

# Biochemical Changes among the Varieties of *Piper betel* L. in west Bengal Climatic Condition throughout the Year

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**Abstract:** The biochemical analysis was taken in the laboratory of Bidhan Chandra Krishi Vishwavidyalaya, Kalyani, WB with the different leaves of *Piper betel* (betelvine) cultivars. Remarkable variations were observed in all the biochemical characteristics tested. Kalipatti possessed highest reducing sugar (557.40 mg/100 g) among all the varieties followed by halisahar sanchi (441.00 mg/100 g), gangarampur sanchi (347.96 mg/100 g) and simurali sanchi (303.06 mg/100 g). April was the best for total carbohydrate which possessed maximum (2439.68 mg/100 g) while July possessed minimum (686.21 mg/100 g) carbohydrate concentration throughout the year. The maximum protein contents were found during July (19.07 mg/g) and minimum found during January (11.14 mg/g). Among kapoori cultivars, swarna kapoori possessed significantly more phenol content (422.86 mg/100 g) as compare to all the remaining kapoori varieties. The lowest phenol concentrations were observed in kapoori dodhipatla (361.10 mg/100 g) among all the varieties. The period of October to January was the best for proline increment throughout the year. The comparable observations were recorded with respect to vitamin C contents in kapoori chinacheppali (111.52 mg/100 g), kapoori pedacheppali (116.34 mg/100 g) and kapoori dodhipatla (118.41 mg/100 g). Interaction between cultivars and time was found significant in all the biochemical characteristics.

**Keywords:** Betelvine, Biochemical, Month, Kapoori, Sanchi.

## INTRODUCTION

Betelvine (*Piper Betel* L.) is used in Indian system of medicine to cure many diseases and disorders. Research in recent years is exploring the scientific basis of the traditional uses of this plant as well as discovering new molecules in betelvine to be used as drug. It has been shown that extract of betel leaves has antioxidant activity due to presence of chevibetol (CHV) and allylpyrocatechol (APC) (Rathee *et al.*, 2006). Similarly the extract of betel leaves has hypolipidemic activity (Gramza and Korczak, 2005), antibacterial activity (Nalina and Rahim, 2007; Bissa *et al.*, 2007; Ramji *et al.*, 2002) and anti-carcinogenic properties due to

presence of hydroxyl-chavicol (Bhide *et al.*, 1991). Betelvines are one of the highly investigated plants and phytochemical studies show that it contains a wide variety of biologically active compounds whose concentration depends on the variety of the plant, season and climate. The aroma of betel leaf is due to the presence of essential oils, consisting of phenols and terpenes. The various phytochemicals found in the betel plants are chavibetol, chavicol, hydroxychavicol, estragole, eugenol, methyl eugenol, hydroxycatechol, caryophyllene, eugenol methyl ether, stearic acid, n-triacontanol, ursolic acid, ursolic acid

3 $\beta$ -acetate (Rastogi and Mehrotra, 1993; Kumar *et al.*, 2010). Chewing betel leaf is supposed to prevent bad breath (halitosis), improve the vocalization, harden the gum, conserves the teeth and sweetens breath. The infusion prepared from the leaves and stems are supposed to be useful in treating indigestion, bronchitis, constipation, congestion, coughs and asthma. The leaf juice is given systemically to treat cough and indigestion in children. The Essential oil isolated from the leaves is supposed to be useful in treating respiratory catarrhs and as an anti-septic.

The harmful effects of pan as described in the ayurvedic texts are that it weakens teeth, impairs health and deadens the taste buds of the tongue. In the Indian subcontinent, where chewing tobacco with pan is a common habit, cancer of the mouth is very common. But the educated Indians are of the opinion that moderate use of betel leaf is not merely innocuous but that it may even be conducive to good health. So, Einsiedlen (Switzerland) has told "Everything is poisonous and nothing is not poisonous, only the dose makes a thing poisonous."

Well drained fertile clay loams are suitable for betelvine, it requires a cool humid atmosphere with considerable humidity and regular supply of moisture in the soil. Training is done by fixing the vine at intervals of 15 to 20 cm along the standards loosely with the help of banana fibre. Training is done at every 15-20 days interval depending upon the growth of vines. Betelvine cultivars are assorted as male or female types; they have been selected and conserved by the farmers for centuries for their uniqueness in taste, flavour, texture, leaf size and shape. Some of the land races of betelvine developed due to their adaptation in new ecological niches (Maiti and Saikia, 2002). So the study of biochemical changes in leaves had been selected to observe the changes in betelvine varieties.

