



Fruit nutrient content and lipoxygenase activity in relation to the production of malformed and button berries in strawberry (*Fragaria* × *ananassa* Duch.)

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ABSTRACT

Fruit nutrient content and lipoxygenase (LOX) activity were determined in strawberry fruit to establish a relationship, if it exists, between nutrients, and LOX activity with the fruit malformation and nubbins or button berry disorders. Nearly 17% fruit were affected by malformation and 10% by nubbins in open-field-grown strawberries. 'Etna' produced higher proportion of malformed (22.7%) as well as button berries (16.9%) and 'Sweet Charlie' the lowest (8.9% and 3.3%, respectively). Dry matter content (%) was lower in malformed (5.2%) and button berries (3.23%) than normal berries (7.41%). The concentration of P and Mg did not differ significantly, but that of N and K was notably higher and of Ca and B was lower in malformed and button berries than normal berries. Consequently, the N/Ca and K/Ca ratios were higher in malformed and button berries. LOX activity was significantly higher in malformed as well as button berries than normal berries, with significant differences among cultivars. The correlations between N, K and malformed and button berries were positive and between Ca and B, and malformed and button berries were negative. Similarly, the correlation between LOX activity and malformed, and button berries were also positive, indicating that excess of N and K, and deficiency of Ca and B are related to the production of malformed and buttons or nubbins in strawberry.

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1. Introduction

Strawberry is one of the most delicious, nutritious, and refreshing fruits. Basically, it is a fruit plant of temperate regions, but it grows profitably well in tropical and sub-tropical climates (Sharma and Sharma, 2004). It suffers from several serious physiological disorders like albinism, phyllody, fasciation but at times occurrence of malformed and nubbins or button berries is quite common. Malformed berries appear misshapen, look unattractive and hence fetch poor price in the market (Sharma and Sharma, 2004). Similarly, button berries or nubbins are misshapen and small sized berries, which are a loss to a grower, and if they occur frequently, the loss may be severe (Garren, 1981).

Malformed and button berries in strawberry occur primarily due to inadequate pollination, and thus placement of beehives helps in reducing it. Similarly, some insects like lygus bug are also associated with these disorders (Garren, 1981). However, while working with

strawberry, we experienced that in spite of placement of beehives in strawberry plantations, the problem of frequent occurrence of malformed and button berries takes place and hence, these disorders appear to be physiological in nature and nutrition plays vital role in their occurrence (Sharma and Sharma, 2004). Among different nutrient elements, the role of major nutrients like N, K, and Ca and minor elements like B appears to be important, as these nutrients are either in excess or deficient conditions in Indian soils. Of these nutrients, Ca and B are known to have association with a number of physiological disorders in several fruits.

As of now, 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). Although, the mechanism by which calcium prevents physiological disorders is not well understood, however it is known that it influences membrane bound enzymes, like 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). LOX is found in tissues of a number of plants, and is responsible for the typical breakdown of linolenic acid. It is also associated with some physiological disorders like bitter pit and crown spot in apples

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(Feys et al., 1980). In addition, LOX plays important role in the ripening process of strawberry fruit (Perkins-Veazie, 1995; Perez et al., 1999; Leone et al., 2003). It has been demonstrated that LOX activity in fruit 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. In addition to above facts, B is becoming a limiting factor in Indian soils and its deficiency in strawberry has been reported to be associated with fruit malformation (Singh et al., 2007). Further, we have already reported that fruit Ca content and LOX have direct relationship with albinism. Hence, we hypothesized that there may exist some relationship between fruit nutrient content and LOX activity with the occurrence of malformed and button berries in strawberry, and thus systematic studies were conducted.

