Postharvest Pepper Conservation with Different Storage Strategies

Dalva Paulus¹*, Gilmar Antônio Nava¹, Ivan Carlos Zorzzi² and Raquel Valmoribida¹

¹Department of Agronomy, Federal University of Technology – Paraná, CEP: 85660-000-Dois Vizinhos, Paraná, Brazil.
²Department of Agricultural Engineering, Federal University of Technology – Paraná, Campus Pato Branco – CEP: 85503-390 – Pato Branco, Brazil.

Authors’ contributions

This work was carried out in collaboration between all authors. Authors DP and GAN designed the study and wrote the first draft of the manuscript. Authors ICZ and DP performed the experiments. Authors ICZ, DP, RV and GAN participated in fieldwork and laboratory analysis. Authors ICZ and RV managed the analyses of the study. Authors ICZ and DP managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aims: The present study was to assess the postharvest quality of Capsicum annum peppers subjected to different concentrations of salicylic acid at room temperature, and using of different kinds of treatments like as plastic films, refrigerated, with different storage periods.

Study Design: For this, two experiments were conducted: Experiment I – salicylic acid concentrations (0; 0.5; 1.0 and 2.0%) and three storage periods (7, 14 and 21 days) were tested; Experiment II – modified atmospheres were used, with low-density polyethylene (LDPE) films with five thickness (20, 40, 60, 80 and 100 µm), polyvinyl chloride (PVC) of 10 µm and control (without film), being stored under refrigeration at 9.5 ± 0.5°C and 90 ± 2% of relative humidity, kept for 7, 14, 21 and 28 days.
1. INTRODUCTION

Pepper production is an important segment of the vegetable sector, both for agriculture and food industry [1]. Pepper of the species *Capsicum annum* L. have different uses in the pharmaceutical industry, to treat arthritis and muscle aches; in cooking, being used mainly as a condiment, all natural or processed, as a paste, dehydrated, in sauces, seasonings and canned [2].

Pepper fruits are non-climacteric, and the intense red fruit coloration is a quality standard characteristic for the market at the time of commercialization [3]. The main problems of preserving the quality of peppers destined to in natura consumption are fast water loss, resulting in wilting and discoloration of the peduncle, which causes the loss of the characteristic green coloration. These factors reduce the value of the product on the market, generating losses due to the disposal of the fruits [4]. Because it is a tropical fruit, the best storage temperature range for peppers lies between 7 and 12°C [5]. Fresh peppers are susceptible to damage by cold (chilling) when stored below 10°C. However, the intensity of cold damage is associated with the stage of fruit ripening and with the variety of pepper [6].

The use of natural or biodegradable compounds, non-toxic, derived from animals or plants, which have fungistatic effect or induce the natural resistance of plants, has been outstanding in the control of postharvest fungi [7].

In this sense, in postharvest, salicylic acid (SA) is a low-cost alternative, a natural phenolic compound with the ability to interfere in the biosynthesis, because it reduces the activity of ACC oxidase, a precursor enzyme for the synthesis of ethylene from plants, thus delaying the ethylene of production [8]. Exogenous application of salicylic acid reduces the respiratory activity, resulting in less energy expenditure and prolonging shelf-life. Rao et al. [9] studied the use of SA in bell peppers, kept at 10 and 25°C, verified a better preservation of the quality of the fruit.

Storage conditions play an important role in maintaining the quality of fruits and vegetables. However, cell metabolism continues during storage, and can influence the appearance, texture, taste, and nutritional value of the product. For this reason, the use of refrigeration and packaging of fruits in polymer packages are techniques that have an important effect on the post-harvest life of plants, reducing physiological processes, such as respiration [10].

Techniques that allow extending the shelf-life of pepper are alternatives so that the production can be transported and reach the consumer with quality, reaching distant markets and opening new cultivation and marketing possibilities. The modified atmosphere with plastic or natural polymers allows to preserving fruits and vegetables in postharvest, reducing water loss, maintaining firmness and color [11] and, thus, improving the visual aspect of the product during commercialization. Studies with the modified

**Keywords: Capsicum annum L.; plastic films; natural phenolic compound.**
atmosphere in peppers that determine the storage period are scarce in literature and of great importance for the production chain of this crop.

