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Manjesh GN

Scientist, ICAR, Directorate on Medicinal and Aromatic Plants Research, Anand, Gujarat, India

Hima Bindu K

Principal Scientist, Division of Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India

Upreti KK

Principal Scientist, Division of Plant Physiology and Biochemistry, ICAR-Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India

Umesha K

Emeritus Professor, Department of Horticulture, College of Horticulture, Bengaluru, Karnataka, India

Mallikarjuna Gowda AP

Senior Scientist, Department of Horticulture, University of Agricultural Sciences, Bengaluru, Karnataka, India

Rekha A

Principal Scientist, Division of Fruit Crops, ICAR- Indian Institute of Horticultural Research, Hesaraghatta, Bengaluru, Karnataka, India

Corresponding Author:**Manjesh GN**

Scientist, ICAR, Directorate on Medicinal and Aromatic Plants Research, Anand, Gujarat, India

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Estimation of chlorophyll contents in male and female genotypes of betel vine (*Piper betle* L.)

Manjesh GN, Hima Bindu K, Upreti KK, Umesha K, Mallikarjuna Gowda AP and Rekha A

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Abstract

Betel vine [*Piper betle* (L)] is valued for its leaves due to its masticatory health benefits. The cultivars in betelvine are mostly the land races which are cultivated locally. The present study aimed to estimate the chlorophyll contents among male and female genotypes collected from different parts of India. The chlorophyll a, b and total chlorophyll contents among male and female clones varied significantly within genders. The females had slightly higher contents of chlorophyll over male clones. The highest total chlorophyll recorded in genotype Hirehalli Local (2.363 mg/g) followed by, IIHR BV 67 (2.082 mg/g) in females. Among male clones Shirpurkata (2.238 mg/g) followed by, clone Kapoori TN (2.180 mg/g) had higher total chlorophyll contents. The general observation prevails that, male leaves has slightly lesser in chlorophyll contents over females. Present study could able to identify the male genotypes (Shirpurkata and Kapoori TN) which are comparable in chlorophyll contents over female clones.

Keywords: Betelvine, chlorophyll, DMSO, gender

Introduction

Betel vine (*Piper betle* L.,) is an evergreen perennial, dioecious creeper belongs to the family Piperaceae and cultivated commercially in South East Asian countries. It is a heritage crop of India and is a cash crop grown mainly by small and marginal farmers in an estimated area of 50,000 ha. It is also being exported to different countries with foreign exchange to the tune of 30-40 million INR (Guha, 2006) [1]. It is widely used as masticatory agent due to the strong pungent aromatic flavor of leaves. The leaves and stalk of betelvine have been used since time immemorial to treat various ailments in *Ayurveda* and different folk medicines. The leaves exhibit antioxidant, anti-inflammatory, anticarcinogenic, antibacterial, antifungal and nematicidal properties and its essential oils known for their beneficial biological activities (Kumar *et al.*, 2010 and Rai *et al.*, 2011) [4, 6]. The previous reports on chlorophyll 'a' content in leaves was reported in cv. Sanchi (1.61 mg g⁻¹ tissue) and chlorophyll 'b' content as highest in Simurali Sanchi (1.00 mg g⁻¹ tissue). The total chlorophyll was highest in Simurali Sanchi (2.45 mg g⁻¹ tissue) by Rahaman *et al.* (1997) [5]. The present study aimed to know the variability for chlorophyll contents among ten male and ten female clones of betel vine.

Material and Methods**Plant material**

A total of twenty clones including ten clones each in male and female types maintained at ICAR- Indian Institute of Horticultural Research (IIHR), Bengaluru were used in the present study (Table 1). The experiment was carried out at ICAR-IIHR, Hesaraghatta, Bengaluru situated at an altitude of 890 meter MSL at 13058' North latitude and 78045' East longitudes.

Procedure for Chlorophyll estimation in different Male and female clones of betelvine

The leaf chlorophyll content among twenty genotypes was analyzed on harvestable leaf from each genotype according to Hiscox and Israelam (1979) [2]. The freshly harvested leaves were collected, washed with distilled water and allowed to dry for 5-10 minutes. The ten leaf discs of 10 mm diameter were cut using a cork borer, and the fresh weight was noted. The leaf discs were immersed in a test tube containing 10 ml of dimethyl sulphoxide (DMSO) and incubated for 24 hour in dark.