2. Materials and methods

2.1. Experimental material and site

The studies were conducted in the Division of Fruits and Horticultural Technology, Indian Agricultural Research Institute, New Delhi-110 012 during 2003–2004. This region has sub-tropical climate and falls in semi-arid zone having hot summers (May–June) and mild winters (December–mid February) with annual rainfall of about 180 mm, restricted mainly during July and August. Soil of the experimental farm was sandy-loam, having pH 8.5, which was low in organic carbon (0.42%), medium in available phosphorus and high in potash. Soil was thoroughly ploughed and raised beds of 25 cm height and 1 m width were prepared at a distance of 50 cm. 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 the beds at a distance of 25 × 30 cm. Plants were uniformly mulched with paddy straw, and maintained at uniform cultural practices during the entire period of experimentation.

2.2. Estimation of nutrient elements in fruit

Mineral nutrient content of normal, malformed and button berries was determined by adopting standard procedures. Samples of malformed, button and normal berries were taken from randomly selected 10 plants/bed, with each sample containing about 500 g (fresh weight) (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. 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 spectrophotometer. Nitrogen was measured by colorimetrically after a Kjeldahl digestion. The concentrations of minor nutrients like, Fe, Mn, Zn, Cu, S and B were determined by atomic absorption spectrometer.

2.3. Observations on malformed and button berries (%)

Proportion of malformed and button berries (nubbins) was calculated by counting the normal, malformed and button berries in randomly selected 10 plants/bed, replicated five times, and represented as percentage.

2.4. Preparation of substrate

The substrate was prepared as per the procedure described for apple by Feys et al. (1980) with slight modifications. First, linoleic 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 N₂ at 4 °C in dark for no longer than 10 days.

2.5. 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 Na₂S₂O₅. The slur was homogenized with Ultra Turrax for 2 min. The pH was adjusted to 7 with a few drops of 10 N 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 Na₂S₂O₅, and the same procedure was followed for homogenization and centrifugation (supernatant 2). Both the supernatants were combined and used as a source of enzyme.

2.6. Measurement of LOX activity

The LOX activity was measured polarographically at 25 °C with a Clark O₂ 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 O₂ uptake and represented as µkatal kg⁻¹ fresh weight or dry weight. All data are a mean of five runs.

2.7. Data design and analysis

Two-year data were not significant. Hence, data were pooled and analyzed, following CRD (Panse and Sukhatme, 1984). The data on percentage (malformed and button berries) were transformed as per Arc Sin values before analysis. The correlations between N vs malformed, and button berries; K vs malformed, and button berries; LOX activity vs malformed, and button berries; B vs malformed, and button berries; Ca vs malformed, and button berries; B vs malformed, and button berries were calculated.

3. Results

3.1. Dry matter (%), mineral content and nutrient ratios of normal, malformed and button berries

Malformed (5.20%) and button berries (3.23%) have significantly lower dry matter content than normal berries (7.41%), with significant variability among cultivars. Although, there was no significant difference in the concentration of phosphorus and magnesium of malformed, button and normal berries (data not shown), but the concentration of nitrogen and potassium was significantly higher and that of calcium was notably lower in malformed and button berries than normal berries (Table 1). The concentration of nitrogen was significantly higher in malformed (1.29 mg g⁻¹ F.W.) and button berries (1.09 mg g⁻¹ F.W.) than normal berries (0.91 mg g⁻¹ F.W.). K content was higher only in malformed berries (1.72 mg g⁻¹ F.W.) than normal berries (1.63 mg g⁻¹ F.W.). In contrast, Ca content was lower in malformed (0.106 mg g⁻¹ F.W.) and button berries (0.114 mg g⁻¹ F.W.) than normal berries (0.140 mg g⁻¹ F.W.). Consequently, the N/Ca nutrient ratio for malformed (12.2) and button berries (8.8) was higher than normal berries (6.5). Similarly, K/Ca nutrient ratio for

