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Biochemical and physiological characterization of corky tissue affected fruit of sapota (*Manilkara achras* (Mill) Fosberg) cv. Cricket Ball

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ABSTRACT

Corky tissue is a physiological disorder, affecting Cricket Ball variety of sapota to the tune of 35% or more, especially in the summer season. Very little work has been reported so far on the biochemistry of this disorder. In the present study an attempt was made to characterize the corky tissue affected fruit in biochemical and physiological view. Studies revealed that the percent of corky tissue incidence had a positive correlation with the number fruit per panicle and size of the fruit. Gibberellic acid treatment has reduced the incidence of corky tissue as compared to control and paclobutrazol treatment has increased the incidence of disorder. In corky tissue affected fruit pulp the reducing sugars, total soluble sugars content and enzyme activity like amylase were reduced and starch content was higher as compared to healthy fruit pulp, hence decreased activity of amylase might affected the conversion of starch into simple sugars. Total carbohydrate, proteins, fatty acids, mineral nutrients were comparatively less in case of affected fruits. This indicates that there was less mobilization of these biochemical parameters into the fruit and consequently affected the physiological development of the fruit and manifested as disorder.

Keywords: Biochemical changes, corky tissue, moisture content, sapota

Sapota is a delicious tropical fruit. Fruit contains carbohydrate (50.4g 100^{-1}g), protein (0.7g 100^{-1} g), fat (1.1g 100^{-1} g), fibre (2.6g 100^{-1} g) and mineral nutrients *viz*. calcium (28 mg 100^{-1} g), iron (2.0 mg 100^{-1} g), phosphorus (27mg 100^{-1} g) and ascorbic acid (6.0mg100^{-1} g) Gopalan *et al*. (1977). Besides these food values, sapota fruits are also used in some ayuverdic preparations. Therefore, there is a high demand for the fresh fruit, as well as for processed fruit products also. In order to meet the growing demand of the fruit both for internal consumption and exports, there is a need to maintain high quality of the fruits. One of the major problems in this direction is the occurrence of certain physiological disorders, apart from the problems of pests and diseases, which reduce the quality of the fruit drastically. Corky tissue (CT) is one such disorder in the Cricket Ball variety of sapota (up to 35%) in certain seasons and severely hampering the quality of fruits. Recently, the increasing global warming, leading to increase in temperature of earth's atmosphere and variations in the rainfall pattern has affected the integrated development of plant and eventually appear as disorders of the fruit crops. Corky tissue (CT) of sapota is a physiological disorder characterized by hard lump in the pulp, slightly desiccated in nature and acidic to taste. This disorder shows no distinct external symptoms and becomes visible only when the fruit is cut open. Under extremely severe conditions, corky skin eruptions are seen (Sulladamath, 2005). As such, the edible quality of the affected fruit is poor and in advanced stages, it becomes poor for human consumption.

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MATERIAL AND METHODS

Sapota fruit of cv. Cricket Ball for all the studies were collected from Experimental Orchard of the Indian Institute of Horticultural Research (IIHR), Hessarghatta, Bangalore. Tagging of fruits was done at 50% fruit maturity of fruits. One to four fruits per panicle were selected. Pre harvest treatment of immature fruit (at 50% maturity) with gibberlic acid (GA_3) - 200ppm and paclobutrozol (PBZ)-1000ppm to the panicle containing two fruits in different combination.

At fully ripen stage; the corky tissue affected fruits were identified by the symptoms after cutting the fruits into two halves. Pulp of corky tissue affected fruits was identified by the symptoms like hard lumps in pulp and off flavor.

The extraction and estimation of soluble sugars and reducing sugars was done by following the DNS (dinitrosalicylic acid) method (Selvaraj and Lodh, 1973). 1g of the fresh sample was extracted in 10ml of warm 80% ethanol. The extract was centrifuged at 10,000 rpm for 10 min. The supernatant was evaporated on water bath to dryness and the residue was dissolved in 10ml of water. This alcohol-free extract was used for the estimation of reducing sugars by using the DNS reagent and for total soluble sugars the hydrolysis of alcohol free extract was done overnight by adding the concentrated Hcl and neutralized with 40% NaOH and this aliquot was used for estimation of total soluble sugars by using the

DNS reagent the absorbance was recorded at the 540 nm for both reducing sugars and total soluble sugars.