The extract was used to record the chlorophyll content by noting the absorbance at 653 and 663 nm, using UV-Vis spectrophotometer (Systronics, India, Model 119). Chlorophyll a, b and total chlorophyll were estimated by substituting the absorbance values in the formulae given below. The estimations were carried out in triplicate and the total chlorophyll was expressed as mg g⁻¹ of tissue.

$$\text{Chlorophyll (a)} \left(\frac{\text{mg}}{\text{g}}\right) = (12.7 \cdot OD@663 \text{ nm}) - (2.69 \cdot OD@645 \text{ nm})$$

$$\text{Chlorophyll (b)} \left(\frac{\text{mg}}{\text{g}}\right) = (22.9 \cdot OD@645 \text{ nm}) - (4.08 \cdot OD@663 \text{ nm})$$

$$\text{Chlorophyll (b)} \left(\frac{\text{mg}}{\text{g}}\right) = (22.9 \cdot OD@645 \text{ nm}) - (4.08 \cdot OD@663 \text{ nm})$$

$$\text{Total chlorophyll} \left(\frac{\text{mg}}{\text{g}}\right) = (20.2 \cdot OD@645 \text{ nm}) + (8.02 \cdot OD@663 \text{ nm})$$

Table 1: List of female and male clones in *Piper betle* L.

| S. No. | Clones | Place of collection | Gender |
|--------|----------------------|---------------------|--------|
| 1 | Meetha Pan | West Bengal | Female |
| 2 | Mysore Local | Karnataka | Female |
| 3 | IIHR BV 67 | Maharashtra | Female |
| 4 | Gangarampur Sanchi | Tamil Nadu | Female |
| 5 | Hirehalli Local | Karnataka | Female |
| 6 | Halisahar Sanchi | West Bengal | Female |
| 7 | Malvi | Madhya Pradesh | Female |
| 8 | Sirugamani-1 | Tamil Nadu | Female |
| 9 | Ghanaghetta | West Bengal | Female |
| 10 | Karapaku | Andhra Pradesh | Female |
| 11 | Kapoori TN | Tamil Nadu | Male |
| 12 | Kapoori Chintalapudi | Andhra Pradesh | Male |
| 13 | Kapoori Cuddapah | Andhra Pradesh | Male |
| 14 | Kapoori Arvi | Maharashtra | Male |
| 15 | CARI-6 | Andaman Islands | Male |
| 16 | Kapoori Bihar | Bihar | Male |
| 17 | Vasani Kapoori | Maharashtra | Male |
| 18 | Shirpurkata | Maharashtra | Male |
| 19 | Yellow leaf | Andhra Pradesh | Male |
| 20 | Swarna Kapoori | Andhra Pradesh | Male |

Results and Discussion

The estimation of chlorophyll-a, chlorophyll-b and total chlorophyll was carried out in all the clones of betel vine and presented in table-2. The chlorophyll-a content was found with an average of 1.158 mg/g among the clones and ranged from 0.724 (Vasani Kapoori) to 1.924 mg/g (Hirehalli Local). It was found significant among the clones, but nonsignificant between the genders.

On an average male clones recorded 1.18 mg/g and females with 1.13 mg/g. The chlorophyll-b content was found with an average of 0.215 mg/g among the clones and ranged from 0.070 (Kapoori Cuddapa) to 0.439 mg/g (Hirehalli Local). It was found significant among the clones, but nonsignificant between the genders. The average content in male clones recorded was 0.20 mg/g and females with 0.23 mg/g. The average total chlorophyll content was 1.373 mg/g among the clones and ranged from 0.807 (Vasani Kapoori) to 2.363 mg/g (Hirehalli Local). It was found significant among the clones, but nonsignificant between the genders was noticed. The average content in male clones recorded was 1.38 mg/g and females with 1.36 mg/g.

The highest chlorophyll-a content noticed in female clone Hirehalli Local (1.924 mg/g) followed by, IIHR BV 67 (1.701 mg/g). Similarly, among males Shirpurkata recorded 1.820

mg/g which was comparable to female clones. The Chlorophyll-b content was highest in female clone Hirehalli Local (0.439 mg/g) followed by, IIHR BV 67 (0.381 mg/g). Similarly in males the highest chlorophyll-b was in Shirpurkata (0.418 mg/g) followed by, Kapoori TN (0.384 mg/g). The total chlorophyll content varied significantly within genders and found higher content in Hirehalli Local (2.363 mg/g) followed by, IIHR BV67 (mg/g) in female clones. Similarly, among male clones Shirpurkata (2.238 mg/g) and Kapoori TN (2.180 mg/g) had higher total chlorophyll contents.

The variations in chlorophyll-a, chlorophyll-b and total chlorophyll was quantified among the clones of betel vine and shown in fig. 1, 2 and 3. The chlorophyll-a, b and total chlorophyll contents were found to differ significantly among the clones but, nonsignificant results were observed between the genders.

The higher content of chlorophyll was observed in female clones in comparison to male clones. Contrasting results were reported by Xu *et al.* (2009) [10] and Xing, (2011) [9] where, *Populus cathayana* Rehd male plants had higher chlorophyll content over female counterparts. The higher content of chlorophyll was observed in female clones in comparison to male clones. Similar findings were recorded by Kumar *et al.* (2006) [3].

The previous report by Shivashankara *et al.* (2012) [7] found the chlorophyll was higher in female and sweet betel vine when compared to Madras type of cultivars. In another report by Usha *et al.* (2009) [8] decipher the difference among the genders of betel leaf varieties Desavari and Bangla and reported total chlorophyll, chlorophyll a and b was higher levels in Bangla and lower levels in Desavari.