Table 1
Dry matter content (%) and mineral content of strawberry fruits^a

Cultivar	Dry matter (%)				Major nutrient content (mg g ⁻¹ F.W.)												Minor nutrient content (mg Kg ⁻¹)			
	NB	MB	BB	Mean	Nitrogen				Potassium				Calcium				Boron			
					NB	MB	BB	Mean	NB	MB	BB	Mean	NB	MB	BB	Mean	NB	MB	BB	Mean
Chandler	7.42	5.21	3.21	5.21	0.86	1.26	1.06	1.06	1.68	1.77	1.71	1.72	0.139	0.106	0.115	0.120	21.6	14.8	12.3	16.2
Etna	7.46	5.30	3.40	3.39	0.98	1.39	1.12	1.16	1.75	1.84	1.76	1.78	0.133	0.100	0.108	0.114	18.5	12.4	10.4	13.8
Sweet Charlie	7.34	5.10	3.08	5.17	0.78	1.06	1.00	0.95	1.45	1.55	1.43	1.48	0.148	0.112	0.120	0.127	24.6	17.4	15.6	19.2
Mean	7.41	5.20	3.23	-	0.87	1.24	1.06	-	1.63	1.72	1.63	-	0.140	0.106	0.114	-	21.6	14.9	12.8	-
CD (0.05)	Fruit type = 0.07; cultivar = 0.03; fruit × cultivar = 0.12				Fruit type = 0.010; cultivar = 0.11; fruit × cultivar = 0.14				Fruit type = 0.12; cultivar = 0.20; fruit × cultivar = 0.32				Fruit type = 0.10; cultivar = 0.07; fruit × cultivar = 0.22				Fruit type = 2.2; cultivar = 1.5; fruit × cultivar = 2.8			

^a MB, malformed berry; BB, button berry; NB, normal berry.

malformed (16.3) and button berries (14.4) was also higher than normal berries (11.7) (Table 2).

Further, most of the minor nutrient elements of normal, malformed and button berries were non-significant (data not shown) except for B. B content was significantly lower in malformed (14.9 mg kg⁻¹) and button berries (12.8 mg kg⁻¹) than normal berries (21.6 mg kg⁻¹), with significant variability among cultivars.

3.2. Occurrence of malformed and button berries

Strawberry plants produced nearly 17% malformed and 10% button (nubbins) berries, with greater variability among cultivars. 'Etna' produced malformed (22.7%) and button berries (16.9%) in higher proportion than 'Chandler' or 'Sweet Charlie' (Table 2). Consequently, 'Etna' produced lowest percentage of normal berries (60.4%) and 'Sweet Charlie' the highest (87.8%).

3.3. LOX activity

LOX activity expressed on per fresh or per dry weight basis, was quite higher in malformed and button berries than normal berries, with significant variability among cultivars. The activity was maximum in 'Etna' and lowest in 'Sweet Charlie' (Table 2).

3.4. Correlations

The correlations between N vs malformed ($r = +0.730$), and button berries ($r = +0.622$); K vs malformed ($r = +0.620$), and button berries ($r = +0.330$); LOX activity vs malformed ($r = +0.742$), and button berries ($r = +0.864$) were positive, and B vs malformed ($r = -0.784$), and button berries ($r = -0.744$); Ca vs malformed ($r = -0.684$), and button berries ($r = -0.633$) were negative.

Table 2
Malformed and button berry production (%), mineral nutrient ratios and LOX activity in strawberry fruit^a

