Pergamon

PII: S0031-9422(96)00192-6

Phytochemistry, Vol. 43, No. 2, pp. 323–326, 1996
Published by Elsevier Science Ltd
Printed in Great Britain
0031-9422/96 $15.00 + 0.00

POLYAMINES, ABSCISIC ACID AND ETHYLENE PRODUCTION IN TOMATO FRUIT

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(*Received in revised form* 22 *February* 1996)

Key Word Index—*Lycopersicon esculentum*; Solanaceae; tomato development and ripening; slow-ripening cultivar; fast-ripening cultivar; polyamines; abscisic acid; ethylene.

Abstract—Tomato fruit of the fast-ripening cultivar Bretón had higher rates of ethylene production than the slow-ripening cultivar Daniela. The initial abscisic acid values were high in both cultivars, but fell during the first six weeks of development. After this time the levels increased, reaching a maximum before ethylene production began to increase. This maximum was much more pronounced in the fast-ripening cultivar. The polyamine/ethylene ratio also differed, with the slow-ripening cultivar having higher levels of putrescine and lower levels of ethylene. In this cultivar, putrescine was the major polyamine throughout, and it increased sharply during the ripening stage. Spermidine levels, on the other hand, decreased gradually, especially during the first two weeks of development. Published by Elsevier Science Ltd

INTRODUCTION

It is well established that ethylene plays an important role in the ripening process of climacteric fruit. It has been suggested that other regulators such as abscisic acid (ABA) and the polyamines, putrescine (Put), spermidine (Spd) and spermine (Spm) might also be important in fruit development and ripening, and attempts have been made to establish the exact role of each and their relation with the ethylene action mechanism [1–3]. In non-climacteric fruit, the polyamines and ABA might play a much more important role because of the lesser importance of ethylene in their development and ripening.

The change in concentration of ABA during fruit development differs according to theories. In some cases, ABA concentration increases as the fruit develops, as in 'Jonagold' apple and avocado [1]. On the other hand in acid lime, sweet cherry and mandarin, ABA levels are high during the first stages of development and then fall, remaining low until the fruit ripen [3–5]. As regards the relationship between ABA and ethylene, any interaction there might be is far from proved, and there are contradictory views. It has even been suggested that both hormones act independently and that ABA has nothing to do with the ripening process [6].

Both polyamines and ethylene have a common intermediate, *S*-adenosylmethionine (SAM), which is a substrate for 1-aminocyclopropane-1-carboxylic acid (ACC) synthase in ethylene synthesis and a substrate for SAM decarboxylase in a pathway which leads to the formation of the polyamines Spd and Spm from Put, by consecutive addition of aminopropyl groups from decarboxylated SAM [2]. In general, ethylene has multiple effects associated with senescence; it initiates fruit ripening, induces chlorophyll loss in leaves, and promotes senescence [7–9]. Polyamines, on the other hand, can be considered as senescence inhibitors. They inhibit the rise in RNase, protease and peroxidase, reduce the rate of senescence of leaf protoplasts and induce DNA synthesis and mitotic activity [2, 10]. In avocado, apple, pear and tomato cv Rutgers fruit, free polyamine levels fall during fruit development [11–15], although they increase in mandarin, Shamouti orange, and tomato landrace Alcobaca containing the recessive allele alc, during fruit maturation and ripening [16–18].

In this work, we study variations in free polyamines, ABA and ethylene, and their possible relationship with differences in the ripening patterns of two tomato cultivars (cv Bretón and cv Daniela, fast-ripening and slow-ripening, respectively).

RESULTS AND DISCUSSION

Fruit growth

A study of the development of the fruit from the two cultivars led us to divide fruit development into four

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physiological stages: a) the first two weeks after fruit set, during which growth occurs through cell division; b) from two weeks after fruit set until the ninth week, during which fruits grow through cell expansion in both Daniela and Bretón cultivars; c) the beginning of ripening, when the fruit changes from green to red (10 weeks after fruit set); and d) the over-ripening stage, when senescence begins (11 weeks after fruit set in Bretón and 12 weeks after fruit set in Daniela).

