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Short communication

Segregation of corky tissue affected fruits of sapota by specific gravity method

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ABSTRACT

Sapota cv. Cricket Ball is a popular commercial variety in India which is plagued by the physiological disorder known as Corky Tissue (CT). This disorder adversely affects the eating quality of the fruit, making it unfit for consumption. This paper describes a technique of dipping sapota fruits in 12.5% salt solutions to segregate the CT affected fruits from healthy fruits based on differences in fruit specific gravity. The healthy fruits with a specific gravity of >1.0895 sank to the bottom, while the fruits affected by CT having a specific gravity of <1.0895 floated on the solution, thus ensuring complete separation of healthy and CT fruits in a single step process. The method is simple, rapid and cost effective and could be applied for culling out CT affected fruits at the field level by the growers and traders.

Key words: Specific gravity separation, corky tissue, salt solution, sapota.

Sapota [*Manilkara achras* (Mill) Fosberg] cv. Cricket Ball is highly prone to the physiological disorder, known as corky tissue (CT) in which the edible quality is greatly reduced, which poses a major constraint for the production of superior quality fruits. The incidence of CT can significantly reduce orchard profitability because the rate of incidence of the disorder is unpredictable. The disorder appears in mature ripe fruits, which shows no external symptoms and detected only after the fruits are cut open (Fig. 1). Corky tissue is characterized by the presence of a hard lump in the pulp, partially desiccated in nature occurring close to the peel of the ripe fruits that is slightly acidic in taste (Sulladmath, 7). The occurrence of CT in sapota cv. Cricket Ball in our country during summer months is around 50% (Shivashankar *et al.*, 6). Rain-fed trees are found to record higher incidence of CT compared to irrigated trees (Shivashankar *et al*., 5). Under extreme dry weather conditions, the rate of incidence of CT could exceed 60%, while irrigated trees show the incidence of the disorder to a lesser degree (Shivashankar *et al*., 6). A characteristic feature of the disorder is that CT is initiated in the pre-harvest phase due to which the intensity of the disorder increases with storage time. The disordered fruits are indistinguishable from healthy fruits by external appearance. At a commercial level, there is no simple method which could be adopted by growers and traders to separate the healthy and corky fruits, especially for niche markets. Hence, the present research work was carried out to bridge this gap. In a previous study, we reported that the fat contents of both seed and pulp were lower in CT fruits compared to healthy fruits (Shivashankar *et al*., 6) due to which changes in the specific gravity of healthy and CT fruits were discernible. Based on this observation, studies were performed to determine if the differences in specific gravity of fruits could be exploited to distinguish between healthy and CT fruits.

Sapota fruits cv. Cricket Ball were harvested at the full maturity stage from trees maintained at the experimental block of the institute during April 2014. Fruits were stored in large trays to ripe naturally under ambient conditions of temperature (32.7°C) and relative humidity (40.4%). As fruits began to ripen, fruits free from symptoms of microbial infection and injuries from insects or physical damage were

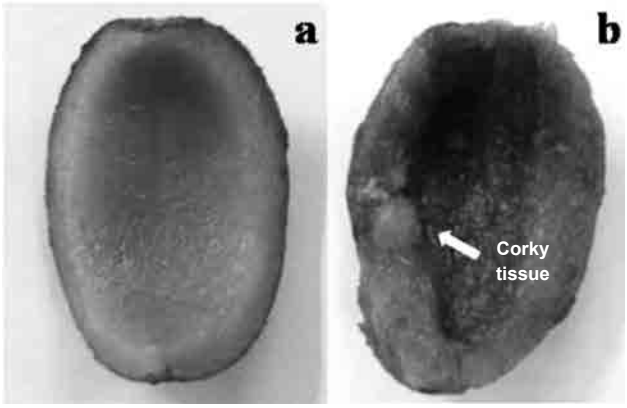

Fig. 1. Healthy (a) and affected (b) fruits of sapota cv. Cricket Ball.

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selected and used for the study. Salt solutions of food-grade sodium chloride ranging in concentrations from 10 to 15% with 0.5% increments were employed for the study. Specific gravity of each of these was determined from conversion table (Wolf *et al*., 8). Selected uniformly ripe fruits were transferred to the salt solutions. The experiment involved 100 fruits per treatment and replicated thrice. The number of sinking and floating fruits in each concentration solution was recorded separately. Thereafter, the fruits in each group were cut open to score for the incidence and intensity of CT. Results were analysed statistically and expressed as mean ± SE.