For extraction and estimation of starch, 1g of the fresh sample was extracted in 10ml of warm 80% ethanol. The extract was centrifuged at 10,000 rpm for 10 min. The residue was retained and air dried well. The dried residue, 5 ml of water and 6.5 ml of 52% perchloric acid were added and stirred for 20 min at 4^0C, the extract was centrifuged at 5,000 rpm for 15 min and the supernatant was used for the estimation of starch by using the DNS reagent and the absorbance was recorded at 630 nm. And total carbohydrate estimation was done by following the methodology of Hodge and Hofrieter (1962).

Extraction and estimation of total soluble protein was done by the standad described method and free fatty acids were estimated by the method of Jayaraman (1981).

Amylase activity was determined by DNS method. The product of amylase activity was allowed to react with DNS reagent, which formed an orange-red colour product, when starch was used as the substrate. Maltose, the reducing sugar, which was formed by the action of amylase, was determined calorimetrically at 540 nm (Bernfeld, 1955).

For phosphorous and calcium estimation the fruit sample was digested by using Diacid mixture 9:4 (Nitric and Perchloric) and digest of phosphorous analyzed by the Vanadomolybdic yellow colour method and calcium and iron were analyzed by using the methodology of Atomic absorption spectrophotometry (Jackson, 1973).

The data were statistically analyzed by subjecting to ANOVA as described by Sundaraj *et al*. (1972) adopting the Fisher's analysis of variance technique. Critical difference values were compared at 5% levels of significance and wherever 'F' was found significant, treatment means were compared.

RESULTS AND DISCUSION

Physiological study

In the present study, competition of fruits was studied in relation to the development of corky tissue. There was an increasing incidence of corky tissue with increasing number of fruits per panicle (Table 1). Fruit growth is part of the integrated growth of a plant. Fruit acts as a sink for photosynthates, nutrients, water and when a number of fruits are present on the plant, there is a competition between different sinks (Salisbury and Ross, 1986). The study conducted by Ali and Kelly (1992) indicated that inter-fruit competition significantly limits the growth rates in chillie fruit. Reducing the number of flowers or fruits increases fruit size, improves quality, prevents alternate bearing and balances the fruit shoot ratio, leading to an increase in assimilates to fruits and shoots. The practical consequences of thinning include an increase in individual fruit weight, fruit maturity enhancement and better flower bud formation (Costal and Vizzatto, 2000). The source-sink alteration by fruit removal and shoot trimming had impact on yield, leaf sugar metabolism and composition of berries in grape (Mota *et al.,* 2010).

Table 1: Relationship between number of fruits per panicle and incidence of corky tissue in sapota

Number of fruits per panicle	Corky tissue incidence (%)
1	10
2	32
3	45
4	50
SEm (±)	**0.71**
LSD (0.01%)	**3.06**

Above findings on inter fruit competition and incidence of corky tissue was supported by treating the fruits with growth regulators and data presented in table- 2. Results revealed that big sized fruits were having lesser incidence as compared to small sized one. Same trend was also observed in case of GA_3 and PBZ treated fruits, but GA_3 treated fruits (big and small) showed less incidence as compared to control, in contrasts to this, PBZ treated fruits showed higher incidence. Result also revealed that in case of GA_3 treated fruits, smaller fruits were also having lesser incidence as compared to untreated big fruits, and this may be because of GA_3 treatment, which has increased the sink strength of treated small fruit. And reverse trend was observed in PBZ treated fruits, even big fruits, treated with PBZ were having more incidence as compared to control fruit. It is well established that the growth regulator GA_3 enhances the sink strength and in contrast to this, PBZ reduces the sink strength by inhibiting the biosynthesis of GA_3.

