day-old) stage of the plants for the leaf and the stem characters; the inflorescence characters were taken at the reproductive stage, at the 200 day-old plants. For collection of the biochemical data, leaf samples were collected at the same stage and were processed for isolation and/ or quantification of the parameters.

### Morphological Parameters

Defined descriptors are not available in tulsi. Therefore, based on the visual observations and numerous published reports in similar (generally from the same family) plants, a total of 21 parameters were recorded; out of which 10 were quantitative parameters (Table 2) while 11 were qualitative parameters (Table 3). Each record was taken with five replications (five plants of each ecotype) for better reproducibility.

Table 1. Tulsi accessions collected from various geographical regions of Assam.

| Sl. No. | Places of collection | Accession number |
|---|---|---|
| 1. | Karimganj | Acc #1 |
| 2. | Sivasagar | Acc #2 |
| 3. | Morigaon | Acc #3 |
| 4. | Jorhat | Acc #4 |
| 5. | Narayanpur | Acc #5 |
| 6. | Karbi Anglong | Acc #6 |
| 7. | Tinsukia | Acc #7 |
| 8. | Nagaon | Acc #8 |
| 9. | Nalbari | Acc #9 |
| 10. | Majuli | Acc #10 |
| 11. | Udalguri | Acc #11 |
| 12. | Kamrup | Acc #12 |
| 13. | Biswanath Chariali | Acc #13 |
| 14. | Lakhimpur | Acc #14 |
| 15. | Golaghat | Acc #15 |
| 16. | Cachar | Acc #16 |
| 17. | Dhemaji | Acc #17 |
| 18. | Darrang | Acc #18 |

For better presentation, the quantitative characters were divided into three groups, viz, leaf characters (three parameters: number of leaves per plant, length of mature leaf and breadth of mature leaf), plant characters (four parameters: plant height, intermodal distance, petiole length and number of nodes per plant) and inflorescence characters (three parameters: number of spikes per plant, number of spikelet per spike and number of florets per spikelet). Out of the nine total qualitative characters that were analysed in the study, six were leaf-characters (viz, leaf colour, leaf pubescence, leaf pigmentation, leaf shape, leaf margin and leaf-vein colour), three were stem characters (viz, stem pubescence, stem angularity and stem colour) and two were inflorescence characters (viz, flower colour and calyx laceration). The categorization of the qualitative characters is shown in Table 3.

### Biochemical Parameters

As in case of the morphological parameters, biochemical descriptors also are not available in tulsi. Therefore, based on published reports on related and un-related herbaceous plants, four biochemical parameters, namely, the chlorophyll-a content, chlorophyll-b content, carotenoids content and crude oil content, were recorded in the study. As in case of the morphological parameters, biochemical parameters were also taken in five replications for better reproducibility.

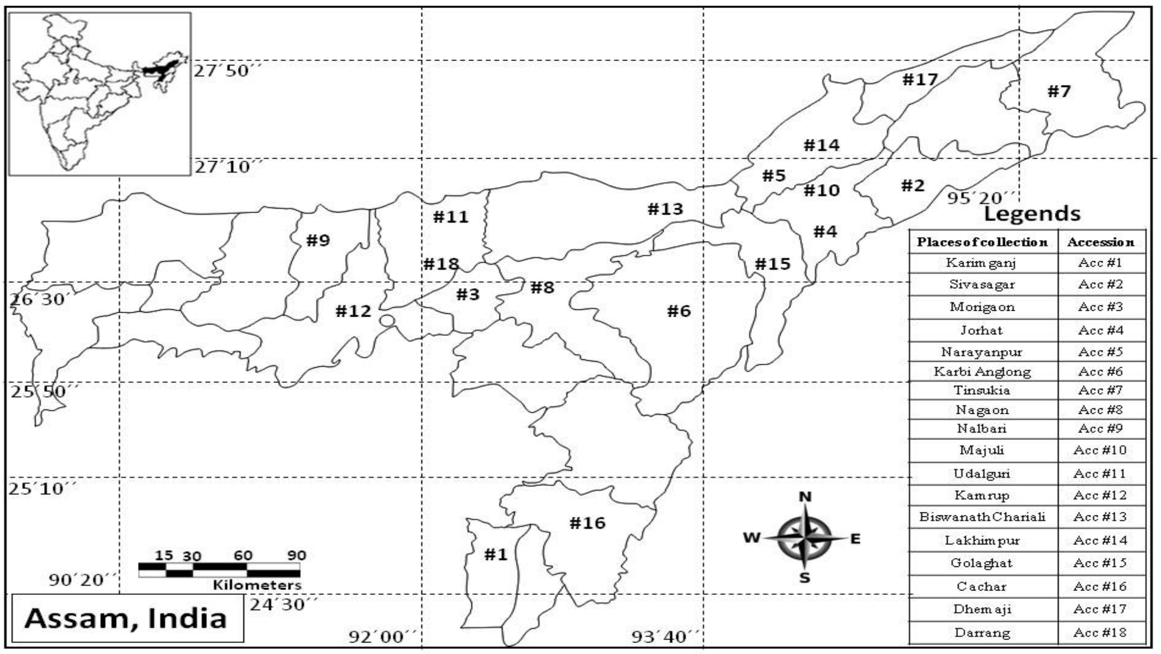


Figure 1. Map of Assam showing various locations of tulsi accessions.

