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ScienceDirect

Scientia Horticulturae 112 (2007) 215–220

SCIENTIA HORTICULTURAE

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Pre-harvest foliar application of calcium and boron influences physiological disorders, fruit yield and quality of strawberry (*Fragaria* × *ananassa* Duch.)

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Received 31 July 2006; received in revised form 15 November 2006; accepted 6 December 2006

Abstract

Studies were conducted in 'Chandler' strawberry to determine if pre-harvest foliar application of Ca, B or their combination influences physiological disorders, fruit yield and quality or not. For this, treatments consisted of (i) five sprays of calcium as $CaCl_2$ (first spray was performed at the petal fall stage and later at 7 days interval), (ii) three sprays of boron as boric acid (first spray at the beginning of flowering and later at 15 day interval), (iii) combination of (i) and (ii), and (iv) plants sprayed with water served as the control. Results indicated that fruit harvested from plants, which were sprayed either with Ca or Ca + B had significantly lesser incidence of albinism (6.7 and 6.5%), and grey mould (1.3 and 1.2%) than those harvested from plants sprayed either with B alone or in control. Although, B alone could not influence the incidence of albinism and grey mould, but it reduced fruit malformation (3.4 and 3.1%) significantly. Further, Ca, B or their combination had not influenced the individual berry weight, but marketable fruit yield differed significantly. The lowest marketable fruit yield (149.3 g/plant) was recorded in plants under control, and the highest (179.2 g/plant) in plants sprayed with Ca + B. Similarly, such fruit were firmer; had lower TSS, higher acidity and ascorbic acid content at harvest than those in control. Similarly, such fruit after 5 days storage were firmer and brighter, and have significantly lower TSS (7.9 and 7.8%); higher ascorbic acid content (43.7 and 45.0 mg/100 g pulp) and acidity (1.08 and 1.07%) than those in control or those receiving B alone. Incidence of grey mould was significantly lesser in fruit, which received Ca (2.2%) or Ca + B (1.9%) than those, which received either B (8.1%) alone or those in control (8.4%). Our studies indicated that pre-harvest foliar application of Ca + B is quite useful for reducing the incidence of disorders and getting higher marketable yield in 'Chandler' strawberry.

Keywords: Albinism; Boron; Calcium; Fruit yield; Gray mould; Malformation; Strawberry

1. Introduction

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most delicious fruits of the world, which is a rich source of vitamins and minerals, and has fabulous flavour and tantalizing aroma. In India, it was introduced in the early 1960s, but due to several reasons, it could not be popularized (Sharma and Sharma, 2004). However, in recent years, several day-neutral varieties have been introduced and different agro-techniques were standardized at various research stations (Sharma et al., 2004). As a result, strawberry has become the most favourite fruit crop among the growers, especially near towns and cities, which has resulted a phenomenal increase in its area and production. In India, many varieties of strawberry are grown, but 'Chandler' has become the most popular dessert variety due to its high production potential, and production of attractive red medium-sized fruit of better quality (Asrey and Singh, 2004). Under proper management, it grows profitably well, but in the recent years, authors of this paper have observed the production of albino and malformed fruits in larger proportion in its plantations, which not only affect the fruit yield, but the quality as well. The available literature reveals that nutrients like calcium and boron may be related with the occurrence of albinism and fruit malformation in strawberry (Lieten, 1989, 1998, 2002; Sharma et al., 2006).

The involvement of Ca in the regulation of fruit maturation and ripening processes is well established (Ferguson, 1984). It has been established that fruit low in Ca content are sensitive to

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0304-4238/$ – see front matter
doi:10.1016/j.scienta.2006.12.019

many physiological and pathological disorders, and such fruit have usually short shelf-life (Conway et al., 1992; Fallahi et al., 1997). Thus, calcium is applied before and after harvest to prevent physiological disorders, delay ripening and to improve quality of various fruits crops, including strawberry (Poovaiah, 1986; Cheour et al., 1990; Asrey et al., 2004; Chaiprasart et al., 2006; Dunn and Able, 2006; Hernandez-Munoz et al., 2006). However, little is known on the efficiency of foliar application of Ca in improving fruit quality or yield in strawberry. Further, boron (B) is an essential nutrient element and its deficiency reduces pollen germination and growth of pollen tubes, which consequently results in the development of malformed fruits, which lowers crop yield and deteriorates fruit quality (Guttridge and Turnbull, 1975; Lieten, 1989, 1998, 2002; Sharma and Sharma, 2004). In semi-arid regions, the soils are light, having low organic content and B deficiency usually occurs during growing season under low rainfall (Wojcik and Lewandowski, 2003). However, application of boron in soil, in some cases, causes phytotoxicity as there is a narrow range between B deficiency and toxicity for many fruit crops including strawberry (Gupta, 1979). Moreover, its foliar spray is more effective than soil application, but little is known about its effects on strawberry yield and quality.