Physical–chemical parameters associated with ripening

In Bretón tomatoes, firmness and acidity began to decrease 9 weeks after fruit set and continued to do so until the fruit were totally ripe. These changes began after 10 weeks and were less pronounced in Daniela, thus explaining its denomination as slow-ripening (Table 1). The optimum time for picking was 10 weeks after fruit set for Bretón tomatoes and 12 weeks for Daniela, a time at which firmness and acidity values were similar in both cultivars (Table 1). At 12 weeks, Daniela tomatoes were just beginning to change colour, while Bretón tomatoes were already pink.

Ethylene production

Both cultivars exhibited a climacteric rise in ethylene production (Fig. 1), which began during the last phases of fruit development and coincided with the beginning of ripening. However, it is of interest that maximum ethylene production occurred 11 weeks after fruit set in Bretón tomatoes with high rates of evolution (8.0 nl g^{-1} hr^{-1}) while in Daniela, ethylene production peaked between weeks 9 and 11, and with lower values (maximum around 3.0 nl g^{-1} hr^{-1}).

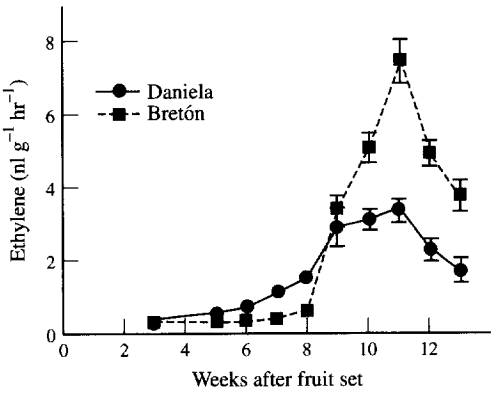

Fig. 1. Evolution of ethylene production in Daniela (●) and Bretón (■) tomatoes during development and ripening. Mean±s.e. of the separate measurements made with 20 fruits.

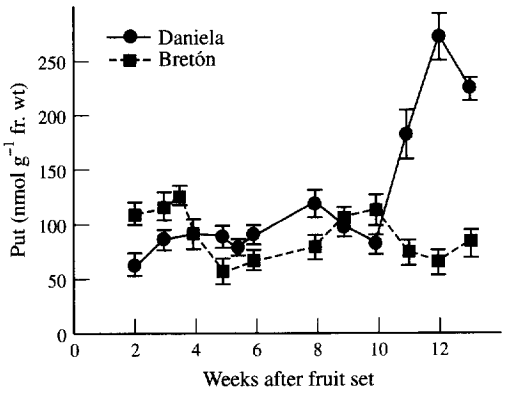

Fig. 2. Putrescine levels in Daniela (●) and Bretón (■) tomatoes during development and ripening. Mean±s.e. of three extractions made in a homogeneous mixture from 20 fruits (each extract was quantified in duplicate).

Polyamine levels

Put, Spd and Spm were present in the pericarp of both cultivars, although Spm was only just detectable. The levels of Put varied between 50 and 100 nmols g^{-1} fr. wt during the first eight weeks from fruit set. After the 10th week these levels fell slightly in Bretón but increased 2.5 fold in Daniela (Fig. 2). Spd levels evolved in a similar way in both cultivars, decreasing during the first five weeks of development from 90 nmol g^{-1} fr. wt to 40 nmol g^{-1} fr. wt. These values remained constant until the 12th week after fruit set before falling to 15 nmol g^{-1} fr. wt in both varieties. There was a correlation between Spd levels in tomato mesocarp and the rate of cell division. At fruit set, when cell division occurs, high levels of Spd were found and these levels diminished as the fruit matured and the rate of cell division decreased, as has been found in other fruits, such as avocado, apple and pear [11–14].