Studies with natural phenolic compounds, such as salicylic acid and plastic films, are important measures for preserving color and quality of the pepper [12], which justifies conducting studies in this area.

The increase in consumption of pepper and the need to reach distant markets from the production site make it necessary to assess the techniques that allow maintaining the postharvest quality of the vegetable. The objective of this study was to assess the postharvest quality of peppers subjected to different concentrations of salicylic acid at room temperature and the use of different plastic films under refrigeration, with different storage periods.

2. MATERIALS AND METHODS

2.1 Plant Material and Growing Conditions

The experiments were conducted in the Horticulture Laboratory of the Federal University of Technology of Paraná (UTFPR), Campus Dois Vizinhos, Paraná – Brazil. Peppers of the “BRS Mari” cultivar were harvested in the Olericulture of the UTFPR, from a single harvest in 2016. The fruits were homogenized according to ripening and coloring, and those with physical damage, overly ripe and excessively green were discarded. 20 fruits were used per experimental unit (3 green fruits – scale 1; 7 yellow fruits – scale 2; 10 orange fruits – scale 3) with an average total weight of 104 g per experimental unit (5.2 g fruit-1).

2.2 Treatments and Experimental Design

In case of experiment 1, randomized block design (RBD) was used, arranged in a factorial scheme (4×3), with four salicylic acid concentrations (control; 0.5; 1.0 and 2.0%) and three storage periods (7, 14 and 21 days), at room temperature (average of 15°C ± 4.0°C and 81% ± 14% of relative humidity, RH) and four repetitions, being each experimental unit composed of a Styrofoam container with 20 fruits, which were not disinfected. Salicylic acid was diluted in distilled water heated to 45°C; followed by immersing the fruits for three minutes in the solutions and leaving them to dry on germitest paper for 10 minutes on a countertop in the shade.

For Experiment II, the experimental design used was RBD, arranged in a factorial scheme (7×4), with seven different modified atmospheres (control; with low-density polyethylene (LDPE) films – 20, 40, 60, 80, 100µm of thickness and polyvinyl chloride (PVC) with 10µm of thickness) and four storage periods (7, 14, 21 and 28 days), with four repetitions, being each experimental unit composed of a Styrofoam container with 20 fruits. The Styrofoam containers with the fruits were placed in a Biochemical Oxygen Demand (B.O.D) chamber with an average temperature of 9.5 ± 0.5°C and 90 ± 2% of RH.

2.3 Evaluated Parameters

The fruits of both experiments were assessed in the same day after the treatments were applied (day 0) and, subsequently, every seven days of storage (7, 14, 21 and 28 days) for both experiments. The following parameters were analysed: soluble solids content (SS, °Brix), obtained by direct reading in Hanna® bench refractometer model HI 96801, (corrected to a temperature of 20°C) and titratable acidity (TA) content, through titration of 10 mL of juice + 90mL of distilled water with the NaOH 0.1 N solution, up to pH 8, expressing the results in total percentage of organic acids. The SS/TA ratio was obtained through the division of the aforementioned contents of each experimental unit, respectively. The evaluation of the incidence of rot in fruits was performed by visual analysis, individually for each fruit, and expressed as a percentage of fruit with symptoms of diseases (apparent mycelium), and/or fruits with soft deterioration were observed in each evaluation period; subsequently, the number of deteriorated fruits was divided by the total number of fruits in the sample, and the values were expressed as a percentage of deteriorated fruits (%) and total of deteriorated fruits (%).

Fresh fruit weight loss was obtained with a precision scale of two decimal digits, from the difference of weight observed between the moment the experiment was installed and the end of each pre-established storage period, and the obtained values were also expressed as a percentage. Individual background color score of the epidermis of each fruit that composed the experimental units was obtained through individual comparing, under fluorescent lamp, with subjective scale of six color levels, as
follows: 1 – green fruit; 2 – yellow; 3 – yellowish-orange; 4 – orange; 5 – reddish orange; and 6 – deep red (ripe fruit). The average color score of each sample was obtained by the equation:

$$ACS=\frac{\sum_{i=1}^{n}p_i}{20}$$

where:

- ACS = Average color score
- n = number of fruits (total of 20 fruits)
- p = corresponding weight of the color scale by fruit

2.4 Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA) through the F test, and the quantitative and qualitative variables were subjected to regression analysis and to the means grouping Scott-Knott’s test, \((P=0.05)\), using the Statistical Analysis System Studio [13].