Cultivar	Malformed berries (%)	Button berries (%)	Mineral nutrient ratios								Lipoxygenase activity ((katal kg ⁻¹))							
			N/Ca ratio				K/Ca ratio				Fresh weight basis				Dry weight basis			
			NB	MB	BB	Mean	NB	MB	BB	Mean	NB	MB	BB	Mean	NB	MB	BB	Mean
Chandler	18.8	10.3	6.6	12.0	7.3	8.6	12.1	16.7	14.9	14.6	0.416	0.525	0.532	0.491	0.635	0.780	0.810	0.742
Etna	22.7	16.9	7.4	14.6	10.7	10.9	13.2	18.4	16.3	16.0	0.431	0.547	0.558	0.512	0.670	0.810	0.842	0.774
Sweet Charlie	8.9	3.3	5.5	10.1	8.3	8.0	9.8	13.8	11.9	11.8	0.374	0.465	0.478	0.439	0.580	0.682	0.710	0.657
Mean	16.8	10.2	6.5	12.2	8.8	-	11.7	16.3	14.4	-	0.407	0.512	0.523	-	0.628	0.757	0.787	-
CD (0.05)	1.9	1.2	Fruit type = 0.12; cultivar = 0.18; fruit × cultivar = 0.26				Fruit type = 0.12; cultivar = 0.16; fruit × cultivar = 0.22				Fruit type = 0.20; cultivar = 0.28; fruit × cultivar = 0.33				Fruit type = 0.18; cultivar = 0.22; fruit × cultivar = 0.32			

^aMB, malformed berry; BB, button berry; NB, normal berry.

4. Discussion

Strawberry plants produced nearly 17% malformed and 10% button berries, which appear to be quite high. Although, information about these disorders is available in the literature, however, none has conducted systematic studies on the occurrence either for malformed or button berries in different cultivars. Moreover, all studies have been conducted in greenhouse-grown strawberry. Thus, it is first official report on occurrence of malformed and buttons or nubbins in field-grown strawberries. Studied cultivars differed greatly in terms of producing malformed and button berries. 'Etna' produced higher percentage of malformed and button berries and 'Sweet Charlie' the minimum, which may be attributed to genetic variability existing among the cultivars (Sharma et al., 2006).

Dry matter content of malformed as well as button berries was lower than normal berries indicating that such berries were more hydrated. It may be due to increased competition between leaves and fruits for different nutrients during the period of excessive vegetative growth (Sharma and Sharma, 2003b). Increased vigour is usually associated with overuse of N and K, as a result, vigorous cultivar 'Etna' produced malformed fruit in higher proportion than 'Sweet Charlie', a dwarf cultivar under Indian conditions (Sharma and Sharma, 2003a).

Further, among nutrients, nitrogen and potassium were notably higher and calcium was lower in such berries with higher ratios for N/Ca and K/Ca. Higher nutrient ratios in malformed and button berries suggest that such fruits were physiologically riper and more senescent than normal fruit (Marcelle, 1989; Sharma and Sharma, 2003a,b). Similarly, fruit Ca and B content were lower in malformed and button berries than normal berries, indicating that low Ca and B may also be responsible for the production of malformed and button berries, which is supported from the

negative correlations between Ca vs fruit malformation ($r = -0.684$), Ca vs button berries ($r = -0.633$), and B vs fruit malformation ($r = -0.784$), and B vs button berries ($r = -0.744$). This is further supported by the observation that LOX activity was greater in malformed and button berries than normal berries. Similarly, positive correlation between LOX and fruit malformation ($r = +0.742$), and LOX and button berries ($r = +0.864$) indicated that LOX activity also has direct relationship with the production of malformed and button berries in strawberry (Sharma et al., 2006). This supports the contention that calcium deficiency and boron deficiency may be responsible for the production of malformed and button berries in strawberry. This also supports the findings that Ca may be involved in senescence due to which, LOX activity might have increased in malformed and button berries as reported by Sharma et al. (2006) in relation to albinism in strawberry. Although, there is no report in the literature to support the contention that Ca is involved in the production of either malformed or button berries in strawberry, however B plays significant role, which is also not well documented (Singh et al., 2007). Although, Garren (1981) has reported that excess N or B deficiency may be related to fruit malformation or button production in strawberry, however, the role of pollination, pollinator, insects like lygus bug, and adverse weather conditions cannot be ruled out.

5. Conclusion

It appears from the study that high N and K; low Ca and B and high LOX activity is related to the production of malformed and button berries in strawberry.

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