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Variability studies in *Madhuca longifolia* var. *latifolia* flowers from Northern Western Ghats of India

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ABSTRACT

The aim of the present study was to check the sugar content variability within the flowers of *Madhuca longifolia* var. *latifolia* from Northern Western Ghats of India. For this study, vast survey was conducted during March-April of 2016 and total 99 accessions were collected for morphological and sugar analysis of flowers. From the study, it was revealed that accession ML01 has the highest amount of sugar (70.29 %) content compared to other accessions. Accession ML57 showed the highest amount of 100 flower weight. The present study will be useful for identifying high sugar containing germplasm from Northern Western Ghats. The mass multiplication of promising germplasms with the development of grafting techniques would pave ways for generating employment with economic stability to the tribal community of Western Ghats.

1. Introduction

As the population of world increasing day by day, global demand for biofuel is also increasing. One of the products of biofuel is bioethanol which is produced by fermentation. Raw materials containing sugars that can be transformed into simple sugars can be used as substrates for fermentation. Commercially, beet, sugarcane molasses and small quantities of sweet sorghum, Jerusalem artichoke tuber and fruit juices are used directly as fermentable material. Although the huge demand for bioethanol the production is comparatively low. Therefore, there is need to find out alternative crop products which contain high sugar for producing ethanol using fermentation.

Madhuca longifolia var. *latifolia* commonly called as *M. longifolia*, butter tree or Mahua in India, which belongs to the family Sapotaceae (Singh *et al.,* 2000). The name *Madhuca* emanate from Sanskrit language (Madhu=Honey) due to its flower which is sweet in nature. It is a large deciduous tree growing mostly under dry tropical and sub-tropical climatic conditions and found up to the elevation of 1200m (Hiwale 2016). Flowers appear during March and April month and usually blooming depend upon environmental conditions. The plant is famous in tribal people because of its flower which is mostly used for liquor preparations. Mahua flowers are renewable, cheap carbohydrate substrate which comes from the non-agricultural environment such as a forest. Due to its sweet nature of flower, it is also used for the preparation of different kinds of Indian food dishes including kheer, puri, halwa etc. (Mishra and Pradhan 2013). The flowers of Mahua are medicinally important for various diseases and act as an aphrodisiac, galactagogue and carminative. Mahua flowers are rich in total sugars, out of which reducing sugar present in high amount.

Although the wide use of Mahua flowers most of the study was carried out on ethanol production using flower sugar. No report yet published on variability in flowers of *M. longifolia*. Keeping in this view the objective of the present study was to determine the variability in *M. longifolia* flowers and its sugar content from Northern Western Ghats of India.

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2. Materials and Methods

Survey and Flower Collection

The survey and collection of *M. longifolia* flowers were carried out during March-April 2016. The flowers were collected from the forest of different districts of Northern Western Ghats of India (Table 1). Primarily the precise location (longitude and latitude) and diameter at breast height (DBH) of plants were recorded (Suryawanshi and Mokat 2019a). Then approximately 2kg fresh flowers were collected in polybags. The collected flowers were carefully brought in the laboratory as early as possible and kept it in the refrigerator until further use.

Flower analysis:

Determination of Total Solids and Moisture content:

For determination of moisture, accurately weighed (5 g) of flowers was taken in Petri dishes and dried in hot air oven at 80° C till constant weight achieved and measured the dry weight of the sample. The moisture content in flower was simply measured by using following formula (Nielsen 2010).

$$\text{Moisture } (\%) = \frac{\text{weight of wet flowers} - \text{weight of dry flowers}}{\text{weight of wet flowers}} \times 100$$

The dry matter remains after the moisture removal referred as total solid. The total solid content in flower was calculated by removing moisture from wet flower.

$$\text{Total Solids } (\%) = \frac{\text{weight of oven dry flowers}}{\text{weight of wet flowers}} \times 100$$

Table 1. Accessions collected from 9 districts of Northern Western Ghats

Districts	No. of accessions
Raigad	28
Thane	18
Palghar	12
Nashik	20
Pune	16
Ahmednagar	4
Dhule	1
Total	99

Determination of Ash content:

To determine the ash content, 1 g of the flower was placed in a crucible and combusted in a Muffle Furnace (Tempo) at 550 ºC. The ash was cooled in desiccators and measured the weight to check the ash percentage with following formula (Nielsen 2010).

$$\text{Ash } \% = \frac{(\text{Weight of crucible} + \text{ash}) - \text{Weight of crucible}}{\text{Weight of flower} \times \text{dry matter coefficient}} \times 100$$

Where, dry matter coefficient= Total solid (%) / 100

Determination of Sugars:

Total reducing sugar (TRS), Reducing (RS) and Non reducing sugar (NRS) present in dry flower juice extract was measured by titration method as suggested by Lane-Eynon method (Lane and Eynon 1934)

Determination of pH:

Dried flowers (50 g) were weighed on an analytical weighing balance. The leachate was prepared by submerging the flowers in 250 ml of distilled water and kept on a shaker for 30 min for shaking. The pH of leachates was measured by dipping the electrode of pH meter in the solution (Make- Mettler Toledo).