Table 2. Quantitative characters recorded from tulsi accessions.

| Leaf characters | Plant characters | Inflorescence characters |
|---|---|---|
| Number of leaves per plant | Plant height | Number of spikes per plant |
| Length of mature leaf | Intermodal distance | Number of spikelet per spike |
| Breadth of mature leaf | Petiole length | Number of florets per spikelet |
| | Number of nodes per plant | |

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### Biochemical Methods

All the biochemical parameters were recorded following the standard biochemical techniques. Chlorophyll was estimated using the spectrophotometric method, given by Arnon (1949) and the final concentration of chlorophyll was calculated in milligram per gram of freshweight. Carotenoids were also extracted and estimated from the plant leaves using the standard procedure (Pepkowitz 1943) and the pigments were separated using calcium hydroxide columns. For extraction and estimation of the crude oil from the tulsi leaves, the standard procedure given by Soxhlet (1879) was followed. The percent oil content was calculated as follows:

$$\text{Percent oil content} = \frac{\text{Volume of oil extracted x 100}}{\text{Weight of the sample}}$$

Table 3. Qualitative characters recorded from tulsi accessions.

| Parameters | Classes | Accessions (Acc) |
|---|---|---|
| 1) Leaf colour | i) Yellowish green | Acc #2, #13 |
| | ii) Green | Acc #1, #3, #4, #10, #12, #16, #17, #18 |
| | iii) Dark green | Acc #5, #6, #7, #14 |
| | iv) Purple | Acc #8, #9, #11, #15 |
| 2) Leaf pubescence | i) Glabrous | Acc #1, #5, #6, #10 |
| | ii) Slight pubescence | Acc #3, #4, #7, #8, #9, #11, #12, #14, #15, #16, #17, #18 |
| | iii) Heavy pubescence | Acc #2, #13 |
| 3) Leaf pigmen-tation | i) Un-pigmented | Acc #1, #2, #5, #6, #11, #13, #14 |
| | ii) Slight pigmented | Acc #3, #7, #10, #12, #16, #17, #18 |
| | iii) Medium pigmented | Acc #9 |
| | iv) Heavily pigmented | Acc #8, #15 |
| | v) Slight changing to heavily pigmented | Acc #4 |
| 4) Leaf shape | i) Ovate | Acc #1, #3, #5, #8, #14, #15, #17, #18 |
| | ii) Rounded ovate | Acc #4 |
| | iii) Lanceolate | Acc #2, #6, #7, #9, #10, #11, #12, #13, #16 |
| 5) Leaf margin | i) Entire | Acc #3 |
| | ii) Serrate | Acc #1, #2, #5, #6, #8, #9, #10, #11, #12, #13, #14, #15, #16, #17, #18 |
| | iii) Sinuate | Acc #4, #7 |
| 6) Leaf vein colour | i) Light green | Acc #1, #5 |
| | ii) Green/Dark green | Acc #2, #3, #6, #10, #13, #16, #17, #18 |
| | iii) Purple | Acc #4, #7, #8, #9, #11, #12, #14, #15 |
| 7) Stem pubescence | i) Slight | Acc #1, #4, #6, #8, #9, #10, #11, #12, #14, #15, #16, #17, #18 |
| | ii) Heavy | Acc #2, #3, #5, #7, #13 |
| 8) Stem angularity | i) Cylindrical | Acc #1, #3, #4, #6, #7, #8, #9, #10, #11, #12, #14, #15, #16, #17, #18 |
| | ii) Triangular | Acc #2, #5, #13 |
| 9) Stem colour | i) Green | Acc #1, #2, #5, #6, #10, #13, #16, #17, #18 |
| | ii) Purple | Acc #8, #9, #13 |
| | iii) Green changing to purple | Acc #3, #4, #7, #11, #14, #15 |
| 10) Flower colour | i) White | Acc #2, #5, #13 |
| | ii) Light violet | Acc #1, #8, #9, #11, #15, #16, #17, #18 |
| | iii) Violet | Acc #3, #4, #6, #7, #10, #12, #14 |
| 11) Flower laceration | i) Lacerated | Acc #2, #5, #10, #12, #13 |
| | ii) Non-lacerated | Acc #1, #3, #4, #6, #7, #8, #9, #11, #14, #15, #16, #17, #18 |