## MATERIALS AND METHODS

The purpose of the experiment was to characterize the betelvine varieties with biochemical

characteristics such as carbohydrate, soluble protein, phenol, proline etc.

### Site of Experiment

For the present study, location having well drained fertile clay loam soils and cool, humid atmosphere with irrigation facilities was selected from All India Coordinated Research Project (AICRP) on Betelvine at Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India.

The experiments were carried out with kapoori and sanchi cultivars in a randomized block design (RBD) with five replications inside the different conservatories (boroj) from the years 2012 to 2014.

### Plant material used

The different betelvine cultivars namely kapoori and sanchi from many sites of All India Coordinated Research Project (AICRP), Kalyani, WB were used for the present study. Fresh leaves of kapoori and sanchi were selected for examination in the laboratory of Bidhan Chandra Krishi Vishwavidyalaya, WB as follows-

#### (A) Kapoori type

1. Kapoori chinacheppali
2. Kapoori dodhipatla
3. Kapoori pedacheppali
4. Swarna kapoori

#### (B) Sanchi type

1. Gangarampur sanchi
2. Halisahar sanchi
3. Simurali sanchi
4. Kalipatti

Reducing sugar was estimated with the methodology used by Patil and Gaikwad (2011). 100 mg of the sample was extracted with hot 80% ethanol twice and collected the supernatant. The supernatant was evaporated by keeping it on a water bath at 80°C. 10 ml water was added and dissolved the sugars.

0.5 ml of aliquots was taken in a test tube and the volume was made up to 2 ml with distilled water. Tube containing 2 ml distilled water served as a blank. One ml of alkaline copper tartrate reagent was added to each tube and placed the tubes in boiling water for 10 minutes. After cooling the tubes, 1 ml of arsenomolybdate reagent was added and the volume in each tube was made up to 10 ml with water. The absorbance of blue colour was read at 620 nm after 10 min. From the graph drawn, the amount of reducing sugars present in the sample was calculated and represented as mg reducing sugar per 100 g sample.

The total carbohydrate was determined with the method used by Edewor and Theresa (2013). 100 mg leaf sample was hydrolysed by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cooled to room temperature. The extract was neutralised with solid sodium carbonate, volume was made up to 100 ml and centrifuged. The 0.5 ml aliquot was taken and volume was made up to 1 ml by adding distilled water in each tube including blank. Four ml anthrone reagent was added to each tube and heated for eight minutes in a boiling water bath. The tubes were cooled rapidly and optical density of green to dark green colour was measured at 630 nm. The amount of carbohydrate present in the sample was calculated from the graph and represented as mg sugar per 100 g sample.

Protein was estimated with the methodology proposed by Sadasivam and Manickam (1996). 500 mg of finely cut leaves samples were grinded with addition of 10 ml buffer. Extracts were centrifuged at 5000 rpm for 10 minutes and used the supernatants for protein estimation. 0.2 ml of supernatant was taken into a test tube and the volume was made up to one ml in each tube including blank with distilled water. Five ml alkaline copper solution was added to each tube, mixed well and allowed to stand for 10 minutes. After a proper mixing 0.5 ml folin-ciocalteau reagent

(FCR) was added, mixed well and incubated at room temperature in the dark for 30 minutes for blue colouration. The intensity of blue colour was measured in a spectrophotometer at 660 nm. From the graph prepared of standard BSA (bovine serum albumin) concentration the protein content of sample was calculated and represented as mg protein per g leaf sample.

The total phenols were determined by Folin-Ciocalteau reagent method described by Rekha *et al.* (2012). For that purpose leaf sample was mixed in 10-time volume of 80% ethanol and centrifuged at 10000 rpm for 20 minutes. The supernatant was collected and saved. The extraction was repeated with 5 times volume of 80% ethanol, centrifuged again and the supernatant was pooled to the earlier one. It was then evaporated to dryness and the residue was dissolved in 5 ml water. From the dissolved solution 0.2 ml aliquot was taken in test tube and the volume was made up to 3 ml with water. 0.5 ml folin-ciocalteau reagent (FCR) was added and then after 3 minutes 2 ml of 20%  $\text{Na}_2\text{CO}_3$  was added. The solution in the tubes was mixed thoroughly and placed in the boiling water bath for exactly 1 minute. It was then cooled and absorbance was read at 650 nm against the reagent blank. Using standard curve of catechol, concentration of phenol was calculated and expressed as mg phenols per 100 g leaf sample.

