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Estimation of chlorophyll and mucilage canal in different genotypes of betel vine (*Piper betle*)

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Abstract
Betel vine (*Piper betle*) is heart-shaped deep green, human healthy leaves, perennial plant an important cash crop of India for livelihood security in rural area particularly small as well as marginal betel growers of "baree community" in Maharashtra. In the present study, an attempt has been made for the estimation of chlorophyll, total mucilage no. in different betel vine genotypes leaf from the germplasm preserve at oilseed research station, Jalgaon. The chlorophyll of from five different germplasm of betel vine was estimated. The JBL 06(2.59 ppm) having significantly superior chlorophyll concentration than the others, while higher no. of mucilage canals found in Kapoori and JBL 01 (04) cultivar with only one mucilage canal found in Jalgaon Red the local cultivar of betel vine. Both the parameter which are estimated are responsible for the antioxidant property of betel vine. Several species of Piper (e.g. *P. betle* L. and *P. nigrum* L.) are distinguished by mucilage canals in petiole and axis.

Keywords: Piper betel leaves, chlorophyll, mucilage canals

Introduction
Betel vine is a perennial rooted climber belongs to the family *Piperaceae*. Its scientific name is *Piper betle* L. (Srichana *et al*. 2009) [20]. Betel leaf plays an important role since ancient culture. Its use in India dates back to 400 BC. The *Piper betle* leaf commonly known as 'paan' or 'Nagvalli. Have significance of leaves has been explained in relationship to every sphere of human life including social, culture, religious and very much relevant even in modern days.As per ancient books of Ayurveda, Charaka, Sushruta Samhitas, and Kashyapa have importance in human consumption, toward the 13 th century, European traveler Marco Polo recorded that the kings and nobles chewing the betel leaf after meal in India (Toprani and Patel, 2013) [21]. India is the largest producer of betel leaves in the world (Arulmozhiyan *et al*., 2005) [2]. It is cultivated in India on about 75,000 ha area with an annual production worth about Rs. 1000 million (Dasgupta, 2011, Vijayakumar and Arumugam, 2012) [6, 23], with an export 6159 Million Tone worth 3.55 million USD in 2020-21(APEDA, Statistical report 2021). The betel vine is called as 'green gold of india' as about 20 million people derive their livelihood directly or indirectly from production, processing, handling, transportation and marketing of betel leaves in India. They have various properties like antioxidant, antifungal, antidiabetic, antimicrobial, anti-inflammatory, anti fertility, antinacepative and radio protective properties, (Sripradha, 2014) [19]. The particular properties of *Piper betel* leaves are antimicrobial and antileshmian properties. Piper betel leaves have long been studied for their diverse pharmacological actions (Sarkar *et al*., 2008) [16]. The chlorophyll and the mucilage canals is one of the important parameter for its quality judgment. So the study is carried for sack of estimation of them.

Materials and Method
The laboratory experiment was conducted under state plan project at Betel vine Research Project, Jalgaon, Maharashtra which is under administrative control of Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahemadnager, (M.S.).This centre was situated in agro-climatic Zone III B of Maharashtra state at latitude and longitude (21.00770N, 75.56260E) with 770 to 800 cm. average rainfall having medium to deep black cotton soil and PH 6.8-7.5. Where more than 25 Betel vine crop was grown and preserve under shed of shevari (*Sesbania sesban*), shisam (*Dalbergia sissoo*), drumstick (*Moringa oleifera*) in month of III rd week of June 2018. Young cutting from one and half year's old healthy vine was used as planting material.

The cuttings were planted in 5*1 m for each treatment plot size, maintaining 80 cm row to row, 10 cm plant to plant distance in three replication. After establishment of the cutting the field was fertilized with only FYM at the rate of 5 tons per hectare per year. Followings genotype is selected out in a randomized block design with three replications. All the observations recorded with statistical methods by using Panse and Sukhatme.

Sr. No.	Name of Cultivar collected
1	JBL 01 (Promising lines in germplasm)
2	JBL 06 (Promising lines in germplasm)
3	JBL 22 (Promising lines in germplasm)
4	Kapoori (cultivated all over Maharashtra State)
5	Jalgaon red (Local)

Lab. instruments

Spectrophotometer-Spectronic 20 Colorimeter (Geaesys 545TM Spectrophotometer, New York, USA) was used for analysis purpose. All the glassware's of Borosil make were used for analysis of product. All the chemicals used were Analytical M/S Reagent (AR) and Guaranteed Reagent (GR) grade for analytical work which was manufactured by Merk, India Ltd/ Glaxo India Ltd.

