Changes in Polyamines and Ethylene during the Development and Ripening of Eggplant Fruits (*Solanum melongena*)

Silvia del C. Rodriguez,[†] Beatriz López,[‡] and Alicia Raquel Chaves*,[§]

Instituto de Ciencias Químicas (ICQ), Facultad de Agronomía y Agroindustrias-UNSE,
Avenida Belgrano (S), 1912 (4200) Santiago del Estero, Argentina, and Centro de Investigación y Desarrollo
en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas-UNLP-CONICET,
47 y 116 (1900) La Plata, Argentina

The levels of free polyamines in the endocarpium of eggplants (*Solanum melongena*) cv. Black Nite were studied during fruit growth and ripening. Other parameters such as weight and volume variation, respiratory intensity, ethylene production, and sugar content were determined. The polyamines found were putrescine and spermidine, with a higher amount of the former. No spermine, agmatine, or cadaverine were found during the development and ripening period of eggplant. At the beginning of fruit development, the levels of putrescine and spermidine observed were 1.67 nmol/g of fresh tissue. Putrescine levels peaked at 17.4 nmol/g of fresh tissue on the ninth day after petal fall, decreasing later to the initial levels. No significant changes in spermidine were observed during the growth period of the fruit. Beginning 9 days after petal fall, there was a rapid increase in fruit weight and volume, which coincided with the maximum content of free polyamines. At the same growth stage, total sugar content was maximum. Ethylene production decreased rapidly from 14.23 to 1.5 μL/kg·h and remained low during the whole growth period.

Keywords: *Polyamines; putrescine; spermidine; eggplant fruit; Solanum melongena; ethylene*

INTRODUCTION

Eggplant fruits are nonclimacteric and are purple, white, or striped. Purple fruits are commercially more important, particularly in Asia and Mediterranean countries (Nothman, 1986). They grow well in tropical regions since the plant needs warm temperatures to develop. In irrigated areas of province of Santiago del Estero, Argentina, with mean temperatures between 20 and 30 °C, the harvest begins in December and continues until May. The fruit reaches its commercial size about 13 days after petal fall.

Free polyamine levels have been determined in several fruits: avocado (Winer and Apelbaum, 1986), apples (Biasi et al., 1988), tomatoes (Saftner and Baldi, 1990), tangerines (Nathan et al., 1984), and pears (Toumadje and Richardon, 1988). The free polyamines found most frequently were putrescine, spermidine, and spermine, and their concentrations varied from one fruit to another.

The presence of free polyamines and ethylene production are related, and they could control fruit ripening and storage characteristics. Fruit growth regulation has not been fully explained. It is unclear whether the mechanism is controlled by the polyamines themselves, their precursors, and/or related metabolites (Kakkar and Ray, 1993).

In general, a relation has been found between the synthesis of polyamines and the inhibition of ethylene biosynthesis, and this has been interpreted as a result of metabolic competition by the same precursor, i.e., *S*-adenosylmethionine (Escribano and Merodio, 1994).

Polyamines are present during the development of fruits such as avocado, mandarin, apple, litchi, and olive, and their levels decrease in the mature fruit (Kakkar and Ray, 1993). Studies of the influence of polyamines during fruit development revealed peak levels in putrescine and spermidine during the early growth stages and then decreasing values as the fruit becomes completely ripe. Smith (1982) has reported that polyamines naturally present in vegetables play an important role in cell division regulation and in plant growth and found that the maximum level of putrescine coincides with the expansion of fruit weight and volume. In tomatoes, the high putrescine levels and the rapid cell proliferation that occurs during the early stages of fruit growth suggest an influence of polyamines on cell division (Teitel et al., 1985; Kakkar and Ray, 1993).

At the present, polyamine evolution and their possible participation in the control of growing and ripening of eggplant fruits are unknown. In this paper, we report on a study of polyamine evolution during growth and normal ripening of eggplant fruit and the relationship to ethylene production, respiratory activity, and sugar content.