Among different diseases, *Botrytis* fruit rot (grey mould) caused by *Botrytis cinerea*, is the most devastating disease of strawberry, which causes great losses and assumes serious concern with the increasing environmental temperature (Bulger et al., 1987; Wilcox and Seem, 1994; Hancock, 1999). Increasing the calcium content in the cell wall of fruit tissue can help to delay softening and mould growth and thus may decrease the gray mould incidence and physiological disorders (Poovaiah, 1986; Naradisorn et al., 2006). Thus, the aim of the present studies was to determine the effect of foliar application of calcium and boron on physiological disorders, fruit yield and quality of 'Chandler' strawberry grown on sandy-loam soil with low organic content and low status of available boron under semi-arid climate.

2. Materials and methods

2.1. Experiment design and treatments

The studies were conducted at the research farm of the Central Institute of Post Harvest Engineering and Technology, Abohar, Punjab, India during 2003–2004 and 2004–2005 cropping season. The experiment was laid out in randomized block design (RBD), with four replications. Soil of the experimental farm was sandy-loam (Ustic Haplocambid), having pH 8.5, which was low in organic carbon (0.42%), medium in available phosphorus, high in potash and low in boron (0.23 mg Bkg^{-1}). Soil analysis was done by following standard procedures (Tandon, 2000). Soil was thoroughly ploughed and raised beds of 25 cm height, 5 m in length and 1 m width were prepared at a distance of 50 cm. Healthy and disease free runners of 'Chandler' strawberry were planted at a spacing of 25 cm × 25 cm on raised beds during first week of October in both years. Irrigation was provided with micro-sprinkler system during early stage of plant establishment, which was replaced by drip system after 20 days of planting. Each treatment consisted of 80 plants in a plot size of 500 cm × 100 cm. All necessary cultural practices (Singh et al., 2005) and plant protection measures were followed uniformly for all the treatments during the entire period of experimentation.

The following treatments were applied: (i) five spray of calcium as $CaCl_2$ @ 2.0 kg Ca ha^{-1} $spray^{-1}$. The first spray was performed at petal fall stage and others at weekly intervals; (ii) three spray of boron @ 150 g B ha^{-1} $spray^{-1}$ (8% B as boric acid, Ranbaxy, Chandigarh, India) at the beginning of flowering and other sprays at 15-days interval; (iii) spray of B and Ca in combination of (i) and (ii); (iv) plants sprayed with water served as the control. All these sprays were done in the morning (9–10 a.m.) using a hand pressure sprayer at 1,000 l ha^{-1}. During both years, berries were harvested every 3–4 days from 17 February to 28 March.

2.2. Measurements and observations

Data on different parameters were measured on all harvest dates. Fruit yield was calculated by taking marketable fruits, which were free from skin injury, albinism and malformation and Botrytis rot symptoms. Randomly selected 100 normal fruit of each harvest were taken to calculate mean berry weight. The titratable acidity (TA), total soluble solids (TSS), firmness, external fruit colour and fruit rotting were determined at harvest and also after 5 days of storage at 10 °C and 90% RH. Acidity, TSS, ascorbic acid content were measured as per AOAC (1989).

Firmness was determined on 25 fruit samples from each replicate with texture analyzer (TA-Hdi, Stable Micro Systems, UK) with the 2 mm diameter stainless steel probe. Fruit were tested equatorially at their maximum diameter with speed of cross-head 50 cm min^{-1}. The force was expressed in Newton (N).

The fruit colour in terms of L, a, b values was determined using Hunterlab miniScan XE Plus colourimeter (HAL, USA, Model45/0-L), in which, 'L' denotes the lightness or darkness, 'a' green or red, and 'b', blue or yellow colour of the samples. Before measuring the colour of samples, the colourimeter was standardized with black and white calibration tiles provided with the instrument.

Incidence of albinism and malformation fruit were determined at each harvest by counting all albino, malformed and normal fruit and represented as percentage (%) (Sharma et al., 2006). Incidence of grey mould (*Botrytis cinerea*) was determined in 50 randomly selected fruit from each treatment replication. Physiological loss in weight (PLW) of fruit after 5 days of storage at 10 °C and 90% RH was measured on 25-fruit samples from each plot.