Chlorophyll analysis

One gram of leaf sample was finely cut and gently mixed with a clean pestle and mortar. To this homogenized leaf material, 20ml of 80% acetone and 0.5gm $MgCO_3$ powder was added. The materials were further grind gently. The sample was then put into a refrigerator at 4 °C for 4 hours. Thereafter, the sample was centrifuged at 500 rpm for 5 minutes. The supernatant was transferred to 100 ml volumetric flak. The final volume was made up to 100 ml with addition of 80% acetone. The color absorbance of the solution was estimated by a spectrophotometer using 645 and 663nm wavelength against the solvent. Acetone (80%) was used as a blank. (Kamble *et al*., 2015) [8].

Formula: Chl a= 11.75×A662.6 – 2.35×A645.6
Chl b=18.61×A645.6 – 3.96× A662.6

Where, Ca and Cb are the chlorophyll a and chlorophyll b, A is absorbance. 2.5 Statistical Analysis Data generated during the course of investigation were analyzed using randomized block design (RBD) technique according to Snedecor and Cochran (1994).

Mucilage canal analysis

The mucilage canals are the anatomical feature in betel vine leaf dorsal side, in mesophyll cells responsible for not only storage of water, food but also thickening of membrane to protect the cells. So above different five cultivar of betel vine was estimated for no. of mucilage canals by using double staining methods (Johansen, 1940) [9] Leaves were fixed in formalin–acetic acid–alcohol (FAA) for 24–48 h depending upon whether the species had soft or hard leaves. Composition of FAA (for 100 mL) was 90 mL of 50% ethanol, 5 mL of glacial acetic acid, and 5 mL of formalin 37–40%. Fixed material was dehydrated through a graded ethanol series and embedded in paraffin wax, sections (5 µm thickness) were cut using a Leica RM2245 rotary microtome (Leica Biosystems, Buffalo Grove, Illinois, USA). Staining-dehydration and mounted with DPX (Sigma-Aldrich Co.). The sections were observed using a Nikon SMZ-800 Stereoscopic light microscope. (Johansen,1940) [9].

Result and Discussion

The chlorophyll of from five different germplasms of betel vine was estimated. The JBL 06(2.59 ppm) having significantly superior chlorophylls concentration than the others (table.no.1). (Kamble *et al*., 2015) [8] reported chlorophyll content in different leaves Mango, Guava, Neem and Ashoka as 6.48, 12.54, 36.18 and 6.48 mg/l respectively. Also, (Guha 2006) [5] reported that chlorophyll content in *Piper betel* leaves 0.01 to 0.25%. Betel leaves are potential non-toxic natural antioxidant (Arambewela *et al*.,2006) [3]. Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants was estimated by (Mirza *et al*.,2013 and Srichaikul *et al*., 2011, Venugopalan *et al*.,2008) [12, 18, 24]. Green plants have different characters because of the presence of various pigments like chlorophyll, carotenoid, other pigments and water content which together constitutes the spectral characters of a plant body was studied by (Philip and Shirly 1978) [14] and Jan-Chang Chen, (2007) [7], Kamble *et al*.,2015) [8] analyzed chlorophyll content of different leaves and reported that chlorophyll a concentration was higher than that of chlorophyll b (Kubade *et al*.,2021) [10]. The previous report by (Shivashankara *et al*.,2012) [17] 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) [22] 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 1: Estimation of chlorophyll from various cultivars of betel vine

Sr. No	Name of cultivar	Mean value of chlorophyll (ppm. concentration)
1	JBL 01	1.85
2	JBL 06	2.59
3	JBL 22	1.08
4	Kapoori	2.20
5.	Jalgaon Red	1.92
	SE+-	0.11
	CD@5%	0.33
	CV	3.56

Table 2: Estimation of mucilage canals from various cultivars of betel vine

Sr. No	Name of cultivar	Mean Mucilage canals (No.)
1	JBL 01	04
2	JBL 06	03
3	JBL 22	03
4	Kapoori	04
5	Jalgaon Red	01
	SE+-	0.18
	CD@5%	0.59
	CV	13.97

Higher no. of mucilage canals found in Kapoori and JBL 01 (04) cultivar with only one mucilage canal found in Jalgaon Red the local cultivar of betel vine.(table No.2) (Metcalfe and Chalk 1950) [11] mentioned that stem and petiole of *Piper betle* L. show spherical secretory cells with suberized walls, containing mucilage.

Conclusion

The importance of the betel leaf as discussed above prove that leaf has a great potency to act as natural antioxidant also leaf possess the broad spectrum chlorophyll.

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