## RESULTS AND DISCUSSION

### Variations of the Morphological Characters

A wide range of variations in all the morphological parameters, both qualitative and quantitative, were observed among the screened ecotypes of tulsi. The variations in the quantitative characters are presented in Fig 2 and in Table 4. Among the leaf characters, while the number of leaves per plant varied from 70 (Acc #1, Karimganj) to 12 (Acc #13, Biswanath Chariali), the length of mature leaves varied from a maximum of 6.2 cm (Acc #13, Biswanath Chariali) to a minimum of 2.1 cm (Acc #3, Morigaon; Acc #5, Narayanpur and Acc #6, Karbi Anglong) and the breadth of mature leaves varied from a maximum of 3.5 cm (Acc #11, Udalguri and Acc #13, Biswanath Chariali) to 1.3 cm (Acc. #1, Karimganj). Among the plant characters, the plant height showed a considerable variation from 39.34 cm (Acc #15, Golaghat) to 12 cm (Acc #12, Kamrup) and the internodal distance varied from 4 cm (Acc #4, Jorhat) to 1.28 cm (Acc #12, Kamrup). While the petiole length varied from a maximum of 2.3 cm (Acc #3, Morigaon) to a minimum of 0.84 cm (Acc #12, Kamrup), the number of nodes per plant showed a wide range, showing a value from 24 (Acc #8, Nagaon) to 6 (Acc #2, Sivasagar). In the same time, the inflorescence characters also revealed considerable variation among the three parameters recorded. While the number of spikes varied from 21.4 (Acc #11, Udalguri) to 5.6 (Acc #5, Narayanpur), number of spikelet per spike varied from 17.8 (Acc #3, Morigaon) to 10.4 (Acc #18, Darrang) and number of florets per spikelet from a maximum of 6 (Acc #11, Udalguri; Acc #12, Kamrup; Acc #15, Golaghat and Acc #18, Darrang) to a minimum of 4 (Acc #2, Sivasagar; Acc #3, Morigaon; Acc #4, Jorhat; Acc #7, Tinsukia and Acc #13, Biswanath Chariali). Using this dataset as input in the PAST software, a distance matrix was generated (Supplementary table 1) and the phylogeny was analysed (Fig 3) to check the closeness/ distantness of the collected tulsi ecotypes. The distance matrix showed that the distance between a pair of ecotypes showed a considerably wide range; the highest distance was found between Acc #1 (Karimganj) and #13 (Biswanath Chariali; 59.007), and the lowest distance was found between Acc #8 (Nagaon) and #15 (Golaghat; 6.056). In the phylogenetic map, it was found that the accessions were grouped into three major clusters and one outlier. While the cluster ‘A’ consisted of Acc #11 (Udalguri), #12 (Kampur) and #13 (Biswanath Chariali), cluster ‘B’ consisted of Acc #2 (Sivasagar), #3 (Morigaon), #4 (Jorhat), #5 (Narayanpur), #8 (Nagaon), #10 (Majuli) and #15 (Golaghat), and the cluster ‘C’ consisted of Acc #6 (Karbi Anglong), #7 (Tinsukia), #9 (Nalbari), #14 (Lakhimpur), #16 (Cachar), #17 (Dhemaji)and #18 (Darrang). Acc #1 (Karimganj) was an outlier, however, sharing more similarity to the cluster ‘A’. The clusters, ‘B’ and ‘C’ were approx. 24% distant from each other; while the outlier, Acc #1 (Karimganj) was approx. 30% distant from both of them. Cluster ‘A’ was approx. 35% distant from all of them. Both cluster ‘B’ and ‘C’ again had two sub-clusters each. The distance matrix and the phylogenetic map showed a similar patter, as expected.

The accessions were grouped according to the observed features of the qualitative characters; considerable variations were also found in them (Table 3). Among the six leaf characters, four types of leaf colour were found; yellowish green, green, dark green and purple. The maximum number of samples (eight) showed the green colour, while dark green and purple colour was shown by four samples each, and two samples showed yellowish green colour. Considering the pubescence of the leaves, 12 out of the 18 accessions showed slight pubescence, four showed no pubescence (glabrous) and two showed heavy pubescence of the leaves. The leaf pigmentation showed five groups; while seven accessions each were found to be un-pigmented or slightly pigmented, two were heavily pigmented and one accession was medium pigmented; one accession showed a peculiar kind of pigmentation, i e, while no pigmentation was seen on the young leaves, mature leaves gained pigmentation. The leaf shape was either ovate or rounded ovate or lanceolate; only one accession had rounded ovate, eight had ovate and nine accessions had lanceolate leaves. Leaf margins also showed three groups; entire-type, showed by single accessions, serrate-type, showed by 15 accessions and sinuate-type, showed by two of the accessions. The leaf vein-colour was found to be either light green, showed by two accessions or green, showed by eight accessions or purple, showed by eight accessions.