Twenty-five leaves with petiole from each plot were collected after last harvest for analysis of leaf Ca and B content. Similarly, 25 medium-sized fruit of equal weight and same ripening stage were collected at each harvest for determining fruit Ca and B content. Leaves and fruit were washed in distilled water and bottled dry. Leaf samples were dried in a forced-draft oven at 70 °C and ground. Fruit samples were homogenated and then heated in a water bath at 50 °C. Finally, these samples were ashed and then dissolved in 0.5% HCl. Calcium and boron content in the samples were determined by Spectroscopy.

2.3. Data analysis

The data of both years were pooled as the year interaction was non significant and subjected to analysis, following standard procedures (Panse and Sukhatme, 1984). Data in percentage units (incidence of albinism, malformed fruits and grey mould) underwent arcsin transformation before subjected to analysis. Differences between means were evaluated using Duncan's Multiple Range Test at $P \leq 0.05$.

3. Results

3.1. Effect on physiological disorders, grey mould and fruit yield

Pre-harvest foliar application of Ca, B or Ca + B has influenced the occurrence of physiological disorders, like albinism and fruit malformation; incidence of grey mould and fruit yield of 'Chandler' strawberry at harvest significantly (Table 1). Fruit harvested from plants, which were sprayed either with Ca or Ca + B have lesser incidence of albinism (6.7 and 6.5%), and grey mould (1.3 and 1.2%) than those harvested from plants either B alone or not at all sprayed (control). Boron alone could not influence the incidence of albinism and grey mould, but in combination with Ca, it reduced the production of malformed fruit significantly (3.4 and 3.1%), whereas application of Ca could not do so (Table 1). Further, individual application of Ca or B or Ca + B could not influence the individual berry weight, but marketable fruit yield was significantly different. The lowest fruit yield (149.3 g/plant) was obtained in plants under control, and the highest (179.2 g/plant) in plants sprayed with Ca + B (Table 1).

3.2. Effect on quality parameters at harvest

Calcium alone or in combination with B has significantly influenced the fruit firmness, and quality parameters of 'Chandler' strawberry at harvest. These treatments have slightly affected external fruit colour, but not significantly ($P \leq 0.05$) (Table 2). At harvest, fruit which were harvested from plants sprayed either with Ca alone or in combination with B, were firmer (1.55 and 1.62 N); had lower TSS (7.1 and 7.2%), higher acidity (1.15 and 1.12%) and ascorbic acid content (51.3 and 51.6 mg/100 g pulp) than those harvested from plants under control. However, B alone could neither influence firmness, fruit colour nor any of the quality parameters significantly over control. Fruit harvested from plants sprayed either with Ca or Ca + B had higher values of L (36.2 and 36.5), a (37.1 and 38.3), b (46.3 and 47.5) than those harvested from plants sprayed with B alone or in control (Table 2).

3.3. Effect on Ca and B content in fruit and leaf tissues

Pre-harvest foliar application of Ca or B alone or their combination has significantly increased their respective content in the leaves and fruits of 'Chandler' strawberry (Table 3). Spray either of Ca or Ca + B has increased Ca content in leaves (16.3 g kg^{-1} and 16.8 mg kg^{-1}) and fruit (198 mg and 207 mg kg^{-1}) compared to those in control. Similarly, plants sprayed either with B alone or Ca + B had increased B content in the leaves (42.1 and 41.8 mg kg^{-1}) and fruit (2.7 and 2.8 mg kg^{-1}) than those in control (Table 3).

Table 1
Effect of foliar application of Ca and/or B on physiological disorders, grey mould and fruit yield of 'Chandler' strawberry fruits

Treatment	Albinism incidence (%)	Fruit malformation (%)	Grey mould (%)	Individual berry weight (g)	Marketable fruit yield (g/plant)
Calcium spray	6.7b	10.9a	1.3b	11.9a	168.4c
Boron spray	14.8a	3.4b	4.8a	11.4a	161.3b
Calcium + Boron spray	6.5b	3.1b	1.2b	11.7a	179.2d
Control (water spray)	15.1a	12.4a	5.2a	11.2a	149.3a

Means within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$.

Table 2
Fruit firmness, colour and quality of 'Chandler' strawberry as influenced by pre-harvest spray of Ca and/or B at harvest

Treatment	Firmness (N)	Colour			TSS (%)	Acidity (%)	Ascorbic acid content (mg/100 g pulp)
		L	a	b			
Calcium spray	1.55b	36.2a	37.1a	46.3a	7.1a	1.15b	51.3b
Boron spray	1.32a	35.8a	36.2a	44.9a	7.7b	1.02a	49.1a
Calcium + boron spray	1.62b	36.5a	38.3a	47.5a	7.2a	1.12b	51.6b
Control (water spray)	1.30a	35.2a	36.5a	45.8a	7.8b	0.99a	48.5a

Means within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$.