Table 1. Fruit firmness and titratable activity of Bretón and Daniela tomato fruits during ripening

Weeks after fruit set	Firmness* (kg cm^{-2})		Acidity† (g 100 g^{-1})	
	Bretón	Daniela	Bretón	Daniela
8	17.5±0.4	21.0±0.6	1.11±0.10	0.90±0.05
9	16.3±0.5	19.1±0.7	0.92±0.05	0.86±0.01
10	13.8±0.3	17.0±0.5	0.70±0.06	0.82±0.03
11	11.2±0.2	15.8±0.3	0.68±0.03	0.74±0.03
12	10.0±0.2	13.9±0.2	0.68±0.02	0.65±0.01

*Results are given as mean±s.e. of two measurements per fruit for 20 fruits.
†Results are given as mean±s.e. of the separate measurements made on 20 fruits.

Daniela shows higher free Put levels and lower climacteric ethylene production in ripening fruits than Bretón (Figs 1 and 2). The fruits of Daniela also ripen more slowly and keep longer than the fruits of Bretón (Table 1). Since the application of polyamines (Put and Spd) has been shown to inhibit ethylene production in a variety of plant tissue [19] including tomato pericarp [18], the high level of free Put in ripe tissue may be responsible for the long-keeping quality of Daniela fruit. High free Put levels have also been observed in the pericarp of the tomato landrace Alcobaca with the recessive allele alc [18] and in the tomato Liberty [15]. Both these cultivars ripen slowly and have prolonged keeping qualities. Furthermore, when inhibitors of polyamine biosynthesis were applied to pericarps of Liberty, free polyamine levels decreased and ethylene production increased [20]. The high concentration of Put in Daniela may compete for SAM required for ethylene production. Also polyamines may directly inhibit ACC synthase and ACC oxidase which control the last two steps in ethylene production. Thus, the effects of free polyamines and ethylene are interrelated and the high levels of Put in Daniela cultivar may account, at least in part, for the ripening, low ethylene production rate, and storage characteristics of these tomato fruits.

ABA levels

The initial ABA concentration was high in both cultivars (2.5 nmol g^{-1} fr. wt) falling to 1 nmol g^{-1} fr. wt six weeks after fruit set (Fig. 3). Subsequently, in Bretón, the ABA level increased five-fold during weeks 7 and 8, reaching values of 4.2 nmol g^{-1} fr. wt and then fell sharply (Fig. 3). However, these levels only increased two-fold in Daniela between weeks 7 and 9, reaching a value of 1.5 nmol g^{-1} fr. wt (Fig. 3). These results point to the marked differences between the cultivars. The decrease in ABA was extremely marked in Bretón tomatoes, in which the ABA content fell by 3.8 nmol g^{-1} fr. wt in only two weeks, while it fell by only 1 nmol g^{-1} fr. wt in Daniela over four weeks.

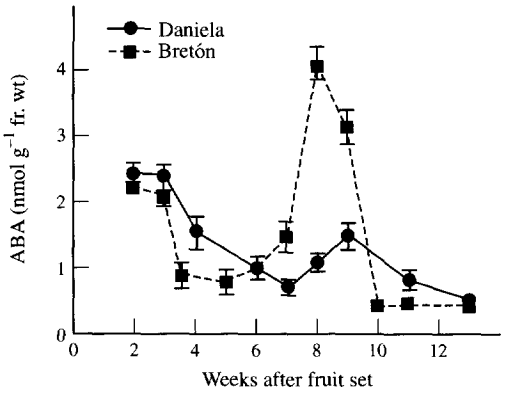

Fig. 3. ABA content in Daniela (●) and Bretón (■) tomatoes during development and ripening. Three extractions were made from a homogeneous mixture of tissue from 20 fruits. Four dilutions were made of each extract and each dilution was quantified in duplicate. Data are mean±s.e. of these 24 measurements.

We have previously suggested that the effects of free polyamines and ethylene are interrelated. Similar conclusions are reached when the abscisic acid and its relation with ethylene production were analysed. In both cultivars, ABA levels increased before the rise in ethylene production took place, although this increase in ABA was greater in Bretón than Daniela (Fig. 3), as was the increase in ethylene production (Fig. 1). These results suggest that ABA might act as a promoter of ethylene biosynthesis, the interaction of both hormones stimulating fruit ripening and senescence. This suggestion is supported by studies of the response of tomato pericarp sections subjected to exogenous ABA [21] and studies of fruit development and ripening in sweet cherry, avocado and apple [3].