MATERIALS AND METHODS

Plant Material. Eggplant fruits (*Solanum melongena*) cv. Black Nite were grown on a farm at Colonia María Luisa, Banda district, province of Santiago del Estero, Argentina. Eggplants were harvested by hand every 48 h beginning 3 days after the petals fell until the fruits reached commercial size (500 cm³ volume). Fruits were analyzed immediately after

* Corresponding author (tel, 54-221-4249287/4254853/4890741; fax, 54-221-4254853; e-mail, mcanon@isis.unlp.edu.ar).
† Professor FAA.UNSE.
‡ Member of Research Career of CONICET. Professor FAA.UNSE.
§ Member of Research Career of CONICET. Professor FCE.UNLP.

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harvest. The harvest began on December 15. Fruits were weighed, and their volume was determined by water displacement.

Determination of Respiratory Activity and Ethylene Production. Carbon dioxide production was measured by placing the fruits in 1-L jars once they were carried to the laboratory. After 60 min a sample (1 mL) of internal atmosphere of the jar was taken with a syringe and fed to a KONIC gas chromatograph fitted with a thermal conductivity detector and a Porapack Q column at 30 °C. Helium was used as a carrier gas, with a flow rate of 6 mL/min. The results were expressed as mL of CO_2/kg·h.

Ethylene was measured in a KONIC gas chromatograph (GC) fitted with a flame ionization detector and Porapack Q column at 30 °C. A 1-mL sample was taken from the jar and injected into the GC. Nitrogen was used as carrier gas, with a flow rate of 16 mL/min. Results were expressed in μL of C_2H_4/kg·h.

Extraction and Determination of Sugars. Five fruits were selected and the samples prepared by cutting a 1-cm thick slice from the widest part of each fruit. Each slice was then divided radially into four portions, taking two opposite ones for analysis. Portions were weighed and crushed for 3 min with reagent-grade ethanol (95%) in a 1:2 (w:v) product:alcohol ratio. After 5 min, the samples were vacuum-filtered and centrifuged at 3000g for 8 min. Supernatants were stored at −20 °C until used. Sugars were determined in a HPLC (KONIC) fitted with a refraction index detector and a Lichrosorb NH_2 5 column. Acetonitrile/water (80:20) was used as isocratic mobile phase with a flow rate of 1 mL/min. Column temperature was 30 °C. The injection volume was 20 μL. Sugars were identified and quantified using 0.04 mg/mL standard solutions of glucose, fructose, maltose, and saccharose.

Extraction and Determination of Polyamines. Fruit samples were prepared as was indicated for the sugar extraction. Tissue (20 g) was taken from eggplant portions, finely homogenized for 2 min with a solution of 5% perchloric acid in a 1:1 w:v ratio, kept for 1 h under refrigeration with periodic stirring, and centrifuged at 5000g for 8 min. The supernatant, containing free polyamines, was placed in plastic jars and kept in a freezer at −20 °C until used.

For analysis, samples (200 μL) were taken and mixed with 1.5 μL of a 1 mM solution of 1,6-hexanediamine as internal standard. The solution was stirred before adding 400 μL of saturated Na_2CO_3 solution. After this, 800 μL of dansyl chloride (5 mg/mL in acetone) was added, and the mixture was kept for 1 h on a shaker at 60 °C in the dark. Then, 200 μL of proline (0.1 mg/mL in water) was added, and the mixture was sonicated for 4 min. The dansylpolyamines were extracted with 750 μL of ethyl acetate, and the volume was reduced under nitrogen in the dark. The residue was resuspended in 500 μL of acetonitrile/water (75:25). The polyamines present were analyzed with a Waters HPLC equipped with a fluorescence detector (350-nm excitation and 495-nm emission) and a reverse-phase column (Beckman, Ultrasphere ODS 5 μm, 4.6 mm × 25 cm) at 30 °C. As elution solvent a mixture of 75:25 acetonitrile/water was used with a flow rate of 2 mL/min. The injection volume was 20 μL. The polyamines were identified using 1 mM standard solutions of putrescine, spermidine, spermine, cadaverine, and agmatine and were quantified using the internal standard method.