Table 3
Effect of foliar application of Ca and/or B and their subsequent concentration in leaf and fruit tissues of 'Chandler' strawberries

Treatments	Leaf calcium concentration (g kg^{-1})	Leaf boron concentration (mg kg^{-1})	Fruit calcium concentration (mg kg^{-1})	Fruit boron concentration (mg kg^{-1})
Calcium spray	16.3b	25.7a	198b	1.1a
Boron spray	12.5a	42.1b	135a	2.7b
Calcium + boron spray	16.8b	41.8b	207b	2.8b
Control (water spray)	12.4a	26.2a	137a	0.9a

Means within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$.

Table 4
Effect of pre-harvest application of Ca and/or B on fruit firmness, colour and quality of 'Chandler' strawberry after 5 days storage at 10 °C and 85% RH

Treatments	Firmness (N)	Colour			TSS (%)	Acidity (%)	Ascorbic acid content (mg/100 g pulp)	Grey mould (%)
		L	a	b				
Calcium spray	1.27b	34.1b	35.1b	33.2b	7.9b	1.08b	43.7b	2.2a
Boron spray	1.01a	27.2a	27.5a	23.4a	8.2a	0.95a	40.2a	8.1b
Calcium + boron spray	1.33b	34.8b	36.2b	34.7b	7.8b	1.07b	45.0b	1.9a
Control (water spray)	0.91a	26.8a	25.4a	22.3a	8.5a	0.91a	39.1a	8.4b

Means within the column with the same letter are not significantly different by Duncan Multiple Range Test at $P \leq 0.05$.

3.4. Effect on fruit firmness, external colour and quality parameters during storage

Pre-harvest application either of Ca or Ca + B have significantly influenced fruit firmness, external colour, quality parameters (TSS, acidity, ascorbic acid content) and grey mould incidence in 'Chandler' strawberry during 5 days storage at 10 °C and 90% relative humidity (Table 4). However, B alone could not influence these parameters significantly over control. Fruit harvested from plants sprayed either with Ca or Ca + B were firmer (1.27 and 1.33 N) than those harvested from plants sprayed either with boron (1.01 N) or those under control (0.91 N). Further, fruit which received Ca or Ca + B at pre-harvest stage have higher values of L (34.1 and 34.8), a (35.1 and 36.2) and b (33.2 and 34.7) than those, which received B alone or those in control. Similarly, such fruit have significantly lower TSS (7.9 and 7.8%); higher ascorbic acid content (43.7 and 45.0 mg/100 g pulp) and acidity (1.08 and 1.07%) during storage. Similarly, the incidence of grey mould was significantly lesser in fruit, which received Ca (2.2%) or Ca + B (1.9%) than those, which received either B (8.1%) alone or those in control (8.4%). However, physiological loss in weight (PLW%) of fruit during storage was not influenced significantly with any of the pre-harvest sprays (data not presented).

4. Discussion

In our study, pre-harvest foliar application of Ca, B and Ca + B has influenced albinism, and fruit malformation disorders, incidence of grey mould and fruit yield of 'Chandler' strawberry at harvest (Table 1). Similarly, fruit receiving Ca or Ca + B were firmer, brighter (higher L, a, b values) and have better quality attributes (Table 2). Lesser incidence of albinism and grey mould in fruit receiving Ca or Ca + B might be due to the fact that Ca slows down the ripening and senescence processes in many fruit including strawberry (Ferguson, 1984; Poovaiah, 1986; Sharma et al., 2006). Hence, albino fruit has been produced in lesser proportion in plants, which received Ca or Ca + B, and because the fruit receiving Ca were firmer, hence less affected by grey mould incidence. Wojcik and Lewandowski (2003) and Naradisorn et al. (2006) had also reported that fruit receiving Ca are much firmer and less affected by grey mould. It is also evident that fruit receiving Ca or Ca + B were having higher concentration of Ca both in leaves and fruit (Table 3) which support that presence of higher Ca concentration might have contributed for better firmness of fruit which directly or indirectly had influenced albinism and grey mould incidence. Similarly, better firmness of fruit receiving Ca or Ca + B may be attributed to the effect of Ca on middle lamella of cells/tissues as it acts as binding agent between the cell walls, as a result, it might have resulted in higher firmness in such fruit, which also resulted in lesser incidence of grey mould. Ca treatment could not exert any influence on fruit malformation, however, fruit harvested from plants receiving B or Ca + B had lesser incidence of fruit malformation, which may be attributed to significant role of B on pollen germination, pollen tube growth, which results in better fruit-set and subsequent fruit growth. Presence of lesser proportion of malformed fruit which received B alone or Ca + B can be very well correlated with higher concentration of B both in leaves and fruit in our study (Table 3). Malformed fruit have reported to be produced in larger proportion in B deficient soils, primarily because plants remain B deficient, and as reported above, B is considered essential for proper pollen germination, pollen tube growth and subsequent fruit growth in strawberry (Guttridge and Turnbull, 1975; Lieten, 1989, 1998, 2002; Sharma and Sharma, 2004).