Organoleptic properties:

The wet and dry flowers were used for the organoleptic study. Organoleptic study of *M. longifolia* flowers were performed on color, odour, taste, texture and shape (Arya and Thakur 2012).

3. Results and discussion

Survey and field collection:

Explorations were undertaken in 7 districts namely Raigad, Thane, Palghar, Nashik, Dhule, Ahmadnagar and Pune districts of Maharashtra (Fig. 1) Total 99 accessions of the flower of *M. longifolia* were collected during the study. DBH values of selected trees were documented and average DBH was found to be 62.80cm.

During the survey latitude and longitude of the trees were recorded using GPS. *M. longifolia* trees were found abundantly in the forest of Palghar and Thane. According to tribal of the surveyed area, the flowers are mostly used for daily food purposes. As Mahua flower contains sugar the

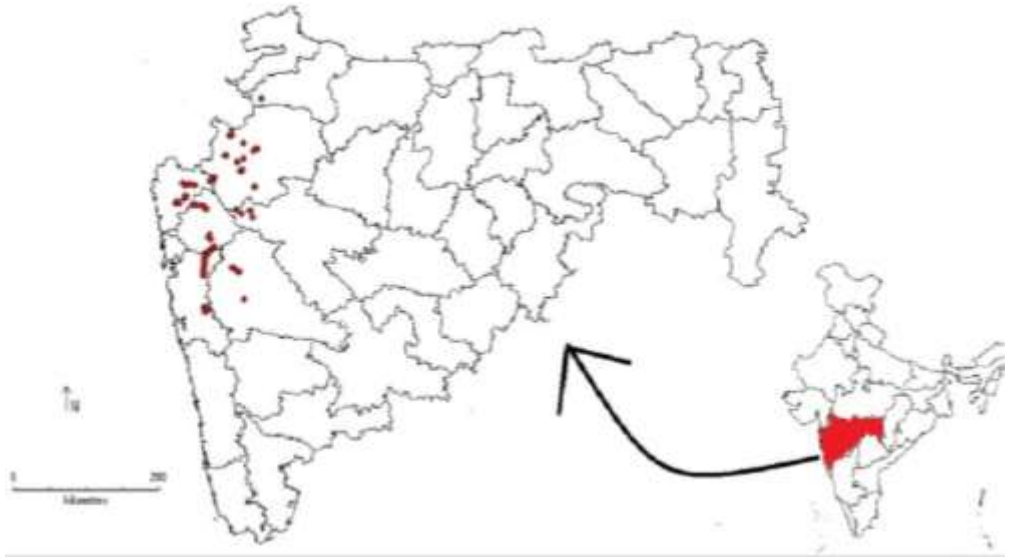

Fig. 1. Map of selected trees of *M. longifolia* var. *latifolia* from Northern Western Ghats.

wet flowers used as energy food during summer. Traditionally liquor production using dried Mahua flowers is famous in the tribal zone. The dried flower sold in the local market at 25-30 Rs per Kg (0.33 USD/Kg). Although, Mahua flower has been utilized extensively by tribal very few commercial products are available.

Organoleptic properties:

M. longifolia wet flowers are mostly pale yellow in color (Fig. 2) and after drying the color changes from brown, dark brown to black color. The color of the flower is important parameter because it attracts the insects for pollination (Oberrath and Böhning-Gaese 1999). The corolla is fleshy and sweet in nature. The aromatic essential oil was also isolated from the flowers of Mahua (Suryawanshi and Mokat 2019b). The organoleptic characters of *M. longifolia* flower are shown in table 2. The organoleptic characters offer a scientific basis for the further use of *M. longifolia* flowers in different systems of medicine (De Medeiros *et al.,* 2015).

Table 2. Organoleptic features of *M. longifolia* var. *latifolia* flowers

Particulars	Flower	
Condition	Wet	Dry
Color	Cream	Brownish
Odour	Sweet	Sweet
Taste	Sweet	Sweet
Texture	Smooth	Rough
Shape	Round	Flat

Flower analysis:

In the present investigation, it was found that the moisture content of dried flowers from different regions of Western Ghats of Maharashtra ranges from 5.30 to 21.68 %, respectively. Furthermore, the average moisture content 14.33 % was found in flower which is higher than previously published report who determined 11.40 % mean moisture content in *M. longifolia* var. *latifolia* flowers (Jayasree *et al.,* 1998). Total solid of the flower is an important parameter of analysis because it contains the sugar. The average total solids present in flowers was recorded as 85.67 %. The average wet flowers moisture content in studied flower is 70.6 % while dried flower moisture contains 14.33 %. The ratio of moisture in wet flowers to total solid is 10:7, while in dry flower it is 10:1. The results are corroborated with Jayasree *et al.*, 1998. The average ash content of Mahua flowers was 3.21 % which is comparatively lower than previous reports (Kureel *et al.,* 2010; Fowler *et al.*, 1920). The high ash content present in ML76 (5.22 %) is may be due to the high mineral content in the soil. The ash content is generally used to measure the functional properties of food (Hofman *et al.,* 2002). The 100 flowers weight ranged from 49.91 to 174.96 g. The accession ML57 contains the highest number of 100 flowers weight compared to others. The high flower weight with low moisture content will be a good source for the production of ethanol using *M. longifolia* var. *latifolia* flowers. The results showed that pH of Mahua flowers is acidic in nature which ranges from 4.46 to 5.31. Flower pH is an important factor for ethanol production during fermentation (Swain *et al.,* 2007; Behera *et al.*, 2010; Mohanty *et al.*, 2009). Most ethanol is produced from *Saccharomyces cerevisiae* which grows on acidic media and as a Mahua flower juice is already acidic in nature which can give optimal growth of *S. cerevisiae* (Benerji *et al.,* 2010; Gedela *et al.,* 2017).

Fig. 2. a, Buds and **b**, Flowers of *M. longifolia* var. *latifolia.*

Table 3. Variability parameters of *M. longifolia* var. *latifolia* flowers.

	pH	Ash (%)	TRS (%)	RS (%)	NRS (%)	100FW (g)
Average	4.73	3.21	62.51	41.46	21.05	105.13
Maximum	5.31 (ML106)	5.22 (ML76)	70.29 (ML01)	47.34 (ML01)	27.56 (ML05)	174.96 (ML57)
Minimum	4.46 (ML20)	0.39 (ML 06)	57.09 (ML25)	36.04 (ML05)	17.11 (ML94)	49.91 (ML89)
SD	0.17	1.05	2.80	2.53	2.13	20.47
SE	0.01	0.11	0.28	0.25	0.21	2.06

TRS= Total Reducing Sugar, RS= Reducing Sugar, NRS= Non Reducing Sugar, 100FW= 100 Flower weight.*Accessions numbers represents in brackets.

Total reducing sugar (TRS) content was in the range of 57.09 to 70.29 %. As an average 40-72 % of TRS were reported by different researchers from different geographical regions (Awasthi *et al.*, 1975; Sutaria and Magar 1955; Belavady and Balasubramanian 1959; Jayasree *et al.*, 1998). Reducing Sugar (RS) ranges from 36.04 to 47.34 % and Non Reducing Sugar (NRS) from 17.11 to 27.56 % in Mahua flowers. Similarly, RS 48-57 % and slightly lower NRS 3-18 % has been previously reported (Sutaria and Magar 1955; Belavady and Balasubramanian 1959; Jayasree *et al.*, 1998). The highest TRS content was recorded in accession ML01 (Raigad), while minimum in accession ML25 (Thane) (Table 3). The results showed that the flowers are a rich source of sugars and contain huge variability. The toxicity study on albino rats found that the flowers are safe for human consumptions (Patel and Naik 2010). Sugar syrup is prepared by the various researcher using dried Mahua flowers (Sutaria and Magar 1955; Shrivastava *et al.*, 1970). Chromatographic analysis showed Mahua flower extract contains sucrose, glucose, fructose, arabinose, maltose and rhamnose (Sutaria and Magar 1955).

As we know the global demand for ethanol is increasing every year, the Mahua flowers can be used to produce an enormous amount of ethanol as it contains a high amount of sugars. The high sugar containing germplasm (ML01) will be useful for mass cultivation. The use of appropriate grafting techniques may increase the early flowering in Mahua seedlings.

Although the flowers have a rich source of sugar in rural areas, these flowers are not very popular for food. A very small quantity of flower is consumed as a fried or cooked in different parts of India. Apart from human consumption, it is also used in cattle feed. Most of the flowers are used in the preparation of liquor by tribals and villagers. Hence it is necessary to use this tree in breeding and commercial cultivation for better economic stability in tribal areas of Western Ghats.

4. Conclusion

M. longifolia is an important tree present in most of the regions of Western Ghats, but has by no means been explicitly investigated for its economic gains. Documentation in variability among commercial traits within species is important criteria for effective and successful tree improvement program. From the present study it is revealed that Mahua flower contains high amount of sugar which can be used as raw material for food processing industries and to manufacture the food products. There is huge variability found in flower sugar content, the accession ML01 reported

to be elite germplasm and it will be considered under breeding program and commercial cultivation. The present investigation will be useful for identification of high sugar containing Mahua germplasm for its domestication purposes in hilly areas of Western Ghats where this plant grows luxuriantly.

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6. Conflict of interest

The authors declare that they have no conflict of interest within themselves and others including the funding agency and the agency where the research was carried out.

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