In conclusion, we think it is safe to suggest that high levels of ABA and ethylene production and low levels of Put are associated with rapid ripening, as occurs in the climacteric cultivar (cv Bretón). This hypothesis is supported by the comparatively low levels of ABA and ethylene recorded in the long-keeping cultivar (Daniela), in which, furthermore, the concentration of Put rose during ripening. The balance between these three plant development regulators could be responsible for the different pattern of ripening observed in each cultivar studied.

EXPERIMENTAL

Plant material. Twenty fruits were harvested weekly after fruit set from greenhouse-grown tomato plants (*Lycopersicon esculentum*) cv Bretón and cv Daniela, in Murcia (Spain). Plants were grown under the same conditions of light, temp. and nutrition. The day on which fruit set was induced by pollination of flowers by bumble bees was considered as day 0. The following determinations were made.

C_2H_4 *production* rate was measured by placing each fruit in a 0.5-l glass holder. 1 ml of holder atmosphere was extracted after 1 hr and the C_2H_4 was quantified using a GC equipped with flame ionization detector, and a 3-m stainless steel column with an inner diameter of 3.5 mm containing activated alumina of 80/100 mesh. Ethylene was expressed as C_2H_4 given off per g of tissue per hr (nl g^{-1} hr^{-1}).

Fruit firmness was determined with a crosshow penetrometer (ROZE Paris), fitted with a 3-mm diameter probe. 2 readings were made on opposite sides of each of 20 fruits. The results are expressed in kg cm^{-2}.

Acidity was determined by potentiometric titration with 0.1 N NaOH up to pH 8.1 using 1 ml of diluted juice in 25 ml H_2O. The results are expressed as g malic acid per 100 g fr. wt.

The pericarp of the 20 fruits collected each week was cut into small pieces, to obtain a homogeneous sample, and frozen in liquid N_2 before being lyophilized and

stored at −20° until the polyamines and ABA were analysed.

Polyamine analysis. Since only free, but not conjugated, polyamines act as endogenous antisenescence agents [10], only free polyamines were analysed. Extracts of pericarp tissue for polyamine analysis were prepared according to ref. [22]. 1,6-Hexanediamine (100 nmol g^{-1} fr. wt of pericarp tissue) was added as int. standard. The homogenate was then centrifuged for 30 min at 20 000 *g* and free polyamines in the supernatant fr. were analysed by the benzoylation method as previously described [23]. Derivatives were analysed by HPLC. The elution system consisted of MeOH–H_2O (16:9) as solvent, run isocratically with a flow rate of 0.8 ml min^{-1}. The benzoyl-polyamines were eluted through a reversed-phase column (LiChroCart 250–4,5 μm) and detected by *A* at 254 nm. A relative calibration procedure was used to determine the polyamines in samples, using 1,6-hexanediamine as the int. standard and standard curves for Put, Spd and Spm. Three extractions of polyamines were made from each sample and each extraction was quantified by the benzoylation method and HPLC in duplicate. The results represent the mean±s.e. of these 6 quantifications.

Determination of abscisic acid. All manipulations were carried out in dim light. ABA was extracted and quantified as previously reported [24]. The extracts were diluted with a 50 mM Tris buffer soln, pH 7.8, containing 1 mM $MgCl_2$ and 150 mM NaCl, and then quantified by an enzyme-linked immunosorbent assay (ELISA), using IgG monoclonal antibody. ABA–bovine serum albumin conjugate was synthesized according to ref. [25]. The ABA content was estimated by comparison with the standard curve prepd for each plate. Three extracts were made from each sample. For each extract, 4 dilutions were prepd (which were quantified in duplicate) and at least 3 of them fell onto the standard curve. The ABA levels were consistent with the dilution made. No interference from impurities was detected when ABA standards were added to diluted extracts of fruits at different stages of growth and maturity.

Acknowledgements—The authors would like to thank I. Hita for his technical assistance. This study was funds by the Comisión Interministerial de Ciencia y Technología, Project ALI 95-0134.

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