Proline estimation was done with the method used by Raja *et al.* (2012). The leaf material (0.5g) was homogenized in 3% aqueous sulphosalicylic acid and the residue was removed after centrifugation. One ml of the homogenized tissues was reacted with 1ml acid-ninhydrin and 1ml of glacial acetic acid in a test tube for one hour at  $100^\circ\text{C}$ . The reaction was terminated in an ice bath and the reaction mixture was extracted with 2ml toluene, mixed vigorously. Toluene layer was separated and warmed to room temperature. The optical density was measured at 520 nm using toluene for a blank. The proline concentration was determined from

a standard curve using D-Proline and expressed as  $\mu\text{M}$  proline per 100 g sample.

Ascorbic acid (vitamin C) was estimated by volumetric method followed by Rekha *et al.* (2012). One gram leaf sample was extracted in 4% oxalic acid and volume was made up to 100 ml, centrifuged and supernatant was collected. Five ml of supernatant and 5 ml working standard solution of ascorbic acid (100  $\mu\text{g}$  ascorbic acid per ml solution) was taken in a beaker separately. 10 ml of 4% oxalic acid was added to those solutions in a beaker one by one and titrated against 2, 6-dichloro phenol indophenols dye. The amount of the dye consumed was equivalent to the amount of ascorbic acid.

### Statistical method implemented

Collected laboratory data was calculated and analysed statistically by Randomized Block Design (RBD) method i.e. two factors RBD. Critical difference values were compared at 5% levels of significance and wherever 'F' was found significant, treatment means were compared.

## RESULTS AND DISCUSSION

This chapter furnishes the characterization of betelvine on the basis of various biochemical characteristics. Results have been described with pooled analysis of the average data collected from both the years.

### BIOCHEMICAL CHARACTERISTICS OF LEAF

#### Carbohydrate

Biochemical changes in betelvine with respect to carbohydrate concentration in leaf is designed and exposed in Table 1. The reducing sugar contents in kapoori chinacheppali (175.12 mg/100 g) and kapoori dodhipatla (176.83 mg/100 g) likewise in kapoori pedacheppali (126.69 mg/100 g) and swarna kapoori (121.15 mg/100 g) were found statistically at par

with each other. Kalipatti possessed highest reducing sugar (557.40 mg/100 g) among all the varieties followed by halisahar sanchi (441.00 mg/100 g), gangarampur sanchi (347.96 mg/100 g) and simurali sanchi (303.06 mg/100 g). Reducing sugar found more in sanchi cultivars than in kapoori cultivars. On the topic of the reducing sugar, January was the best for its increment (437.93 mg/100 g) while during April reducing sugar decreased (117.31 mg/100 g) significantly throughout the year. The reducing sugar during July (279.44 mg/100 g) was less as compare to during October (289.93 mg/100 g). The significant interaction between months and varieties was found in respect of reducing sugar content with the range of 31.96-874.99 mg/100 g in all the varieties throughout the year. Rajeswari and Rajamannar (1991) observed similar results with reducing sugar concentration of 56.537 mg/g dry weight in betelvine variety because present results are on fresh weight basis.

In case of total carbohydrate, the minimum (1080.45 mg/100 g) and maximum (2211.78 mg/100 g) carbohydrate concentration were observed in swarna kapoori and simurali sanchi variety of betelvine respectively. Comparable observations were found in the carbohydrate contents of kapoori chinacheppali (1110.27 mg/100 g), kapoori pedacheppali (1233.55 mg/100 g) and kapoori dodhipatla (1332.48 mg/100 g). Similarly carbohydrate contents in gangarampur sanchi (1558.09 mg/100 g), halisahar sanchi (1801.12 mg/100 g) and kalipatti (1660.06 mg/100 g) also found statistically comparable with each other. Kapoori cultivars verified lesser total carbohydrate concentrations than that of sanchi cultivars. April was the best which possessed maximum (2439.68 mg/100 g) while July possessed minimum (686.21 mg/100 g) carbohydrate concentration throughout the year. The total carbohydrate during January was found significantly high as compare to during October. The total carbohydrate