Experimental Design. The experiments were carried out in a completely randomized design. Each experiment comprises six harvest dates (3, 5, 7, 9, 11, and 13 days after petal fall), and five fruits were used for each harvest date. At least three experiments were carried out. The determinations were done on triplicate extracted samples. The data were subjected to analysis of variance and the means compared using the LSD test, at $P = 0.05$ significance level.

RESULTS AND DISCUSSION

Nine days after petal fall, eggplant fruit weight and volume slowly increased to about 3 and 4 times the
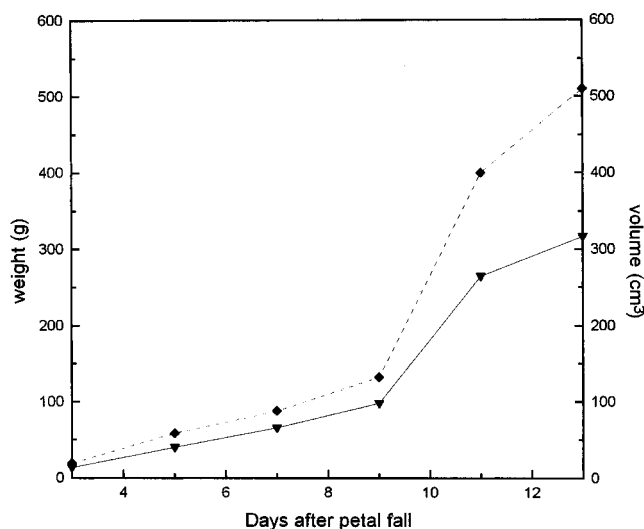
Figure 1. Changes in fruit weight and volume during development and ripening of eggplant, from the third day after petal fall: (···) volume; (—) weight. $LSD_{0.05} = 33.78$ and 34.31, respectively.
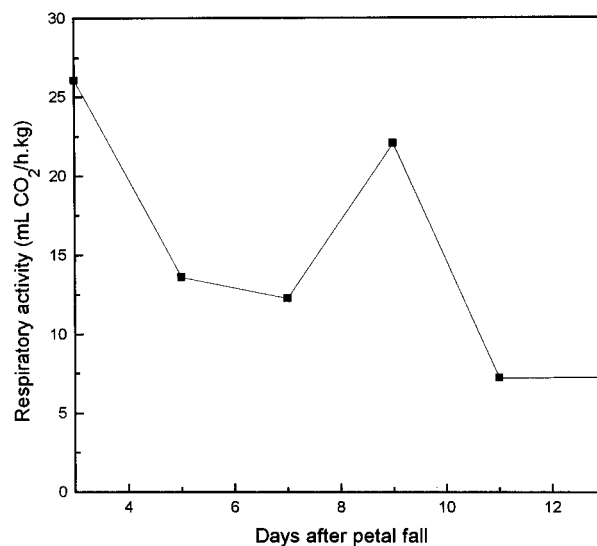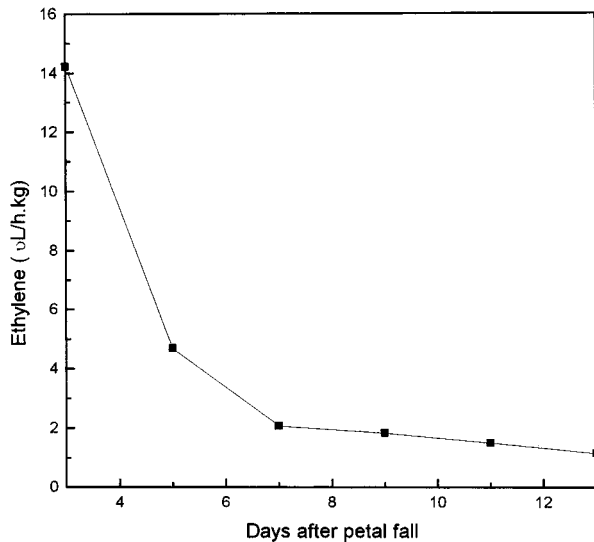