Corcky tissue affected fruits were identified based on the presence of hard lump within the pulp close to skin (Shivashankar *et al*., 5). Fruit weight, seed weight and number of seeds per fruit were recorded. Pulp and seed moisture contents were determined gravimetrically. The intensity of CT disorder was reckoned as percent of the ratio of weight of pulp affected by CT symptom to the total weight of fruit (Shivashankar *et al*., 6). Total fat content in tissue samples was extracted using Soxhlet extractor as described by Osborne and Voogt (4) and determined gravimetrically. One g sample of dry tissue powder packed in a thimble was placed in a Soxhlet extractor and extracted under reflux on a steam bath for 3 h using petroleum ether as solvent (bp. 40-60°C).

The solvent containing the dissolved fats were quantitatively transferred to a dry pre-weighed flask (W_1), evaporated to dryness on a boiling water bath and weighed again (W_2). The difference in the weight was used to calculate the percent of fat in sample using the formula: (W_2-W_1) ×100 / weight of sample. Total soluble solids (TSS) was measured using hand held Erma refractometer, Japan (0-32° scale) and the values were expressed as °Brix after correcting values to 20°C. The reducing sugar was measured using dinitrosalicylic acid (Miller, 3). Fresh tissue (1.0 g) was extracted in boiling 80% (v/v) ethanol, centrifuged at 3,000 ×g for 10 min. at 25ºC, and the supernatant was evaporated to dryness in a boiling water bath. The sugars in the dry residue were then re-dissolved in 2 ml of distilled water. Two-hundred µl of each extract was mixed with 800 µl of water and 500 µl of dinitrosalicylic acid reagent and heated in a boiling water bath for 5 min. The final volume was made up to 20 ml with water and the absorbance was read at 540 nm using a UV-VIS spectrophotometer, Model T80 + (PG Instruments, UK). The concentration of reducing sugar was calculated from a standard curve, and expressed as mg maltose equivalents 100 g^{-1} FW. Total soluble sugar was determined in 80% (v/v) ethanol extracts after acid hydrolysis. One ml of concentrated HCl was added to 10 ml of each extract, mixed, and incubated overnight at 37ºC. The hydrolysate was neutralised with 10M NaOH, using phenolphthalein as the indicator. The final volume was made up to 20 ml with water, and the concentration of total sugars in the extract was measured, as described above.

Seed viability was estimated by measuring TDH activity, using the triphenyl tetrazolium chloride (TTC) assay (ISTA, 1). After removing the seed coat, the embryo was soaked overnight in water. Three ml of 0.1% (w/v) TTC reagent was added to 100 mg of embryos, and incubated for 24 h at 37ºC in a water bath. The tissue was then homogenised in 10 ml of 70% (v/v) methanol, centrifuged at 2,500 ×g for 10 min., and the absorbance of the clear supernatant was read in a spectrophotometer at 485 nm. A standard curve prepared from known concentrations of triphenylformazan was used to express TDH activity in µg formazan produced g^{-1} FW. All the experiments were done in triplicate and the results were subjected to statistical analysis.

Preliminary studies indicated that mature ripe fruits of sapota dipped in salt solutions of varying concentrations sank to the bottom in salt solution containing 10% of salt, while all fruits rose to the top in 15% salt solution. Out of 100 fruits tested in 12.5% salt solution, 21 fruits were found to float while 79 fruits sank to the bottom. On examination of the pulp for the CT symptoms, it was observed that all the 79 sinking fruits were healthy, while all the 21 floating fruits were affected by CT. As against this, in salt solutions ranging from 10.5 to 12.0%, CT affected fruits intensity ranged from 5 to 50% or more were found along with healthy fruits while at salt concentrations higher than 12.5%, a small percentage of healthy fruits were found mixed with CT fruits in varying proportions (Table 1).Thus, it was evident that 12.5% salt solution was the optimum concentration in which both healthy and CT affected fruits could be clearly distinguished. The specific gravity of sapota fruits containing a mixture of both healthy and CT affected fruits was found to vary from >1.0707 to <1.1085. Since the healthy fruits sank to the bottom while the fruits affected by CT floated in the solution containing 12.5% salt, it was evident that the healthy fruits had a specific gravity of >1.0895, while the CT fruits had a specific gravity of <1.0895.