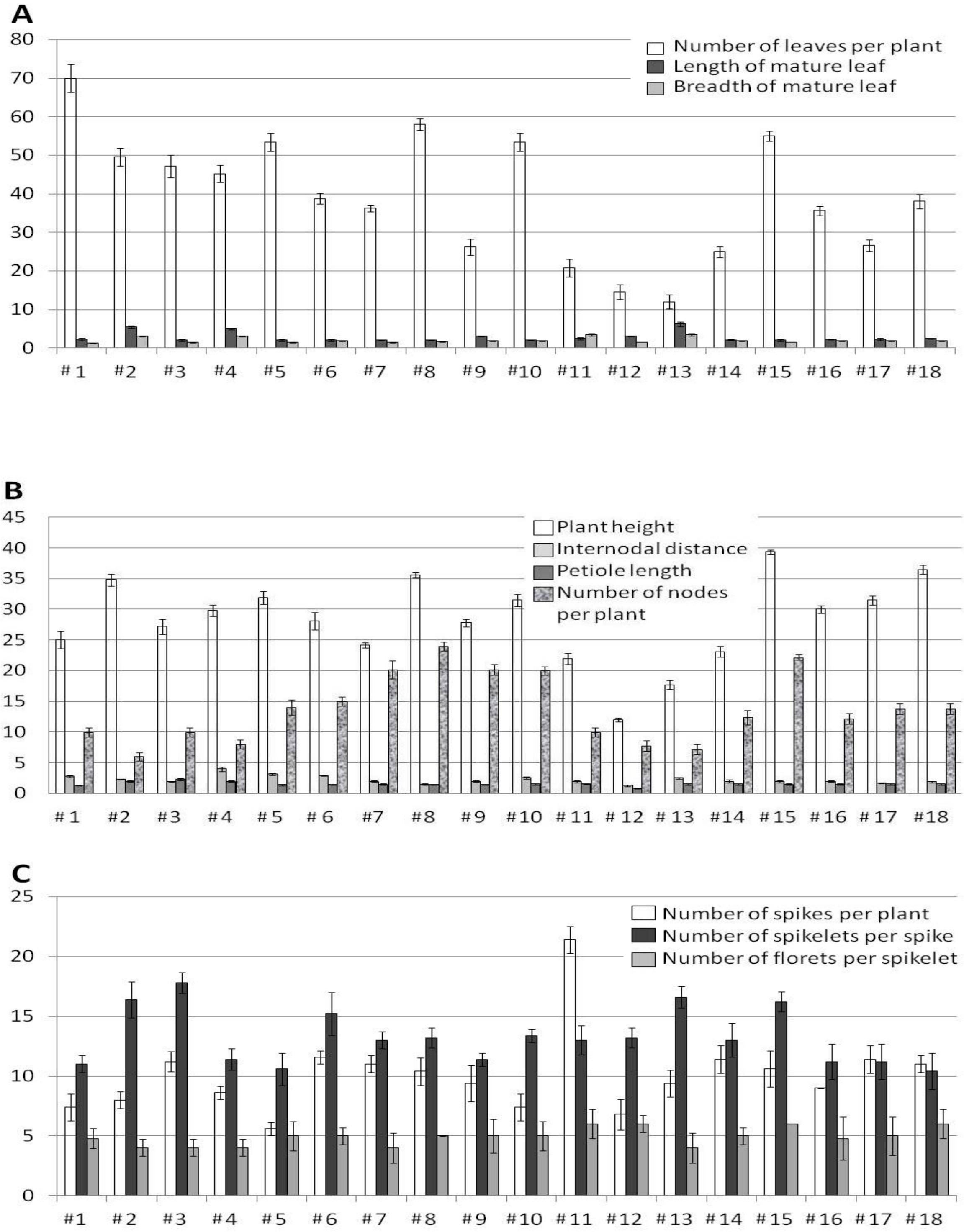


Figure 2. Variations in tulsi characters (#1 to #18). A. Leaf characters (Length and breadth are in cm). B. Plant characters (Plant height, intermodal distance and petiole length are in cm. C. Inflorescence characters.

Three stem characters were screened in the study; among them, considering the stem pubescence, two types were found – slight, showed by 13 accessions and heavy, showed by three. Similarly, the stems were either cylindrical, as in case of 15 of the accessions or triangular, as in case of three accessions.

Considering the stem colour, green-coloured stem was found in nine of the accessions and purple-coloured stem was found in three of them. On the other hand, six accessions showed a peculiar kind of colouration of the stems, the young stems showed green colour which changes to purple as it matures.

Considering the flower-characteristics, the colour of the flowers were either white, showed by three accessions or light violet, showed by eight accessions or violet, showed by seven accessions. Similarly, the flowers were lacerated, as found in case of five accessions or non-lacerated, found in case of 13 accessions.

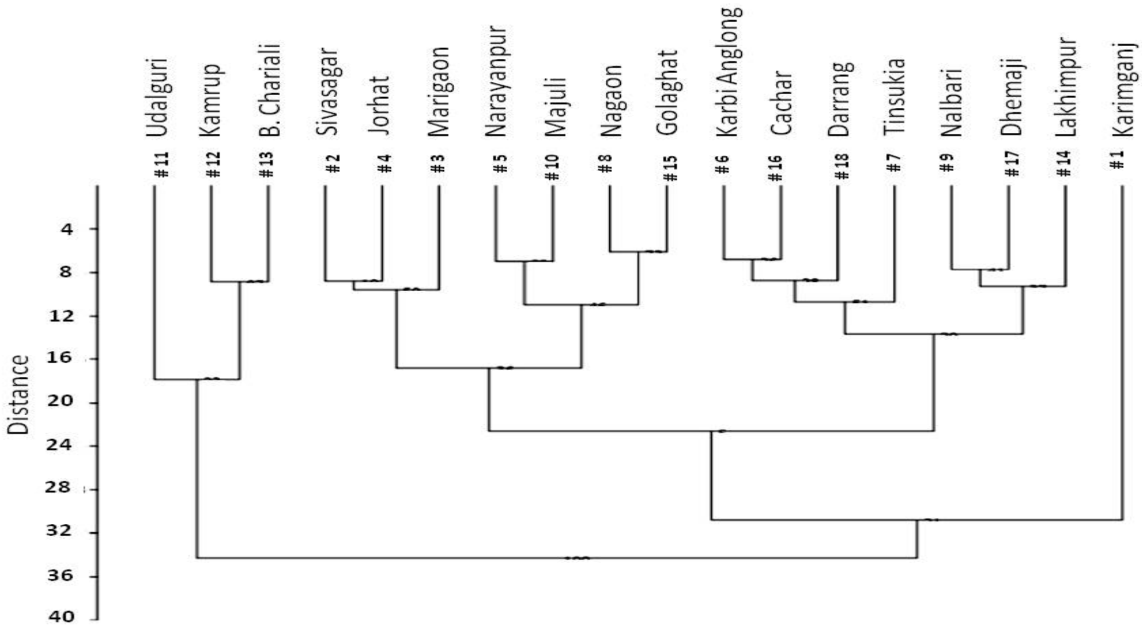


Figure 3. Phylogeny of the tulsi (Acc #1 to #18) based on the quantitative morphological parameters.