Table 2: Chlorophyll content in female and male clones of *P. betle* L.

| Female Clones | Chlorophyll -a (mg/g) | Chlorophyll -b (mg/g) | Total Chlorophyll (mg/g) |
|----------------------|-----------------------|-----------------------|--------------------------|
| Meetha Pan | 0.950 | 0.229 | 1.179 |
| Mysore Local | 0.769 | 0.119 | 0.888 |
| IIHR BV 67 | 1.701 | 0.381 | 2.082 |
| Gangarampur Sanchi | 0.954 | 0.200 | 1.153 |
| Hirehalli Local | 1.924 | 0.439 | 2.363 |
| Halisahar Sanchi | 1.226 | 0.287 | 1.513 |
| Malvi | 1.094 | 0.223 | 1.318 |
| Sirugamani-1 | 0.848 | 0.132 | 0.979 |
| Ghanaghetta | 1.033 | 0.173 | 1.206 |
| Karapaku | 0.847 | 0.097 | 0.944 |
| Male Clones | | | |
| Kapoori TN | 1.796 | 0.384 | 2.180 |
| Kapoori Chintalapudi | 1.002 | 0.132 | 1.134 |
| Kapoori Cuddapah | 0.824 | 0.070 | 0.894 |
| Kapoori Arvi | 1.638 | 0.350 | 1.988 |
| CARI-6 | 1.012 | 0.191 | 1.203 |
| Kapoori Bihar | 1.129 | 0.178 | 1.307 |
| Vasani Kapoori | 0.724 | 0.084 | 0.807 |
| Shirpurkata | 1.820 | 0.418 | 2.238 |
| Yellow leaf | 0.962 | 0.113 | 1.075 |
| Swarna Kapoori | 0.898 | 0.101 | 1.000 |
| Mean(±)Sem | 1.158±0.041 | 0.215±0.008 | 1.373±0.043 |
| CD @5% | 0.117 | 0.022 | 0.122 |
| F-test | S | S | S |
| Male v/s Female | | | |
| Mean±Sem (Male) | 1.18±0.13 | 0.20±0.04 | 1.38±0.17 |
| Mean±Sem(Female) | 1.13±0.12 | 0.23±0.04 | 1.36±0.16 |
| T-test | NS | NS | NS |

S: Significant @5%; NS: Non-significant @ 5%

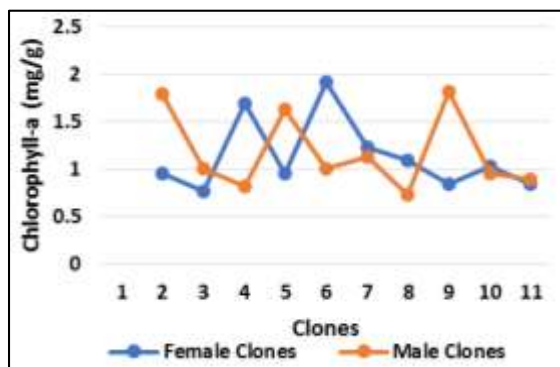


Fig 1: Variation for chlorophyll-a in female and male clones of *Piper betle* (clones as numbered in table-1)

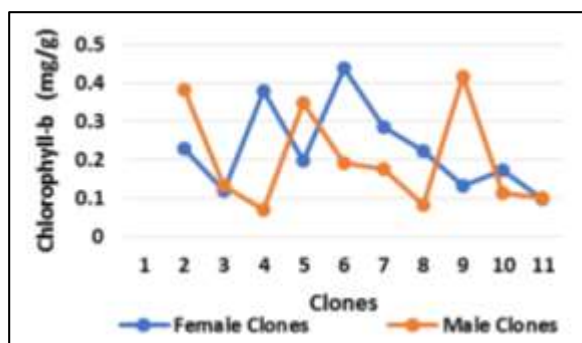


Fig 2: Variation for chlorophyll-b in female and male clones of *Piper betle* (clones as numbered in table-1)

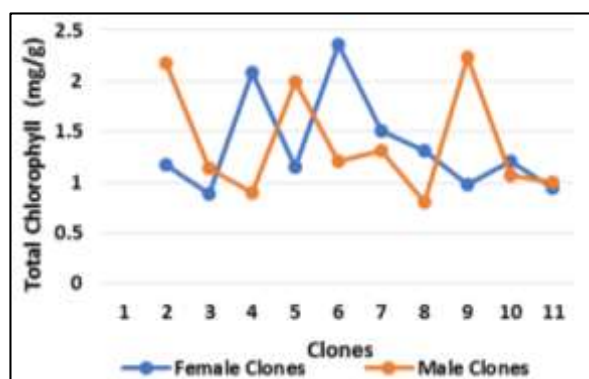


Fig 3: Variation for total chlorophyll in female and male clones of *Piper betle* (clones as numbered in table-1)

Conclusion

The variation in chlorophyll contents was reported and found slightly higher total chlorophyll in females over male counterparts. In general, the male leaves of betel vine genotypes have slightly lesser chlorophyll but, the male genotypes Shirpurkata and Kapoori TN was comparable to females clones.

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