3. RESULTS AND DISCUSSION

3.1 Experiment 1

There was an interaction between salicylic acid (SA) concentrations and storage time for the soluble solids (SS), titratable acidity (TA) and SS/TA ratio variables. There was no interaction for the percentage of deteriorated fruits, fresh fruit weight loss and color scores variables (Table 1).

Regarding storage time (7, 14 and 21 days) and SA concentrations, it was observed that there was no significant difference in the percentage of deteriorated fruits, with average values of 8.45; 6.91; 6.53; 5.43 and 5.51\% for salicylic acid concentrations of 0; 0.5; 1.0; 1.5 and 2.0\%, respectively. Possibly, the SA activated routes of vegetable defenses, because its action is characterized as a sign of routes involved in the Systemic Acquired Resistance (SAR), inducing, thus, the biosynthesis of enzymes that act in the formation of vegetable defense compounds, such as polyphenols and alkaloids, and also pathogenesis-related proteins (PR proteins) [14]. According to the authors, many PR proteins have in vitro antifungal activity, such as chitinase and glucanase, for example.

Concerning fresh fruit weight loss, no significant interaction was observed between SA concentrations and storage time, with quadratic effects over the storage period (Fig. 1A). After seven days of storing the peppers at room temperature and humidity, visually, the fruits of all treatments already had a bad appearance for commercialization, with characteristic wilting due to the severe water and fresh weight loss of the fruits. Vegetable storage in ambient atmosphere promotes an intense exchange of gases, increasing respiration and water loss. Fresh fruit weight loss is a limiting factor both for conservation and for marketing, because it causes commercial devaluation, due to wrinkled fruits and the wilting of the epicarp [15].

The soluble solids content was influenced by SA concentrations, with quadratic adjustment for 0; 0.5; 1.0 and 1.5\% and linear for 2.0\% of salicylic acid (Fig. 1B). The content of soluble solids grew almost linearly in all treatments up to the 7th day of storage but persisting in a growing behavior until 12 to 14 days, with values that varied from 6.1 to 8.1 °Brix, depending on the treatment, and the values were maintained or fell after these storage periods. This increase may be related to fresh fruit weight loss, which causes the concentration of soluble solids in the pulp [16].

Similar results were observed by Rinaldi et al. (2008) [17], who studied different types of bell peppers and storage periods, in which the soluble solids contents varied from 4.6 and 7.4 °Brix over 12 days of storage in a natural environment condition (temperature of 21 ± 2°C and relative air humidity of 70 ± 5\%). SA concentration at 2.0\% kept linear growth and a higher value of soluble solids, while the other concentrations had a decrease of SS from day 14. Thus, based on this variable, which did not show greater weight loss when compared to other treatments, it can be said that salicylic acid at 2.0\% slowed the ripening of the fruits and, when associated with the plastic film, drastically prevented water loss, allowing to store pepper for at least 28 days. It can be said that salicylic acid is suitable for commercial use, if it is considered in terms of cost and safety in consumption. The use of edible coating is an alternative post harvest preservation method for fresh fruits and vegetables that has been gaining an increasing interest because of the ecological considerations and the trends toward convenience foods [18].

For titratable acidity (TA), the SA concentrations of 0; 0.5 and 1% had quadratic adjustment. In the first seven days of storage, there was an increase in TA values; possibly justified by the process of generating free radical (galacturonic acids) with origin in the hydrolysis of the cell wall constituents [19], as well as by the concentration in the fruit due to their high fresh weight loss. From the 7th day of storage, the TA contents began to decrease.
Table 1. Summary of the variance analysis for soluble solids content (SS), titratable acidity (TA), SS/TA ratio, percentage of deteriorated fruits (P.D.F.), fresh fruit weight loss (F.F.W.L.) and color scores (C.S.) in peppers subjected to treatment with salicylic acid (SA) concentrations in different storage periods