Figure 2. Changes in CO_2 production during development and ripening of eggplant fruits, from the third day after petal fall. $LSD_{0.05} = 3.10$.

initial value, respectively (Figure 1). After the ninth day, both parameters increased rapidly. This variation followed the curve of simple growth. Eggplants must be harvested as soon as they reach the desired size, with the skin smooth and bright before fruits get hard or show areas with unusual color (Pantastico, 1979). In our experiments, the eggplants reached commercial size after 13 days of development with a mean volume of 510 ± 70 cm^3 and a weight of 317 ± 36 g.

At the beginning of development, CO_2 production by fruits was 26.08 ± 1.02 mL/kg·h and decreased by 52% after 5 days (Figure 2). At day 7, respiration increased rapidly reaching a maximum ($P < 0.05$) on day 9 (22.01 $\pm$ 1.31 mg/kg·h). The respiratory intensity rapidly decreased ($P < 0.05$) by day 11 (7.25 $\pm$ 1.03 mg CO_2/kg·h), after which it remained constant.

Initial ethylene production was 14.23 ± 2.23 μL/kg·h and decreased to 2.08 ± 1.66 μL/kg·h by day 7 (Figure 3). Thereafter the ethylene rate remained constant at very low values (1.43 ± 0.94 μL/kg·h) until commercial maturity (day 13). Then, ethylene production did not

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Figure 3. Variation in ethylene production during development and ripening of eggplant fruits, from the third day after petal fall. $LSD_{0.05} = 1.06$.
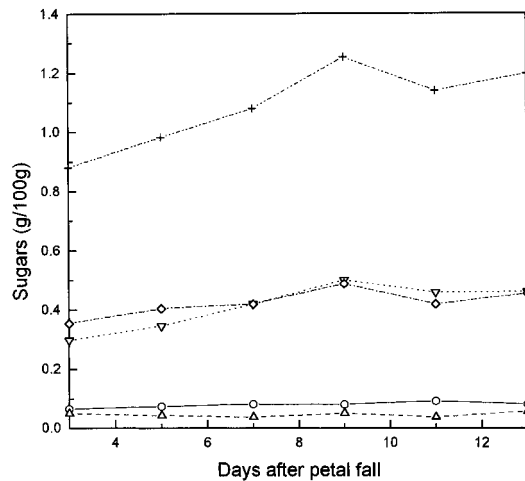

Figure 4. Changes in sugar content of eggplant fruits during their development and ripening, from the third day after petal fall: ($\triangle$) maltose, $LSD_{0.05} = 0.01$; ($\bigcirc$) sucrose, $LSD_{0.05} = 0.02$; ($\diamondsuit$) fructose, $LSD_{0.05} = 0.03$; ($\triangledown$) glucose, $LSD_{0.05} = 0.02$; (+) total sugars, $LSD_{0.05} = 0.04$.

present any change that might be associated with the ripening process.

Glucose, fructose, sucrose, and maltose were detected during fruit growth. The concentration of glucose was the same as that of fructose (Figure 4). Likewise, sucrose and maltose concentrations were coincident but 6 times as low as those of the first two. Kozukue et al. (1978) found glucose and fructose as the main sugars in eggplants (cv. Hyonaga) but did not detect sucrose. Esteban et al. (1992) studied three eggplants cultivars: Semi-round Striped, Purple Long, and Black Round, and during fruit growth, they observed an accumulation of reducing sugars up to about 6 weeks after fruit set, a period in which those eggplant fruits reach their sensorial characteristics. These authors did not find nonreducing sugars, and this fact was attributed to lack of inactivation of invertase during sugar extraction.

