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Fruit calcium content and lipoxygenase activity in relation to albinism disorder in strawberry

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Abstract

Nutrient elements and lipoxygenase (LOX) activity were determined in strawberry fruit to establish a relationship, if it exists, between nutrient ratios (N/Ca and K/Ca), and lipoxygenase activity with albinism disorder. About 33% strawberry fruit were affected by albinism. Etna had highest incidence of albinism (48.6%) and Sweet Charlie the lowest (16.2%). Dry matter content (%) was lower in albino fruit (5.23%) than normal fruit (7.36%). The concentration of N, P, and Mg did not differ significantly, but that of K (1.87 mg g^{-1} fresh weight) was notably higher and of Ca (0.105 mg g^{-1} fresh weight) was lower in albino fruit than normal fruit. Consequently, the nutrient ratios, N/Ca (9.78) and K/Ca (16.96) were higher in albino fruit than normal fruit. Cultivars differed widely in respect to dry matter (%), mineral content and nutrient ratios. LOX activity determined on dry weight or fresh weight basis was significantly higher in albino fruit than normal fruit, with significant differences among cultivars. Positive correlations existed between nutrient ratios and albinism incidence ($r = +0.338$), LOX activity and albinism incidence ($r = +0.412$), and LOX and nutrient ratios ($r = +0.448$). Thus, it appears from the study that calcium and LOX activity may not the basic cause of albinism in strawberry, but these may be involved in senescence or fruit ripening process, as LOX activity was lower in albino than in normal fruit.

Keywords: Albinism; *Fragaria* x *ananassa*; Lipoxygenase; Nutrient element; Nutrient ratio

1. Introduction

Strawberry (*Fragaria* x *ananassa* Duch.) is considered as one of the most delicious, nutritious, and refreshing fruits of the world. Basically, it is a short-day plant of temperate origin, but scientists have bred a number of day-neutral varieties, as a result, it grows profitably well in tropical and sub-tropical climates (Sharma and Sharma, 2004). It suffers from various physiological disorders, but albinism is considered as the most important. Fruit suffering from albinism appear bloated and develop white or pink areas on fruit surface, with pulp remaining pale. Affected fruit have poor flavour and they tend to be acidic. Due to lack of firmness, albino fruit are susceptible to fruit-rot during storage or transportation (Lieten and Marcelle, 1993; Sharma and Sharma, 2004).

Calcium is considered as one of the most important nutrient elements in controlling the metabolism of plant cells. Its role in preventing various physiological disorders is well known (Bangerth, 1979). In addition, it is also known as retardant of fruit ripening and senescence processes (Ferguson, 1984). Though, the mechanism by which calcium prevents physiological disorders is not well understood, however it is known that it principally acts on middle lamella of cell wall and play its role of cross-linking (Poovaiah, 1985, 1988; Marcelle, 1989), where it may influence membrane bound enzymes, like lipoxygenase (LOX) (EC1.13.11.12), that catalyzes the hydro-peroxidation of polyunsaturated fatty acids (Leshem et al., 1982; Perkins-Veazie, 1995; Perez et al., 1999). Although, LOX is found in tissues of a wide variety of higher plants, there are many questions with regard to its functions in plant lipid metabolism (Perkins-Veazie, 1995; Perez et al., 1999).

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However, scientists world over largely agree that it is responsible for the typical breakdown of linolenic acid, and thus responsible for some physiological disorders (e.g. bitter pit and crown spot in apples) (Feys et al., 1980). Some investigators have confirmed a relationship between LOX expression and ripening process in strawberry (Perkins-Veazie, 1995; Perez et al., 1999; Leone et al., 2003). Further, it has been demonstrated that LOX activity in fruits during storage is low if K/Ca ratio in fruit is low and vice versa (Marcelle, 1989), which demonstrates the participatory role of calcium in fruit senescence. Hence, studies were conducted to establish some relationship, if existing, between calcium, lipoxygenase activity and albinism disorder in strawberry.

2. Materials and methods

The studies were conducted in the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi 110012 during 2002–2004. Two hundred runners, each of Chandler, Etna and Sweet Charlie cultivars were procured from IARI, Regional Research Station, Shimla (H.P.) and were planted on raised beds in open field at a distance of 25 cm × 30 cm. Plants were uniformly mulched with paddy straw.

2.1. Estimation of nutrient elements in fruit

Standard procedures were adopted for determining the mineral nutrient content of normal and albino fruit. After ashing, the residue was dissolved in nitric acid to a final solution concentration of 0.16 M. Phosphorus was determined by colorimetrically, potassium by flame emission, calcium and magnesium by atomic absorption. Nitrogen was measured by colorimetrically after a Kjeldahl digestion. Samples of albino and normal fruit were taken from randomly selected 10 plants/bed, with each sample containing about 500 g (fresh weight) as described by Lieten and Marcelle (1993). From each fruit, 3–4 mm thick longitudinal slices were taken, reducing the total weight of the sample to 120–130 g.