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>TA</th>
<th>SS/TA</th>
<th>P.D.F.</th>
<th>F.F.W.L.</th>
<th>C.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>0.274*</td>
<td>0.001 ns</td>
<td>1.423*</td>
<td>0.985*</td>
<td>3.649*</td>
<td>0.103 ns</td>
</tr>
<tr>
<td>SA concentrations</td>
<td>4</td>
<td>1.230*</td>
<td>0.004 ns</td>
<td>3.014*</td>
<td>0.797 ns</td>
<td>5.579 ns</td>
<td>0.105 ns</td>
</tr>
<tr>
<td>Storage periods</td>
<td>2</td>
<td>1.654*</td>
<td>0.094*</td>
<td>69.444*</td>
<td>29.200*</td>
<td>922.451*</td>
<td>8.076*</td>
</tr>
<tr>
<td>SA concentrations*storage periods</td>
<td>8</td>
<td>1.348*</td>
<td>0.009*</td>
<td>2.303*</td>
<td>0.451 ns</td>
<td>74.574*</td>
<td>0.070 ns</td>
</tr>
</tbody>
</table>

DF - Degrees of freedom; * Significant by the F test (P <0.0001); ns – no significant.

Fig. 1. Fresh fruit weight loss (%) (A), soluble solids (°Brix) (B), titratable acidity (TA) (C) and SS/TA ratio (D), in peppers subjected to treatment with salicylic acid (0; 0.5; 1.0; 1.5 and 2.0 %), in the periods of 0, 7, 14 and 21 days of storage.

It was found out that the control SA treatment showed a linear adjustment for the SS/TA ratio, with mean value of 13.79 at 14 days of storage. The SA concentration of 0.5% had a significant effect on SS/TA ratio, with quadratic adjustment. SA concentrations of 1.0, 1.5 and 2.0 did not influence the SS/TA ratio (Fig. 1D). The higher the SS/TA ratio, the sweeter will be the fruits and the greater will be their ripening stage, indicating high respiratory rate with high consumption of organic acids, which are generally the first substrates to be consumed during aerobic respiration [20].

For color score (Fig. 2), a quadratic behavior of regression was observed, and the fruits increased the biosynthesis of orange and red pigments on the background color of the epidermis, characteristics of green fruits over the storage period, even though pepper is not a climacteric fruit [3], showing their normal ripening. The initial average color of the fruit...
epidermis when the experiment was installed (day 0) was of 1.5.

3.2 Experiment 2

Regarding the modified atmosphere factor, a significant interaction was observed between it and storage time for the variables of fresh fruit weight loss, SS, TA and color scores. There was no interaction for the percentage of deteriorated fruits and SS/TA ratio (Table 2). For the percentage of deteriorated fruits, a significant effect in plastic films (Table 3) and quadratic effect were observed, on the average of the tested films, for the storage period (Fig. 3A).

The control treatment showed lower proportion of deteriorated fruits (0.3%), not differing from LDPE plastic films. The treatments with LDPE did not differ among themselves and allowed a reduction in the amount of deteriorated fruits up to 14 days of storage (Fig. 3A), when compared to PVC, which had higher percentage of deteriorated fruits (12.84%) (Table 3). Differing from what was observed by Morgado et al. (2008) [21], who, in studies with stretchy PVC film with 17µm of thickness, observed that this film protected bell peppers against rotting up to 33 days of refrigerated storage at 5°C. PVC is known to have greater permeability to water vapor when compared to LDPE [6], not explain, therefore, the occurrence of rotting when PVC was used.

From day 14, an expressive increase was observed in the proportion of deteriorated fruits in all plastic films (Fig. 3A), which restricted the flow of water vapor generated by the fruits to the external environment, when compared to the control treatment; this is due to the accumulation on the inside of the package, around the fruits, consequently favoring the development of fungi [22].

Pepper behaved linearly for the variables fresh fruit weight loss throughout storage, and those covered with LDPE had smaller losses at 14 days, 1.4% on average, and no difference between thicknesses was observed. At this same storage time, the control and the PVC treatments showed the highest losses, 43 and 13%, respectively (Fig. 3B). LDPE had lower permeability to water vapor than PVC [6], having drastically reduced water loss of peppers. The reduction of the weight loss in wrapped fruits was due to the fact that the modified atmosphere allows the formation of a microenvironment with high relative humidity, which reduces fruit perspiration [16].