In our experiments, total sugar content was initially 0.88 ± 0.01 g/100 g of fresh tissue and increased gradually during the growing period, reaching a maximum ($P < 0.05$) of 1.25 ± 0.01 g/100 g after 9 days. This corresponded with the maximum respiratory activity
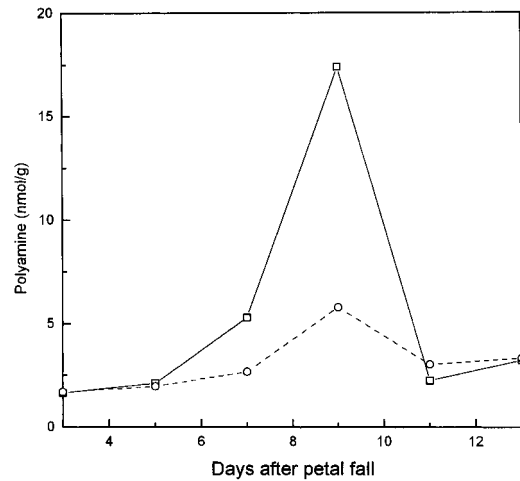
Figure 5. Evolution of polyamines during the development and ripening of eggplant fruits, from the third day after petal fall: ($-$) putrescine; ($\cdots$) spermidine. $LSD_{0.05} = 3.37$.

and the beginning of the exponential growth rate phase. At the end of this stage, a slight increase in total sugar content was observed ($P < 0.05$).

From days 3 to 13 glucose and fructose contents increased by 60% and 40%, respectively (Figure 4). Sucrose and maltose contents remained almost constant throughout the growing period with average concentrations of 0.08 ± 0.01 and 0.05 ± 0.01 g/100 g, respectively.

Polyamines Content. Two free polyamines (putrescine and spermidine) were detected during eggplant development and ripening. Other polyamines such as spermine, cadaverine, or agmatine were not detected in any stage. Very low polyamine concentrations were observed during the development of eggplant fruits cv. Black Nite. Levels were between 2 and 20 nmol/g of fresh tissue (Figure 5). In contrast, for other fruits such as peppers (Serrano et al., 1995), cherimoya (Escribano and Merodio, 1994), and pears (Toumadje and Richardson, 1988) the levels were much higher, about $10-100$ times greater than our results with eggplant.

From days 3 to 5, putrescine and spermidine concentrations were the same (1.67 ± 0.20 nmol/g of fresh tissue), and then putrescine increased around 3 times by day 7. Maximum putrescine levels ($P < 0.05$) of 17.44 ± 0.74 nmol/g of fresh tissue were measured on the ninth day, followed by a large decrease at day 11. This abrupt rise in putrescine levels coincides with the start of expansion of fruit weight and volume. The following decrease could be associated with the ripening process.

Spermidine levels tripled by the ninth day, although this increase was not significant ($P > 0.05$). The final concentration values were similar to those of putrescine.

In summary, eggplant fruit cell expansion started after the ninth day, as revealed by the quick increase of fruit weight and volume. At that moment the content of polyamines, particularly putrescine, was maximum. In parallel with these changes, there was an increase in CO_2 production and a significant increase in total sugar concentration. The concentration of polyamines was low during the first stage of active cell division ($3-7$ days after petal fall) and then increased, particularly putrescine, when cell expansion phase started (ninth day), arriving at a maximum and later decreasing.

These results differ from those found in climacteric fruits such as avocado, where high levels of putrescine and spermidine were found during the first phase of

development (Winer and Apelbaum, 1986). Similar results were obtained by Toumadje and Richardson (1988) for pears where free polyamine levels in the unripe fruits were high over the cell division period and diminished during growth, as has also been observed in peppers (Serrano et al., 1995). In eggplant fruits, polyamine levels diminished during the exponential growth phase and ripening stage.

Kakkar and Rai (1993) suggested that putrescine was directly involved in cell division. They cited work by Teitel et al. (1985) who found a correlation in tomatoes between high levels of putrescine and cell division in the early growth stages. In avocado and tomato an inverse relationship between polyamine levels and ethylene production was observed. We found no relationship between the decrease in polyamine concentration and the production of ethylene in eggplant fruit. Ethylene rates were very low and did not change during the period associated with ripening. On this account, eggplants behaved as nonclimacteric fruits.

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