**Table 1: Biochemical changes in betelvine (*Piper betle* L.) leavesthroughout the year with respect to carbohydrate (mg/100 g)**

Reducing sugar									
Varieties	KAPOORI				SANCHI				Mean
Months	Kapoori chinacheppali	Kapoori dodhipatla	Kapoori pedacheppali	Swarna Kapoori	Gangarampur sanchi	Halisahar sanchi	Simurali sanchi	Kalipatti	
July	118.89	162.70	137.47	105.45	455.96	245.76	363.32	645.93	<b>279.44</b>
October	154.98	166.26	128.03	131.49	280.17	624.74	309.06	524.73	289.93
January	293.11	326.40	209.31	148.33	563.00	695.01	393.25	874.99	<b>437.93</b>
April	133.49	51.96	31.96	99.34	92.72	198.48	146.62	183.93	<b>117.31</b>
Mean	175.12	176.83	126.69	121.15	347.96	441.00	303.06	557.40	
		Factors		C.D.	SE(d)	SE(m)			
		Variety		13.77	6.95	4.91			
		Month		9.74	4.91	3.48			
		Interaction		27.54	13.90	9.83			
Total carbohydrate									
July	289.51	472.23	464.48	406.79	794.50	1111.08	1156.68	794.42	<b>686.21</b>
October	521.14	569.96	551.18	571.59	807.55	1111.30	962.29	1186.78	785.22
January	1546.27	2164.44	1946.43	1514.90	2391.15	2339.99	2845.65	1913.38	<b>2082.78</b>
April	2084.16	2123.27	1972.12	1828.50	2239.14	2642.11	3882.49	2745.68	<b>2439.68</b>
Mean	1110.27	1332.48	1233.55	1080.45	1558.09	1801.12	2211.78	1660.06	
		Factors		C.D.	SE(d)	SE(m)			
		Variety		24.24	12.23	8.65			
		Month		17.14	8.65	6.12			
		Interaction		48.49	24.47	17.30			

concentrations were ranged from 289.51 to 3882.49 mg/100 g in all the varieties throughout the year with significant interaction between months and varieties. The parallel findings were reported by Khatri *et al.* (1997) with total carbohydrate content of 118.75 mg/g dry weight and Satyabrata Maiti *et al.* (1999) with total sugar content of 47.51 mg/g dry weight in betelvine leaves.

**Soluble protein**

Biochemical changes in betelvine leaves revealed that, the highest concentration of protein was found in Kapoori dodhipatla (17.44 mg/g) among the varieties followed by in Kapoori pedacheppali (16.85 mg/g) and and Kapoori chinacheppali (16.15 mg/g) which were not comparable statistically with each other. The protein content was lowest in Halisahar sanchi (10.83 mg/g) whereas protein contents in Gangaramur sanchi, Simurali sanchi and Kalipatti were found at par with each other. The maximum protein contents in the leaves of

betelvine were found during July (19.07 mg/g) and minimum found during January (11.14 mg/g) throughout the year. During October protein contents were low as compare to during April but the results were statistically at par with each other. Analogous findings were reported by Nema, 1991 with protein contents on dry weight basis of 17.90, 18.38 and 19.95% respectively in mandsaur bangla, kalkatia and mahoba. Overall observations showed that Kapoori cultivars were more proteinous than sanchi cultivars. The protein contents of betelvine varieties varied from 8.56 mg/g in Halisahar sanchi during October to 23.51 mg/g in Kapoori dodhipatla during July in all the varieties throughout the year with significant interaction between months and varieties. The biochemical analysis by Padmanabhan *et al.*, 1995 showed protein content of 3.12% in Kapoori leaves which were found to be more than the popular variety, vellaikodi. Also similar results were found by Khatri *et al.*, 1997 with proteins of 12.49 mg/g and free

**Table 2: Biochemical changes in betelvine (*Piper betle* L.) leaves throughout the year with respect to soluble protein (mg/g)**