2.2. Albinism incidence (%)

The albinism incidence was calculated by counting the normal and albino fruit in randomly selected 10 plants/bed, replicated five times. The albinism incidence was represented as percentage.

2.3. Preparation of substrate

The substrate was prepared as per the procedure described for apple by Feys et al. (1980) with slight modifications. First, linolenic acid (0.1 ml) was dissolved in 1 ml 0.1 N NaOH solution. To this, 150 μL Triton-X-100 was added. The solution was emulsified in an Ultra Turrax for 2 min, and diluted to 50 ml with distilled water. Control emulsion was also prepared in the same way using oleic acid. These emulsions were stored under N_2 at 4 °C in dark for no longer than 10 days.

2.4. Preparation of crude enzyme extract

Crude enzyme extract was prepared at 4 °C, following the method of Feys et al. (1980). Diced strawberry fruit (100 g) was thoroughly mixed in Sorvall mixer for 3 min in 100 ml 0.35 M sodium phosphate buffer (pH 7), containing 1% (v/v) Triton-X-100 and 10^{-2} M $Na_2S_2O_5$. The slur was homogenized with Ultra Turrax for 2 min. The pH was adjusted to 7 with a few drops of 10N NaOH solution. The homogenate was centrifuged at 10,000 × g for 20 min (supernatant 1). The pellet so obtained was suspended in 60 ml 0.25 M sodium phosphate buffer containing 10^{-4} M $Na_2S_2O_5$, and the same procedure was followed for homogenization and centrifugation (supernatant 2). Both the supernatants were combined and used as a source of enzyme.

2.5. Measurement of lipoxygenase activity

The lipoxygenase activity was measured polargraphically at 25 °C with a Clark O_2 electrode as described by Feys et al. (1980) for apple. Incubation mixture contained 2 ml 0.2 M sodium phosphate buffer (pH 7), 0.3 ml substrate emulsion and 0.7 ml fruit extract. Enzyme activity was calculated from initial rates of O_2 uptake and represented as μkatal kg^{-1} fresh weight or dry weight. All data are a mean of five runs.

2.6. Data analysis

The data generated for different parameters of the experiment were pooled and analyzed, following simple RBD for albinism incidence, and factorial RBD for dry matter content and nutrient elements, and split plot design for lipoxygenase activity (Panse and Sukhatme, 1984).

3. Results

3.1. Dry matter (%), mineral content and nutrient ratios of normal and albino fruit

Albino fruit have significantly lower dry matter content (5.23%) than normal fruit (7.36%). Though, there was no significant difference in the concentration of nitrogen, phosphorus, and magnesium of albino and normal fruit, but the concentration of potassium was significantly higher (1.87 mg g^{-1} fresh tissue weight), and that of calcium was notably lower (0.105 mg g^{-1} fresh tissue weight) in albino fruit than in normal fruit (1.57 and 0.131 mg g^{-1} fresh tissue weight, respectively) (Table 1). Consequently, the N/Ca

Table 1
Dry matter content (%) and mineral content of albino and normal fruits in different strawberry cultivars[a]

Cultivar	Dry matter (%)			Mineral content (mg g^{-1} fresh tissue weight)														
				Nitrogen			Phosphorus			Potassium			Calcium			Magnesium		
	NF	AF	Mean	NF	AF	Mean	NF	AF	Mean	NF	AF	Mean	NF	AF	Mean	NF	AF	Mean
Chandler	7.38	5.21	6.30	0.92	1.04	0.98	0.162	0.176	0.169	1.57	1.88	1.73	0.130	0.103	0.117	0.142	0.133	0.129
Etna	7.42	5.30	6.39	0.98	1.08	1.03	0.173	0.186	0.180	1.62	1.92	1.77	0.136	0.112	0.124	0.146	0.138	0.142
Sweet Charlie	7.29	5.13	6.21	0.74	0.96	0.93	0.160	0.169	0.165	1.38	1.55	1.66	0.127	0.100	0.114	0.138	0.130	0.134
Mean	7.36	5.23	–	0.92	1.03	–	0.165	0.177	–	1.57	1.87	–	0.131	0.105	–	0.142	0.134	–
CD (0.05)	Fruit type = 0.08; cultivar = 0.03; fruit × cultivar = 0.11			Fruit type = 0.013 cultivar = 0.11; fruit × cultivar = 0.16			Fruit type = 0.18; cultivar = 0.27; fruit × cultivar = 0.30			Fruit type = 0.11; cultivar = 0.08; fruit × cultivar = 0.13			Fruit type = 0.13; cultivar = 0.06; fruit × cultivar = 0.19			Fruit type = 0.18; cultivar = 0.22; fruit × cultivar = 0.33		

[a] AF: Albino fruit, NF: normal fruit.