Table 2: Incidence of corky tissue in fruits treated with growth regulators in sapota (10 replications)

Treatments	Size of the fruit	
	Big fruit	Small fruit
Control	3	5
GA_3	1	3
PBZ	5	8
LSD (0.01)	**2.84**	
SEm (±)	**0.29**	

The term sink strength can be defined as the competitive ability of an organ to receive or attract assimilates (Wareing and Patrick, 1975; Wolswinkel, 1985)**.** In Japanese pear, the application of GA_{3+4} during the period of rapid fruit growth resulted in a marked increase in pedicel diameter and bigger fruit at harvest was reported by Zhangh *et al.* (2005). The application of GA_3 to grape fruits enhanced fruit growth as well as the endogenous hormones (IAA or

Zeatin) during fruit growth and development (Ma and Liu, 1998; Chen *et al.*, 2000). Alternatively, GA_3 is effective in maintaining cell expansion (Gillaspy *et al*., 1993; Ozga and Dennis, 2003) and therefore exogenous gibberellins have been widely used for fruit enlargement.

Biochemical study

To confirm those effects in a more affirmative way some relevant biochemical studies had been made. The biochemical parameters such as the amount of soluble sugar, starch, total carbohydrates, amylase activity and moisture content of fruit were estimated (Table 3). The moisture percentage was estimated by the gravimetric method. Corky tissue affected pulp showed significantly lower moisture percentage (67 %) as compared to moisture of healthy fruit pulp (76%). The Corky tissue incidence was varied from 18% to 90% with respect to moisture loss of 1.1g to 7.6g from fruit (Fig. 1). Corky tissue affected fruit pulp characterized by hard lump in the pulp, slightly desiccated in nature and acidic to taste.

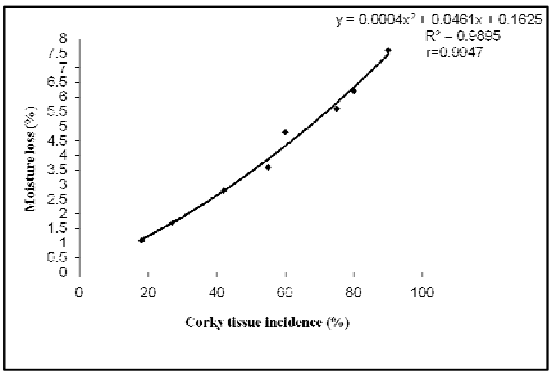

Fig. 1: Extent of water loss from corky tissue affected fruit in sapota

The total soluble sugar and reducing sugars in corky tissue affected fruit pulp were lesser in quantity compared to healthy fruit, but in contrast to this, the starch content of the corky tissue affected fruit was significantly higher as compared to healthier one (Table 3). To support the above findings of harder nature of fruit, less moisture (%), soluble sugar, reducing sugar and higher starch contents of the affected fruit, the amylase enzyme activity was studied both in affected as well as in healthy fruit. The corky tissue affected fruits showed much lower amylase activity compared to healthy fruits (Table 3). It was noticed that nearly about two times decreased in amylase enzyme activity of corky tissue affected fruit pulp as compared to healthy fruit pulp. The lower activity of amylase in corky tissue affected fruit pulp could be due to less moisture content of affected fruit pulp than the healthy fruit pulp. From the results obtained for starch, reducing sugars, total soluble sugars and amylase activity, it clearly shows a decrease in the amylase activity of the corky tissue affected fruit pulp leads to a reduction in the degradation of starch and resulting in lesser content of both total soluble sugars and reducing sugars. High starch content in corky pulp indicates an inhibition of ripening due to incomplete break down of starch therefore fruit pulp becomes harder in corky tissue affected fruit. Similar results were also observed in mango fruit mesocarp by Shivashankar *et al.* (2007). Study conducted by Katrodia and Rane (1989), Lima *et al*. (2001) indicated that the fruit affected with spongy tissue exhibited much lower amylase activity compared to healthy tissue of mango Cv. Tommy Atkins. Lower activity of amylase and other enzymes related to the corbon metabolism in the pulp of spongy tissue affected fruits of Alphonso mango was noticed by the Gupta *et al*. (1985). There was appreciable reduction in non-reducing sugars in spongy tissue as compared to the healthy tissue (Amin 1967) and higher starch content in spongy tissue pulp of mango (Burdon and Moore 1991; Ravindra and Shivashankar 2004). Biochemical studies of spongy tissue affected fruit pulp in mango by Selvaraj *et al.* (2000) indicated that the affected pulp had higher acid to sugar ratio, higher starch, polyphenols and reduction in non-reducing sugars, carotenoids, ascorbic acid and protein.