On the other hand, Hojo et al. (2007) [23], who assessed the use of PVC and cassava starch films in bell peppers, found that PVC reduced the fresh fruit weight loss and allowed the conservation up to the eighth day of storage at ambient conditions. After seven days of storage, the fruits of the control treatment and the ones covered with PVC did not have a good appearance for commercialization, mainly due to intense dehydration, which can be explained by the fast fresh weight loss, with perspiration as the main factor, which is associated to breathing [5]. The fruits wrapped with LDPE, visually, had the potential to be commercialized up to the 18th day.

![Graph](image-url)

**Fig. 2.** Average background color scores of pepper epidermis (average of the treatments with salicylic acid) at 7, 14 and 21 days of storage at room conditions
Table 2. Summary of the variance analysis for soluble solids content (SS), titratable acidity (TA), SS/TA ratio, percentage of deteriorated fruits (P.D.F.), fresh fruit weight loss (F.F.W.L.) and color scores (C.S.) in peppers subjected to treatment with plastic films in different storage periods

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>TA</th>
<th>SS/TA</th>
<th>P.D.F.</th>
<th>F.F.W.L.</th>
<th>C.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Block</td>
<td>3</td>
<td>0.193</td>
<td>*</td>
<td>0.021</td>
<td>*</td>
<td>0.332</td>
<td>*</td>
</tr>
<tr>
<td>Plastic films</td>
<td>6</td>
<td>4.949</td>
<td>*</td>
<td>0.044</td>
<td>*</td>
<td>14.035</td>
<td>*</td>
</tr>
<tr>
<td>Storage periods</td>
<td>3</td>
<td>1.153</td>
<td>*</td>
<td>0.092</td>
<td>*</td>
<td>78.090</td>
<td>*</td>
</tr>
<tr>
<td>Plastic films*storage periods</td>
<td>18</td>
<td>1.043</td>
<td>*</td>
<td>0.041</td>
<td>*</td>
<td>26.106</td>
<td>*</td>
</tr>
</tbody>
</table>

Mean square

DF: Degrees of freedom; * Significant by the F test (P<0.0001); ns – no significant.

Table 3. Percentage of deteriorated fruits and SS/TA ratio subjected to treatment with plastic films (control; LDPE – 20, 40, 60, 80, 100 µm and PVC 10 µm), throughout 28 days of storage

<table>
<thead>
<tr>
<th>Plastic films</th>
<th>Percentage of deteriorated fruits (%)</th>
<th>Ratio SS/TA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.30 b*</td>
<td>13.5* ns</td>
</tr>
<tr>
<td>LDPE 20 µm</td>
<td>6.89 b</td>
<td>14.0</td>
</tr>
<tr>
<td>LDPE 40 µm</td>
<td>3.15 b</td>
<td>14.6</td>
</tr>
<tr>
<td>LDPE 60 µm</td>
<td>1.05 b</td>
<td>14.8</td>
</tr>
<tr>
<td>LDPE 80 µm</td>
<td>4.68 b</td>
<td>13.6</td>
</tr>
<tr>
<td>LDPE 100 µm</td>
<td>3.27 b</td>
<td>13.5</td>
</tr>
<tr>
<td>PVC</td>
<td>12.84 a</td>
<td>13.3</td>
</tr>
<tr>
<td>Mean</td>
<td>4.6</td>
<td>13.9</td>
</tr>
<tr>
<td>C.V. (%)</td>
<td>25.0</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Notes: *Means followed by distinct letters in the column differ by the Scott Knott test at P=0.05.; ns: not significant; C.V.: Coefficient of variance.

The soluble solid contents of the fruits varied throughout storage time with PVC, with quadratic regression adjustment, and no significant effect was observed in the other assessed films (Fig. 3C). SS contents were similar in all treatments up to the 14th day of storage and, only after this period, the control treatment differed from the other treatments, showing the highest values (8.3°Brix). The lowest SS contents of the fruits involved by plastic films in this experiment, after the 14th day of storage, a fact that can be explained by a restriction in permeability to respiratory gases, which generates a reduced respiratory metabolism and, consequently, less non-reducing sugar conversion into reducing ones [5].