The wide range of variation observed among the ecotypes in the morphological characters (both quantitative and qualitative) is a reflection of existence of broad polymorphism among the ecotypes of tulsi in Assam. However, all the characters did not show a similar range of variation; for some of the characters, the range of variation was much wider than the same for some other characters. For example, characters like number of leaves per plant (12 to 70), number of nodes per plant (6 to 24) and number of spikes per plant (5.6 to 21.4) showed a very wide range; on the other hand, number of spikelets per spike (10.4 to 17.8) showed a relatively narrower range. Nevertheless, the variations in all the characters were considerably wide. Being a sexually propagated herb, the wide variation in the tulsi population is expected. Besides, the wide variation among its population could also be accounted for the fact that the south-east Asian region is a known place of origin/ diversity of the plant (Kewalanand 2007). Morphological data has previously been used for study of genetic diversity and taxonomic relationships of many plants (Bult and Kiang 1992, Zviniene and Pank 1996). Meanwhile, *Ocimum* species show high level of variation in morphological traits. Morales *et al.* (1993) reported that there are many cultivars of tulsi (basil), which vary in their leaf size and colour (green to dark purple), flower colour (white, red, lavender and purple), growth characteristics (shape, height and flowering time) and aroma. High level of variation in morphological traits of *Ocimum* species have been reported by other workers as well (Javanmardi *et al.* 2002). Morphological differences had also been reported between Sweet and Genovese basil (tulsi); while Sweet basil exhibited green leaf color, Genovese basil showed purplish white leaves (Nazim *et al.* 2009). The same group also reported differences in their leaf margins. Svecova and his groups (2010) studied 34 cultivars of basil (tulsi) using 13 morphological descriptors including plant-, leaf- and inflorescence characters; they observed that the studied basil cultivars displayed a wide diversity of morphological characteristics. In a similar study of identification of *O. basilicum* L. accessions, carried out by Carovic-Stanko *et al.* (2011), it was concluded that morphological markers provide an inexpensive and reliable method for routine screening of a large number of accessions, as well as to map phylogeny among tusli germplasms.

Table 4: Variations in the quantitative morphological characters of tulsi ecotypes.

| Acc | Leaf parameters | | | Plant parameters | | | | Inflorescence parameters | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number of leaves/ plant | Length of mature leaves (cm) | Breadth of mature leaves (cm) | Plant height (cm) | Internodal distance (cm) | Petiole length (cm) | Number of nodes/ plant | Number of spikes/ plant | Number of spikelet/ spike | Number of florets/ spikelet |
| #1 | 70 | 2.28 | 1.3 | 25 | 2.82 | 1.3 | 10 | 7.4 | 11 | 4.8 |
| #2 | 49.6 | 5.5 | 3.02 | 34.8 | 2.28 | 2 | 6 | 8 | 16.4 | 4 |
| #3 | 47.2 | 2.1 | 1.52 | 27.2 | 1.98 | 2.3 | 10 | 11.2 | 17.8 | 4 |
| #4 | 45.2 | 5.02 | 3.04 | 29.8 | 4 | 1.98 | 8 | 8.6 | 11.4 | 4 |
| #5 | 53.4 | 2.1 | 1.5 | 31.9 | 3.18 | 1.4 | 14 | 5.6 | 10.6 | 5 |
| #6 | 38.8 | 2.1 | 1.84 | 28.1 | 2.9 | 1.48 | 15 | 11.6 | 15.2 | 5 |
| #7 | 36.2 | 2.08 | 1.52 | 24.2 | 1.96 | 1.5 | 20.2 | 11 | 13 | 4 |
| #8 | 58 | 2.08 | 1.7 | 35.58 | 1.5 | 1.5 | 24 | 10.4 | 13.2 | 5 |
| #9 | 26.2 | 3.04 | 1.82 | 27.82 | 1.96 | 1.48 | 20.2 | 9.4 | 11.4 | 5 |
| #10 | 53.4 | 2.08 | 1.82 | 31.5 | 2.54 | 1.52 | 20 | 7.4 | 13.4 | 5 |
| #11 | 20.8 | 2.48 | 3.5 | 21.96 | 1.98 | 1.52 | 10 | 21.4 | 13 | 6 |
| #12 | 14.6 | 3 | 1.52 | 12 | 1.28 | 0.84 | 7.8 | 6.8 | 13.2 | 6 |
| #13 | 12 | 6.2 | 3.5 | 17.68 | 2.52 | 1.52 | 7.2 | 9.4 | 16.6 | 4 |
| #14 | 25 | 2.08 | 1.8 | 23.08 | 2 | 1.52 | 12.4 | 11.4 | 13 | 5 |
| #15 | 55 | 2.08 | 1.52 | 39.34 | 1.98 | 1.48 | 22.2 | 10.6 | 16.2 | 6 |
| #16 | 35.6 | 2.32 | 1.8 | 30 | 2 | 1.5 | 12.2 | 9 | 11.2 | 4.8 |
| #17 | 26.6 | 2.28 | 1.9 | 31.5 | 1.74 | 1.52 | 13.8 | 11.4 | 11.2 | 5 |
| #18 | 38 | 2.4 | 1.92 | 36.5 | 1.88 | 1.52 | 13.8 | 11 | 10.4 | 6 |