Table 2
Albinism incidence (%), mineral nutrient ratios and lipoxygenase activity in albino and normal strawberry fruit[a]

Cultivar	Albinism incidence (%)	Mineral nutrient ratios						Lipoxygenase activity (μ katal kg^{-1})					
		N/Ca ratio			K/Ca ratio			Fresh wt. Basis			Dry wt. Basis		
		NF	AF	Mean	NF	AF	Mean	NF	AF	Mean	NF	AF	Mean
Chandler	35.3	7.21	9.64	8.43	11.91	17.14	14.53	0.411	0.530	0.471	0.624	0.740	0.682
Etna	48.6	7.08	10.10	8.59	12.08	18.25	15.17	0.426	0.552	0.489	0.678	0.798	0.738
Sweet Charlie	16.2	5.82	9.60	7.71	10.87	15.50	13.19	0.369	0.472	0.421	0.595	0.690	0.643
Mean	33.4	6.70	9.78	–	11.62	16.96	–	0.402	0.518	–	0.632	0.742	–
CD (0.05)	4.8	Fruit type = 0.18; cultivar = 0.13; fruit × cultivar = 0.21			Fruit type = 0.16; cultivar = 0.14; fruit × cultivar = 0.19			Fruit type = 0.21; cultivar = 0.23; fruit × cultivar = 0.26			Fruit type = 0.22; cultivar = 0.25; fruit × cultivar = 0.26		

[a] NF: normal fruit, AF: Albino fruit.

(9.78) and K/Ca (16.96) nutrient ratios for albino fruit were much higher than the nutrient ratios of normal fruit (6.70 and 11.62, respectively) (Table 2). Further, dry matter (%), mineral nutrient content and nutrient ratios were also significantly influenced by cultivars, and the interaction between fruit type × cultivar.

3.2. Albinism incidence

Irrespective of cultivar, nearly 33% fruit were affected by albinism, with greater variability among cultivars. Etna produced albino fruit (48.6%) in higher proportion and Sweet Charlie with the lowest proportion (16.2%) (Table 2). Consequently, Etna produced lowest percentage of normal fruit (51.4%) and Sweet Charlie the highest (83.8%).

3.3. Lipoxygenase activity

Lipoxygenase activity expressed on per fresh or per dry weight basis, was significantly higher (0.518 and 0.632 μkatal kg^{-1}, respectively) in albino fruit than normal fruit, which was significantly influenced by cultivars. The activity was maximum in Etna and lowest in Sweet Charlie (Table 2). The interaction, fruit type × cultivar for lipoxygenase activity was also significant.

3.4. Correlation between nutrient ratios and lipoxygenase activity with albinism incidence

The correlations between LOX activity and albinism incidence ($r = +0.412$), LOX and nutrient ratios ($r = +0.448$) and nutrient ratios and albinism incidence ($r = +0.336$) were significantly positive.

4. Discussion

Nearly 33% strawberry fruit were affected by albinism, a serious disorder, which was first described by Ulrich (1971) in green house grown 'Elsanta' strawberry, and later by Sharma and Sharma (2003a) in open-field grown strawberries. Further, cultivars differed greatly in terms of producing albino fruit. Etna produced maximum percentage of albino fruit and Sweet Charlie the minimum, which may be attributed to genetic variability existing among the cultivars, as reported by Sharma et al. (2003) and Sharma and Sharma (2003a,b).

Dry matter content of albino fruit was lower than normal fruit. Lower dry matter content in albino fruit than normal fruit indicated that albino fruit are more hydrated, which may be due to increased competition between leaves and fruits for different nutrients during the period of excessive vegetative growth, because luxuriant growth favours albinism in strawberry (Sharma and Sharma, 2003b).

Among nutrients, potassium was notably higher and calcium was lower in albino fruit than normal fruit, which might have resulted in higher ratios for N/Ca and K/Ca for albino fruit, thereby giving some indications that albino fruit are physiologically riper and more senescent than normal fruit, though they have poor colour development (Marcelle, 1984; Sharma and Sharma, 2003a,b). This is further supported by the observation that LOX activity was greater in albino than normal fruit. Lieten and Marcelle (1993) have also observed lower LOX for albino than normal/healthy fruit. Similarly, Cutting et al. (1992) have also shown that calcium content of avocado decreased and K/Ca ratio increased as the fruit matured. This supports the contention that calcium deficiency is not basically responsible for albinism in strawberry. It has also been demonstrated in Elsanta cultivar by Lieten and Marcelle (1993), which suffers badly from albinism. Further, due to significant positive correlation between LOX and albinism, it appears that lipoxygenase activity may have some relationship with albinism. These observations support the findings of earlier workers that calcium is not directly involved in albinism in strawberry. However, LOX may be involved in senescence due to which, it might have increased in albino fruit (Lieten and Marcelle, 1993). Similarly, LOX has been reported to be involved in ripening process in strawberry fruit (Perkins-Veazie, 1995; Perez et al., 1999; Leone et al., 2003), and thus, albino fruit have lower LOX activity due to poor colour development in them.

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