Table 3: Moisture, amylase activity, starch, total soluble sugars and reducing sugar content of sapota

Fruit status	Moisture (%)	Amylase (mg maltose h^{-1} g^{-1})	Starch (mg g^{-1} dry wt. of pulp)	TSS (mg g^{-1} dry wt. of pulp)	RS (mg g^{-1} dry wt. of pulp)
Healthy	76	7.34	150.65	468.25	167.35
Corky	67	4.33	240.25	257.10	122.00
SEm (±)	**0.37**	**0.13**	**2.88**	**5.91**	**1.72**
LSD (0.01)	**1.42**	**0.52**	**11.75**	**24.04**	**7.03**

Table 4: Total carbohydrate, free fatty acids and protein in healthy and corky tissue affected fruits of sapota

Fruit status	Total carbohydrates (mg g^{-1} dry wt. of pulp)	Free fatty acids (mg g^{-1} dry wt. of pulp)	Protein (mg g^{-1} dry wt. of pulp)
Healthy	816.66	2.427	0.623
Corky	654	1.804	0.462
F test	**	**	**
SEm (±)	**12.68**	**0.04**	**0.02**
LSD (0.01)	**54.80**	**0.17**	**0.09**

The data presented in table- 4 indicated that the total carbohydrates, protein and free fatty acid content of fruit. In corky tissue affected fruit all these parameters were lesser in quantity as compared to healthy fruit. Gupta *et al*. (1985) and Shivashankar *et al.* (2007) observed similar difference in total protein content in affected and healthy mesocarp of spongy tissue affected fruit.

Both the types of fruit pulp (healthy and corky tissue affected fruits) were analyzed for finding their mineral nutrient status. The results revealed that phosphorous (P) and iron (Fe) content of corky tissue affected fruit pulp were significantly lesser in quantity, about 40 percent reduction in corky tissue affected fruit pulp compared to healthy fruit pulp. But there was no much difference in the calcium (Ca) content of corky tissue affected fruit pulp in comparison with the healthy fruit pulp (Fig. 2).

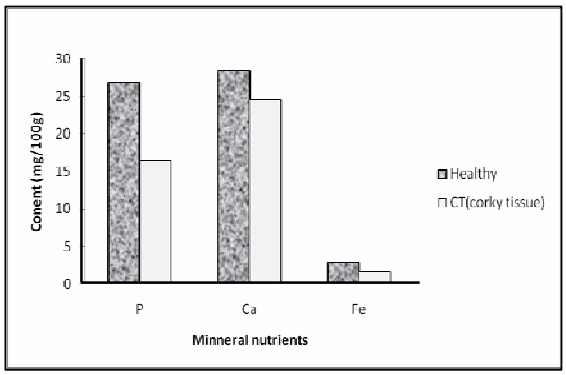

Fig. 2: Minarel nutrient content in corky and healthy fruit pulp of sapota

Sulladmath (2005) showed that the incidence of corky tissue in sapota was directly related to its low Ca and Mg content, although the levels of major and minor nutrients were also lower in corky tissue affected fruit. Sharma and Room Singh (2009) reported that deficiency of Ca and B were the probable causes for the incidence of fruit fitting in the mango.

From the outcome of the physiological and biochemical studies in relation to corky tissue development in sapota, it clearly indicates that the inter-fruit competition will leads to the development of different sized fruits and among these, fruits which were in bigger size were having less incidence of corky tissue as compared to smaller sized fruits. It is due to the weaker sink strength in smaller sized fruits, which leads to the less mobilization of photosynthates, moisture and mineral nutrients to the fruits and this result in physiological imbalance, consequently biochemical changes occurs and this could be appeared as a physiological disorder.

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