Erum *et al.* (2011), comparing various quantitative plant and flower characters, also found that a high variation existed for several characters in tulsi. The distance as shown by the distance matrix and the phylogenetic map, in some cases do depict the geographical distance between/ among the places from where the accessions were collected; however, several accessions also grouped with accessions from geographically distance places. For example, although Acc #13 (Biswanath Chariali) groups with the accessions from Udalguri (Acc #11) and Kamrup (Acc #12), this place is not close to either Udalguri or Kamrup (which was geographically close). On the other hand, Acc #5 (Narayanpur), #8 (Nagaon), #10 (Majuli) and #15 (Golaghat) making the same sub-cluster could be easily explained by those places being close to each other. Erum et al. (2011) found that the Euclidean distance ranged from 3.6 to 7.26 among the nine basil (tulsi) varieties studied. The higher range of the Euclidean distance found in our studies could be due to the large number of quantitative characters taken, and also due to the more number of ecotypes analysed. The possible reason behind the geographical and the phylogenetic distance not showing a similar pattern could be human intervention in propagation of the plant. Because the plant is a household plant and not generally a wild herb; and due to its use in traditional medicines and religious customs, it is commonly grown in majority of the households of Assam, and therefore the propagation of the plant is hugely affected by human intervention. Prevalence of cross pollination in tulsi was proposed to be a possible region of the existence of high variation at the levels of species, subspecies and varieties of tulsi (Krishna 1981). The sexual mode of reproduction of tulsi was previously suggested to be an important reason of the higher genetic polymorphism in population of *Ocimum* (Mustafa *et al.* 2006).

## Variations of the Biochemical Characters

The chemical composition of tulsi is highly complex, containing many nutrients and biological active compounds, which significantly vary with time, cultivation process and storage (Tewari *et al.* 2012). The four biochemical characters that were taken for the study also showed considerable variation among the ecotypes, as shown in Table 5. Among the accessions, the maximum chlorophyll-a content (1.897 mg $g^{-1}$ of fresh weight) was found in Acc #14 (Lakhimpur), and the minimum value for chlorophyll-a content was found in Acc #17 (Dhemaji; 0.271 mg $g^{-1}$ of fresh weight). Similarly, while the highest chlorophyll-b content was found in Acc #4 (Jorhat; 1.089 mg $g^{-1}$ of fresh weight), the lowest value was found in Acc #17 (Dhemaji; 0.115 mg $g^{-1}$ of fresh weight). The total chlorophyll content ranged from 0.387 (Acc #17, Dhemaji) to 2.918 mg $g^{-1}$ fresh weight (Acc #14, Lakhimpur) and the chlorophyll-a: b ratio in the ecotypes, that we had analysed, ranged from 1.517 to 3.415. In an earlier study carried out by Kopsell and Kopsell (2005), the chlorophyll-a content under green house conditions ranged from 0.701-1.40 mg $g^{-1}$ fresh weight, chlorophyll-b content from 0.135-0.191 mg $g^{-1}$ fresh weight and total chlorophyll content ranged from 0.850-1.720 mg $g^{-1}$ fresh weight among the eight basil cultivars studied. Burzo and Mihaiescu (2005), reported chlorophyll-a, chlorophyll-b and total chlorophyll content in the range of 0.733-1.511 mg $g^{-1}$ fresh weight, 0.263-0.398 mg $g^{-1}$ fresh weight and 1.029-2.506 mg $g^{-1}$ fresh weight respectively in the four *Ocimum basilicum* cultivars. Anca-Raluca *et al.* (2011) studied the chlorophyll content in species of the genus *Mentha*, belonging to the *Ocimum* family *Lamiaceae* and reported the chlorophyll-a content in the range of 0.455-2.294 mg $g^{-1}$ fresh weight and chlorophyll-b, 0.178-0.755 mg $g^{-1}$ fresh weight. Thus, it can be seen that wide variation exists in the chlorophyll content in *Ocimum* genus, depending mostly on the cultivar types. Castrillo and his group in 2001 reported the chlorophyll-a: b ratio in the range of 1.41-2.10 in some cultivated and wild species of the *Lamiaceae* family. But in the cultivars of *Ocimum basilicum*, this ratio ranged from 2.47-5.76 (Burzo and Mihaiescu 2005). Vascular land plants (Johnson *et al.* 1993) and algae (Humbeck *et al.* 1988) usually have chlorophyll-a: b ratios in the range of 1.5-4.2 irrespective of the light environments within which they inhabits. Highest chlorophyll-a: b ratio was observed in Acc #15 (Golaghat; 3.415) and lowest in Acc #3 (Morigaon; 1.517). The ratio of chlorophyll-a: b has been a key parameter to judge the shade tolerance of a particular species (Givnish 1988), in that, shade-tolerant species display a lower ratio under shade compared to their counterparts grown under high light environments. It has been shown that shade tolerant species produce a higher proportion of chlorophyll-b relative to chlorophyll-a, which leads to a lower chlorophyll-a: b ratio, to enhance the efficiency of blue light absorption in low light environments (Yamazaki *et al.* 2005). Considering the carotenoid content, Acc #8 (Nagaon) was found to be the richest source of carotenoid (0.899 mg $g^{-1}$ of fresh weight) and Acc #13 was found to be the poorest source of the same (0.314 mg $g^{-1}$ of fresh weight). When the crude oil content was considered, while Acc #9 (Nalbari) was the richest source of crude oil, having 14.33% oil, Acc #17 (Dhemaji) was the poorest source of crude oil, having only 7.00% oil. Interestingly, for all the biochemical characters except for the carotenoid content, the lowest values were shown by Acc #17 (Dhemaji). A similar study on estimation of carotenoid content from some species of *Mentha* plant was carried out by Grzeszczuk and Jadczak (2009), where they reported the total carotenoid content from 0.504 to 0.632 mg $g^{-1}$ of fresh weight among the species.