None of the treatments has influenced individual berry weight, but marketable yield was significantly different among the treatments. It may be ascribed to the fact that while calculating marketable yield, we have taken in to account only the sound/healthy/normal fruit, and those affected by albinism, malformation, and grey mould were discarded. In our study, it is really interesting to note that total fruit yield among the treatments was not significantly different (data not presented), indicating that pre-harvest application either of Ca, B or Ca + B has no remarkable effect on total yield and the significant differences in marketable yield among the treatments were only due to discarding of albino, malformed and grey mould affected fruit. Wojcik and Lewandowski (2003) have also reported that pre-harvest application either of Ca, B or Ca + B has no effect on fruit yield in 'Elsanta' strawberry. Further, plants receiving Ca + B, produced highest marketable yield (179.2 g/plant), primarily because such plants produced least number of albino (6.5%), malformed (3.1%) and grey mould (1.2%) affected fruit. In our study, B or Ca + B application has reduced the incidence of fruit malformation in 'Chandler' strawberry. However, Wojcik and Lewandowski (2003) have not observed such influence, which may be due to different soil and climatic conditions where these studies were conducted.

At harvest, fruit receiving either Ca, B or Ca + B, have lower TSS, higher acidity and ascorbic acid content than those harvested from plants receiving either B or in control. It may be correlated with the firmness of fruit as fruit receiving either Ca, B or Ca + B, were much firmer than control fruit, and hence they have lower TSS, and higher acidity (Hernandez-Munoz et al., 2006). Higher ascorbic acid content in such fruit may primarily be due to the reason that Ca has promotory influence on vitamin C content (Sharma and Sharma, 2004). Studies conducted by us and that conducted by Wojcik and Lewandowski (2003) indicate that B application could not influence the quality parameters in strawberry, but Cheng (1994) had reported that B deficiency in strawberry usually result in poor accumulation of TSS, and vitamin C content.

The concentration of Ca and B both in leaves and fruit increased with the respective application of Ca, B or Ca + B (Table 3), attributing that these nutrients, when applied through foliar means are readily available to plants and then translocated to different parts, where these take part in several vital processes, and affect many pathological and physiological disorders (Conway et al., 1992; Fallahi et al., 1997; Dunn and Able, 2006; Hernandez-Munoz et al., 2006).

In general, irrespective of any pre-harvest treatment, fruit of 'Chandler' strawberry after 5 days storage were less firmer, have higher TSS, lower acidity and ascorbic acid content than at harvest. Fruit, which received either Ca, B or Ca + B at pre-harvest, were firmer; have lower TSS, higher acidity and ascorbic acid content and lower incidence of grey mould after 5 days storage (Table 4). Further, pre-harvest spray either of Ca, B or Ca + B has little effect on external fruit colour, but it was greatly influenced during storage. During storage, fruit harvested from control plants turned darker sharply, whereas those harvested from Ca, B or Ca + B treated plants were comparatively brighter. Similarly, higher values of hue (a) and chroma (b) in such fruit indicate that fruit receiving either Ca or Ca + B were redder and vivid as reported by Hernandez-Munoz et al. (2006). Strawberries soften considerably during storage as a result of degradation of middle lamella of cell wall of cortical parenchyma cells (Perkin-Veazie, 1995). Similarly, cell wall strength, cell to cell contact and cellular turgor may also influence the fruit firmness in strawberry (Harker et al., 1997), which is greatly influenced by Ca application. Thus, fruit which received Ca, B or Ca + B were firmer even after 5 days storage than other fruit, and as reported above, firmer fruit have higher TSS, lower acidity and lesser incidence of grey mould as reported by (Wojcik and Lewandowski, 2003; Hernandez-Munoz et al., 2006) and by us in the present study.

5. Conclusions

The present study revealed that sequential pre-harvest foliar application of combination of Ca and B is quite useful in 'Chandler' strawberry for reducing the incidence of physiological disorders, like albinism, fruit malformation, and disease like grey mould, which helps in getting higher marketable fruit yield with better firmness and other quality parameters.

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