Varieties	KAPOORI				SANCHI				Mean
Months	Kapoori chinacheppali	Kapoori dodhipatla	Kapoori pedacheppali	Swarna Kapoori	Gangarampur sanchi	Halisahar sanchi	Simurali sanchi	Kalipatti	
July	18.16	23.51	23.37	21.65	16.50	14.65	18.14	16.57	19.07
October	15.30	16.30	17.56	13.51	11.04	8.56	12.77	11.34	13.30
January	12.71	13.58	12.40	10.06	11.66	8.64	10.23	9.84	11.14
April	18.43	16.37	14.07	16.38	10.82	11.49	12.42	10.77	13.84
Mean	16.15	17.44	16.85	15.40	12.51	10.83	13.39	12.13	
		Factors		C.D.	SE(d)	SE(m)			
		Variety		1.28	0.65	0.46			
		Month		0.91	0.46	0.32			
		Interaction		2.57	1.29	0.92			

**Table 3: Biochemical changes in betelvine (*Piper betle* L.) leaves throughout the year with respect to phenol (mg/100 g)**

Varieties	KAPOORI				SANCHI				Mean
Months	Kapoori chinacheppali	Kapoori dodhipatla	Kapoori pedacheppali	Swarna Kapoori	Gangarampur sanchi	Halisahar sanchi	Simurali sanchi	Kalipatti	
July	135.83	180.15	212.21	343.64	363.04	593.05	575.98	570.77	371.83
October	372.98	447.92	459.09	449.44	967.68	845.69	1057.77	1330.93	741.44
January	772.38	529.20	454.79	484.11	1431.63	1239.07	822.56	971.71	838.18
April	318.18	287.11	483.34	414.23	537.22	581.07	702.83	378.43	462.80
Mean	399.85	361.10	402.36	422.86	824.89	814.72	789.79	812.96	
		Factors		C.D.	SE(d)	SE(m)			
		Variety		9.54	4.81	3.40			
		Month		6.74	3.40	2.41			
		Interaction		19.07	9.62	6.81			

amino acids of 13.08 mg/g in betelvine leaves, Table 2.

### Phenol

The variation in the phenol content of betelvine varieties throughout the experimental period and among the varieties is presented in Table 3 for biochemical characterization. The comparable variations found in phenol contents of gangarampur sanchi (824.89 mg/100 g), halisahar sanchi (814.72 mg/100 g) and simurali sanchi (789.79 mg/100 g) and kalipatti (812.96 mg/100 g). Among kapoori cultivars, swarna kapoori possessed significantly more phenol content (422.86 mg/100 g) as compare to all the remaining kapoori varieties. The lowest phenol concentrations were observed in kapoori dodhipatla (361.10 mg/100 g) among all the varieties followed by kapoori chinacheppali

(399.85 mg/100 g) and kapoori pedacheppali (402.36 mg/100 g). Kapoori pedacheppali had more phenol contents than that of kapoori chinacheppali but the results were statistically at par with each other. Sanchi cultivars possessed higher concentrations of phenol than that of kapoori cultivars due to which kapoori is more susceptible to atmospheric stress than sanchi. During July, phenol concentration decreased (371.83 mg/100 g) and started to increase from October upto January and then again decreased during April with significant variations throughout the year. Khatri et al. (1997) found total phenol of 48.52 mg/g and Satyabrata Maiti et al. (1999) found total phenol of 27.62 mg/g in the leaves of betelvine on dry weight basis. The phenol contents were ranged from 135.83 to 1431.63 mg/100 g in all kapoori and sanchi

varieties throughout the year with significant interaction between months and varieties, Shivashankara et al., 2012.

**Proline**

The biochemical changes in betelvine accessions with respect to amino acid, proline stated that proline contents of kapoori pedacheppali, swarna kapoori and gangarampur sanchi variety of betelvine were found statistically at par with each other. Among kapoori, significantly more proline contents were observed in kapoori chinacheppali (18.73 µM/100 g) than as compare to in kapoori dodhipatla (15.06 µM/100 g). Proline contents were comparable in halisahar sanchi (21.09 µM/100 g) and simurali sanchi (24.38 µM/100 g) which found more than that of kalipatti (19.10 µM/100 g). The period of October to January was the best for proline increment throughout

the year. There were significant variations observed in proline contents of betelvine accessions throughout the year. The minimum proline concentrations recorded during April (11.49 µM/100 g) throughout the year followed by during July (16.67 µM/100 g) and January (21.87 µM/100 g). The proline contents were varied from 7.66 to 35.67 µM/100 g in all the varieties throughout the year with significant interaction of months and varieties, Table 4.