Similar results were verified by Oliveira et al. [16] with camu camu (Myrciaria dubia – Myrtaceae) in a modified atmosphere, and they observed that the control treatment resulted in increased SS content, due to a greater weight loss, which resulted in pulp concentration, increasing, thus, soluble solid contents. Ozden and Bayindirli [18] have investigated changes in SS content that occur during the storage of pepper and observed increases of this variable.

For titratable acidity (TA), up to the 14th day, there was no difference between the treatments. After this period, the fruits of the control treatment had higher TA than the others (Fig. 3D), this increase can be associated to its concentration in the juice, due to the high fresh fruit weight loss when they were wrapped in the films.

The treatments with LDPE film of 40 and 100 µm and PVC were adjusted to the quadratic level for TA (Fig. 3D). In the first 14 days of storage, there was a reduction of TA in all treatments, influenced by the ripening process, which promoted the conversion of organic acids in sugars and, subsequently, in energy in the form of Adenosine Triphosphate (ATP) for maintaining the vital functions of cells [5].

In the literature [10], evaluated the storage of pepper fruits in polymeric films at low temperatures (4.3°C and 10°C), increased levels of total carotenoids, total soluble solids and titratable acidity.

The films did not influence the SS/TA ratio (Tabel 1). A similar result was obtained by Moura et al. [24], assessing umbu fruits (Spondias tuberosa) kept in a modified and an ambient atmosphere,
in which the isolated atmosphere did not influence the SS/TA ratio. SS/TA ratio was highest at 14 days of storage (Fig. 3E). Using PVC and polyethylene terephthalate (PET), [12] observed that storage time, at temperature of 10°C ± 1°C, did not affect SS and TA contents and the SS/TA ratio of pepper, which corroborates with the data obtained in this research.

For color score, a significant interaction was observed between the films (control and LDPE of 20 and 40 µm) over storage time. In the implementation of the experiment (day 0), the peppers showed a 1.65 color score (9 green fruits; 9 yellow fruits; 2 orange fruits). For the three treatments with film, a quadratic evolution pattern was observed in the color of the fruits throughout the refrigerated storage time, with a larger increase of yellow, orange and red carotenoids pigments after 21 days of storage (Fig. 4).

Fig. 3. Total of deteriorated fruits (%) (A); fresh fruit weight loss (%) (B), soluble solids (°Brix) (C); titratable acidity (D) and SS/TA ratio (E) in peppers subjected to treatment with plastic films (control; LDPE – 20, 40, 60, 80, 100 µm and PVC 10 µm), at 0, 7, 14, 21 and 28 days of storage
Fig. 4. Average background color scores of pepper epidermis subjected to treatment with plastic films (control (A); LDPE – 20 µm (B) and LDPE – 40 µm (C), at 7, 14, 21 and 28 days of storage.

The highest color score was observed at 28 days (approximately 3.0) for the control treatment, while in the fruits wrapped by the film, the average score value was about 2.5, showing the beneficial effect of the modified atmosphere, provided by plastic films, by reducing the respiratory metabolism and preventing ripening. However, peppers stored under refrigeration (9.5°C ± 0.5°C) reached, on average, a maximum score of 3.0, thus, they could be stored for a larger period of time, based on this quality aspect, since the deep red color of the fruit of this cultivar is an important characteristic of quality standard for the marked, in natura and processed [3].

4. CONCLUSION

In the environment storage conditions of peppers used in the experiment, salicylic acid at 2.0% does not prevent fruit deterioration, but when associated to LDPE films, it allows the conservation of pepper for up to 28 days. Under room temperature and humidity or under conventional refrigeration, the fruits maintain good commercial quality for up to seven days. LDPE plastic films, complementary to refrigeration, reduce fresh weight loss and do not increase fruit decay by rotting, prolonging in 21 days the storage period of the fruits.

ACKNOWLEDGEMENTS

The authors thank EMBRAPA for the supply of the pepper seeds.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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and Breeding of Chilli Peppers (Capsicum spp.). Springer International Publishing; 2016. DOI: 10.1007/978-3-319-06532-8_2

Peer-review history:
The peer review history for this paper can be accessed here: http://www.sdiarticle3.com/review-history/46328