The crude fat content among the collected ecotypes was found to range from 7% to 14.33%. Acc #9 (Nalbari) showed highest crude fat content (14.33%) and Acc #17 (Dhemaji) showed lowest crude fat content (7%). In a study carried out by Fagbohun and his group (2012), it was found that leaves of *Ocimum gratissimum* had the highest amount of crude fat (7.57%) when compared to other plants, *Melanthera scandens* (6.81) and *Leea guineensis* (7.28).

As in case of the morphological characters, a phylogeny map was also prepared using the biochemical dataset (Fig. 4) using the distance matrix. The map divided the accessions into two main clusters; the cluster 'A' including Acc #1 (Karimganj), #3 (Morigaon), #4 (Jorhat), #7 (Tinsukia), #8 (Nagaon), #9 (Nalbari), #10 (Majuli), #11 (Udalguri), #12 (Kamrup), #14 (Lakhimpur), #15 (Golaghat ), #16 (Cachar), #17 (Dhemaji) and #18 (Darrang), and the cluster 'B' including Acc #2 (Sivasagar), #5 (Narayanpur), #6 (Karbi Anglong) and #13 (Biswanath Chariali). The cluster 'A' had three sub-clusters within it; however, two of them were closer to each other than the other one that included only two samples, Acc #8 (Nagaon) and #12 (Kamrup). Similarly, within cluster 'B' also, Acc #6 (Karbi Anglong) shared less similarity with the rest. Considering the distance matrix (Supplementary table 2) calculated using the PAST software, it was found that while Acc #8 (Nagaon) and #13 (Biswanath Chariali) were the most distant (value: 58.574), Acc #10 (Majuli) and #15 (Golaghat) were the most closely related accessions (value: 1.699). Bompalli and Nallabilli (2013), carried out the biochemical characterization using the leaf protein banding profile of 11 different varieties of *Ocimum sanctum* and using the UPGMA cluster analysis, found that the varieties were separated in two main clusters in the phylogenetic tree and Euclidean distance ranged between 0 and 1 among the cultivars. But in their case, only one parameter was taken into consideration, but in our case three parameters were taken into consideration.

## CONCLUSION

In our study of genetic polymorphism among 18 geographical accessions of tulsi (*Ocimum* spp.) from the state of Assam, India, using 21 morphological and 4 biochemical parameters, it was found that the accessions display a great deal of diversity in all the parameters screened. The study of the qualitative parameters would also be useful for designing a morphological marker-scale that could be used for scoring and further polymorphism studies. We were also able to establish the phylogeny of the accessions from the analyses. The work is a preliminary but pioneering study and would be useful in future works intending to harness the economic and health potential of the herb. However, more detail works involving more parameters and more ecotypes from the inside as well as outside the state of Assam would possibly be useful to finally realize the benefits from the plant.

## ACKNOWLEDGEMENTS

The works was supported by Department of Biotechnology (Government of India)-Assam Agricultural University (AAU) Centre [02(3/58)/2012/1271; October 25, 2012 to B.K.B.]. The authors would like to acknowledge the intellectual support from Dr. K.M. Bujarbaruah, Hon'ble Vice Chancellor, AAU; Dr. S. Baishya, AAU and Dr. R.N. Sarmah, AAU. They would also like to acknowledge the technical help from Mr. Gyan Protim Gogoi and Mr. Priyanku Kumar Dutta.

## REFERENCES

Anca-Raluca, A., Irina, B., Claudia, P., Doina, A., Magda, C. and Maria-Magdalena, Z. 2011. Comparative biochemical and physiological research on taxa of *Mentha* L genus. *Journal of Plant Development,* 18: 41-44.

Arnon, D. I. 1949. Copper enzymes in isolated chloroplasts Polyphenoloxidase in *Beta vulgaris. Plant Physiology,* 24: 1-15.

Balaji, R., Prakash, G., Suganya, P. D. and Aravinthan, K. M. 2011. Antioxidant activity of methanol extract of *Ocimum tenuiflorum* (Dried leaf and stem). *International Journal of J. Pharmaceutical Research and Development,* 3: 20-27.

Bhattacharjee, S. K. 1998. *A Handbook of Medicinal Plant*. Jaipur: Pointer Publishers.

Bompalli, L. K. and Nallabilli, L. 2013. Genetic diversity of *Ocimum* species through biochemical technique and UPGMA CLUSTER analysis. *International Journal of Pharmacy* and *Pharmaceutical Sciences,* 5: 155-159.

Bult. C. J. and Kiang, Y. T. 1992. Electrophoretic and morphological variation within and among natural populations of the wild soybean, *Glycine soja* Sieb and Zucc. *Botanical Bulletin-Academia Sinica,* 33: 111-112.

Burzo, I. and Mihaiescu, D. E. 2005. Contributions to the data concerning physiological and biochemical processes of *Ocimum basilicum* L. *Biologie Vegetală,* 51: 47-52.

Carovic-Stanko, K., Salinovic, A., Grdisa, M., Liber, Z., Kolak, I. and Satovic, Z. 2011. Efficiency of morphological trait descriptors in discrimination of *Ocimum basilicum* L accessions. *Plant Biosystems,* 145: 298-305.

Castrillo, M., Vizcaino, D., Moreno, E. and Latorraca, Z. 2001. Chlorophyll content in some cultivated and wild species of the family Lamiaceae. *Biologia Plantarum,* 44: 423-425.

Choudhury, B. G., Manaswini, B., Kumar, J. P. and Kumar, T. S. 2011. Pharmacognostical and phytochemical investigation of various tulsi plants available in south eastern Odisha. *International Journal of Research in Pharmaceutical and Biomedical Sciences,* 2: 605-610.