With regard to fruit characteristics, the CT fruits had a significantly higher fruit weight compared to healthy fruits. The total number of seeds and seed weight were higher in healthy fruits. Both pulp and seed moisture contents were significantly higher in healthy fruits. Healthy fruits also registered significantly higher seed TDH activity as a result of which, per cent germination and the rate of germination were also

Table 1. Composition of sinking and floating fruits in salt solutions of increasing concentration and density.

Salt solution (%)	Specific gravity	Sinking	Floating
10.0	1.07070	All fruits sink to the bottom	-
10.5	1.07445	Healthy + CT (very low-medium intensity)	CT (high intensity)
11.0	1.07820	Healthy + CT (very low-moderate intensity)	CT (medium intensity)
11.5	1.08195	Healthy + (very low-moderate intensity)	CT (moderate intensity)
12.0	1.08570	Healthy + CT (low intensity)	CT (low intensity)
12.5	1.08947	Healthy only	CT only
13.0	1.09325	Healthy	CT + Healthy
13.5	1.09702	Healthy	CT + Healthy
14.0	1.10089	Healthy	CT + Healthy
14.5	1.10465	Healthy	CT + Healthy
15.0	1.10850	None	All fruits floating

Very low = <5%; Low = 5-20%; Medium = 21-40%; Moderate = 41-60%; High = >60%

significantly higher. The levels of total sugars, reducing sugars, TSS and total fat content were significantly higher in the pulp of healthy fruits. The pH of both pulp and seed were higher in healthy fruits (Table 2). Thus, the data on fruit characteristics strongly bear testimony to the fact that all the 79 fruits sinking in 12.5% salt solution were healthy fruits and all of the 21 floating fruits were affected by corky tissue. While, on contrary to this, at salt concentrations lower or higher than 12.5%, a mixture of healthy fruits and fruits affected by different intensities of CT, ranging from 5 to 50% were found (Table 1). It indicates that, this happened due to differences in specific gravity of fruits with varying intensities of CT. Earlier study had established that sapota seed from healthy fruit exhibited higher TDH activity, faster germination rate and higher percentage of germination (Shivashankar *et al*., 5), while the converse was true for CT seed.

Results of the sugar content in the pulp were also in agreement with our previous findings on healthy and CT affected fruits (Shivashankar *et al*., 5). Thus, the close conformity obtained between the physical and physiological attributes of pulp and seed confirmed that the fruits sinking in 12.5% salt solution were indeed healthy fruits and those floating on the top were CT fruits. From this, it was clearly evident that healthy and CT affected fruits of sapota cv. Cricket Ball could be effectively distinguished and segregated by dipping ripe fruits in 12.5% salt solution in a single step process based on the differences in specific gravity. Earlier, Lanier and Morris (2) applied the differences in fruit density for the successful separation of Muscadine grapes of different maturity grades. The technique standardized for separation of CT affected and healthy fruit based on specific gravity is a simple, sensitive and rapid technique.

Table 2. Physical and biochemical parameters of sunk and floating fruits of sapota cv. Cricket Ball dipped in 12.5% salt solution.

Parameter	Sunk fruits	Floating fruits
Total No. of fruits (100)	79 ± 1.46[a]	21 ± 2.09[b]
Fruit weight (g)	97.8 ± 1.50[a]	101.4 ± 1.77[a]
No. of seeds	2.9 ± 0.14[a]	2.4 ± 0.16[b]
Total seed weight (g)	1.93 ± 0.09[a]	1.47 ± 0.08[b]
Pulp moisture (%)	76 ± 1.49[a]	67 ± 1.80[b]
Seed moisture (%)	47 ± 1.70[a]	36 ± 1.86[b]
TDH activity (µg/g FW)	47.47± 1.28[a]	14.21 ± 0.64[b]
Germination (%)	93 ± 1.84[a]	64 ± 1.14[b]
Germination rate (days)	60 ± 2.06[a]	110 ± 1.70[b]
TSS (%)	19.68 ± 0.88[a]	14.43 ± 0.99[b]
Total sugar (g/100 FW)	12.15 ± 0.34[a]	8.23 ± 0.23[b]
Reducing sugar (g/100 FW)	8.35 ± 0.28[a]	5.47 ± 0.11[b]
Total fat (g/100 FW)	1.5 ± 0.05[a]	0.9 ± 0.07[b]
Pulp pH	6.18 ± 0.14[a]	5.72 ± 0.08[b]
Seed pH	6.65 ± 0.16[a]	6.22 ± 0.07[b]

[a-b]Mean values bearing similar letter in columns did not differ significantly at 0.05%

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