**Vitamin C**

Vitamin C was determined by volumetric method to characterize the betelvine cultivars for their identification and presented in Table 5. Kalipatti possessed lowest (87.99 mg/100 g) amount of vitamin C among all the kapoori and sanchi cultivars followed by simurali sanchi (94.37 mg/100 g) and gangarampur sanchi (109.60 mg/100 g) while highest recorded in

**Table 4: Biochemical changes in betelvine (*Piper betle* L.) leaves throughout the year with respect to proline (µM/100 g)**

Varieties	KAPOORI				SANCHI				Mean
	Kapoori chinacheppali	Kapoori dodhipatla	Kapoori pedacheppali	Swarna kapoori	Gangarampur sanchi	Halisahar sanchi	Simurali sanchi	Kalipatti	
July	13.02	14.54	12.73	11.51	15.39	23.23	25.52	17.43	16.67
October	30.50	21.62	26.81	26.36	22.21	12.39	35.49	27.51	25.36
January	17.18	14.62	16.93	24.90	20.54	35.67	22.24	22.84	21.87
April	14.22	9.46	14.06	7.66	10.61	13.07	14.25	8.61	11.49
Mean	18.73	15.06	17.63	17.60	17.19	21.09	24.38	19.10	
		Factors		C.D.	SE(d)	SE(m)			
		Variety		0.94	0.47	0.34			
		Month		0.67	0.34	0.24			
		Interaction		1.88	0.95	0.67			

**Table 5: Biochemical changes in betelvine (*Piper betle* L.) leaves throughout the year with respect to vitamin C (mg/100 g)**

Varieties	KAPOORI				SANCHI				Mean
	Kapoori chinacheppali	Kapoori dodhipatla	Kapoori pedacheppali	Swarna kapoori	Gangarampur sanchi	Halisahar sanchi	Simurali sanchi	Kalipatti	
July	43.51	54.36	43.43	39.64	41.12	51.47	40.78	41.34	44.46
October	82.88	65.94	101.80	83.39	85.98	82.55	83.45	64.62	81.33
January	166.39	144.58	126.49	125.98	118.42	153.14	100.37	93.70	128.63
April	153.31	208.76	193.66	230.43	192.89	173.50	152.89	152.30	182.22
Mean	111.52	118.41	116.34	119.86	109.60	115.17	94.37	87.99	
		Factors		C.D.	SE(d)	SE(m)			
		Variety		1.22	0.61	0.43			
		Month		0.86	0.43	0.31			
		Interaction		2.43	1.23	0.87			

swarna kapoori (119.86 mg/100 g). Among kapoori cultivars, all the cultivars demonstrated significant variations with respect to vitamin C. The comparable observations were recorded with respect to vitamin C contents in kapoori chinacheppali (111.52 mg/100 g), kapoori pedacheppali (116.34 mg/100 g) and kapoori dodhipatla (118.41 mg/100 g). Among the sanchi betelvine, vitamin C was significantly more in halisahar sanchi (115.17 mg/100 g) than that in gangarampur sanchi, simurali sanchi and kalipatti. Vitamin C contents were very low during July (44.46 mg/100 g) and started to increase from October to January with highest amount of vitamin C (182.22 mg/100 g) during April throughout the year. The vitamin C contents were ranged from 39.64 to 230.43 mg/100 g in all the varieties throughout the year with significant interaction between months and varieties. Contrasting results were found by Padmanabhan *et al.* (1995) with vitamin C content of 9.2 mg/100 g in betelvine.

## CONCLUSION

Biochemical changes of betelvine showed that reducing sugar, total carbohydrate and phenol content of the sanchi type betelvine was higher than that of kapoori type betelvine. The maximum (824.89 mg/100 g) and minimum (361.10 mg/100 g) phenol contents were respectively in the leaves of gangarampur sanchi and kapoori dodhipatla variety. Soluble protein content of leaf of the kapoori cultivars was higher than that of the sanchi cultivars. The highest vitamin C content was in the leaves of swarna kapoori (119.86 mg/100 g) among all the varieties. The period of October to January was the best for proline increment throughout the year. There were significant variations found among the varieties throughout the year.

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