Erum, S., Naeemullah, M., Masood, S. and Khan, M. I. 2011. Genetic variation in the living repository of *Ocimum* germplasm. *Pakistan Journal of Agricultural Research,* 24: 1-4.

Fagbohun, E. D., Lawal, O. U. and Ore, M. E. 2012. The proximate, mineral and phytochemical analysis of the leaves of *Ocimum gratissimum* L, *Melanthera scandens* A and *Leea guineensis* L and their medicinal value. *International Journal of Applied Biology and Pharmaceutical Technology,* 3: 15-22.

Givnish, T. J. 1988. Adaptation to sun and shade: A whole-plant perspective. *Australian Journal of Plant Physiology,* 15: 63-92.

Grieve, M. 1992. *A modern Herbal: The medicinal, culinary, cosmetic and economic properties, cultivation and* *folk-lore of herbs, grasses, fungi, shrubs and trees with their modern scientific uses*. London: Tiger Books International.

Grzeszczuk, M. and Jadczak, D. 2009. Estimation of biological value of some species of mint (*Mentha* L). *Kerva Polonica*, 55: 193-199.

Gupta, V. K., Singh, J., Kumar, R. and Bhanot, A. 2011. Pharmacognostic and preliminary phytochemical study of *Ocimum gratissimum* L (Family: *Lamiaceae*). *Asian Journal of Plant Science*, 10: 365-369.

Humbeck, K., Hoffmann, B. and Senger, H. 1988. Influence of energy flux and quality of light on the molecular organization of the photosynthetic apparatus in Scenedesmus. *Planta*, 173: 205-212.

Javanmardi, J., Khalighi, A., Kashi, A., Bais, H. P. and Vivanco, J. M. 2002. Chemical characterization of Basil (*Ocimum basilicum* L) Found in local accessions and used in traditional medicines in Iran. *Journal of Agricultural and Food Chemistry*, 50: 5878-5883.

Johnson, G. N., Scholes, J. D., Horton, P. and Young, A. J. 1993. Relationships between carotenoid composition and growth habit in British plant species. *Plant, Cell and Environment*, 16: 681-686.

Kewalanand, 2007. Basil. In: Singh, S. (ed.) *Medicinal and Aromatic crops*, 284-297. Jaipur: Aavishkar Publishers.

Kopsell, D. A. and Kopsell, D. E. 2005. Carotenoid and chlorophyll pigments in Sweet basil grown in the field and green house. *Horticultural Science*, 40: 1230-1233.

Krishna, R. 1981. Natural out crossing in sweet basil - *Ocimum basilicum* L. *Indian Perfumer*, 25: 74-77.

Morales, M. R., Charles, D. J., Simon, J. E. and Simon, J. E. 1993. New aromatic lemon basil germplasm. In: Janick, J. (ed.) *New Crops*, 632-635. New York: Wiley.

Mustafa, A. E.-Z. M. A., Badr, A., El-Galaly, M. A., Ahmed, A. M. and Hassan, M. G. 2006. Genetic diversity among *Ocimum* populations in Egypt as reflected by morphological, seed proteins and isozyme polymorphism. *International Journal of Botany*, 2: 261-269.

Nazim, K., Ahmed, M. and Uzair, M. 2009. Growth potential of two species of basil in sandy soil of Karachi. *Pakistan Journal of Botany*, 41: 1637-1644.

Omidbaigi, R., Mirzaee, M., Hassani, M. E. and Moghadam, M. S. 2010. Induction and identification of polyploidy in basil (*Ocimum basilicum* L) medicinal plant by colchicine treatment. *Internanational Journal of Plant Production*, 4: 87-98.

Pepkowitz, L. P. 1943. The stability of carotene in acetone and petroleum ether extracts of green vegetables: (I) The photochemical destruction of carotene in the of presence of chlorophyll (II) The stabilizing effect of Sodium cyanide. *Journal of Biological Chemistry*, 149: 465-471.

Quereshi, S., Purwar, P., Singh, R., Khan, N. A., Mani, A. and Patel, J. 2011. Studies on essential oils and DNA extraction from *Ocimum* species. *Journal of Phytopathology*, 3: 23-27.

Soxhlet, F. 1879. Die gewichtsanalytische Bestimmung des Milchfettes. *Dingler's Polytechnisches Journal*, 232: 461-465.

Svecova, E. and Neugebauerov, J. 2010. A study of 34 cultivars of basil (*Ocimum* L) and their morphological, economic and biochemical characteristics, using standardized descriptors. *Acta Universitatis Sapientiae, Alimentaria*, 3: 118-135.

Tewari, D., Pandey, H. K., Sah, A. N., Meena, H. S. and Manchanda, A. 2012. Pharmacognostical and biochemical investigation of *Ocimum kilimandscharicum* plants available in western Himalayan region. *Asian Journal of Plant Science and Research*, 2: 446-451.

Yamazaki, J., Takahisa, S., Emiko, M. and Yasumaro, K. 2005. The stoichiometry and antenna size of the two photosystems in marine green algae, *Bryopsis maxima* and *Ulva pertusa*, in relation to the light environment of their natural habitat. *Journal of Experimental Botany*, 56: 1517-1523.

Zviniene, N. and Pank, F. 1996. Data processing for numerical taxonomy in genus *Mentha* L growing in Lithuania. *International Symposium on Breeding Research on Medicinal and Aromatic Plants*, 30 June – 4 July, 1996, Quedlinburg, Germany.