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Relationship between Stress Ethylene Production Induced by Gamma Irradiation and Ripening of Cherry Tomatoes

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Additional index words. ACC synthase, ethylene-forming enzyme, ACC, malonyl ACC, synchronization of ripening, *Lycopersicon esculentum*

Abstract. **Changes in ACC metabolism induced by gamma irradiation have been studied during ripening of cherry tomatoes** *(Lycopersicon esculentum* **L. cv. Sweet 100) treated at the mature-green stage. Irradiation caused a sharp and transient dose-dependent increase in ethylene production during the first 24 hours that was associated with an increase in ACC synthase activity. The activity of ethylene-forming enzyme (EFE) was also stimulated but was never limiting. Nine days following irradiation, ACC metabolism was more active in irradiated fruits, with ACC being mainly directed to ethylene [80% at 3 kilogray (kGy; 1 Gray = 100 Rad = 1 J·kg-[1])] rather than to malonyl ACC (MACC). As a consequence, fruit ripening was accelerated. For doses <1 kGy, the time required for 50% of the fruits to reach breaker stage (the onset of climacteric ethylene production) was inversely correlated with radiation dose and the amount of stress ethylene produced during the first 24 hours. At doses >1 kGy, in spite of a continuous stimulation of stress ethylene production, no additional acceleration of ripening occurred. At 3- to 5-kGv doses. fruit ripening was impaired transiently with a fast subsequent recovery. As a result a significant synchronization of fruit ripening (presumed to be caused by enhanced ethylene production) was observed. Chemical, names used: l-amino-cyclopropane-1-carboxylic acid (ACC).**

Climacteric fruits submitted to gamma irradiation exhibit either a delay of ripening (Akamine and Moy, 1983; Urbain; 1986; Thomas, 1988) or an advance (Maxie et al.,. 1966), depending on the fruit species and the developmental stage at which the treatment is applied. In the specific case of tomatoes, irradiation generally delays ripening when the treatment is applied at the preclimacteric stage (Abdel-Kader et al., 1968; Lee et al., 1968; Thomas, 1988), while it can cause an advance when it is applied earlier (Lee et al., 1968). These differential responses may be ascribed to the dual effects of gamma rays: i) impairment of cellular functions and therefore of ripening, with possible recovery (McGlasson and Lee, 1971; Romani et al., 1968); and ii) stimulation of the synthesis of ethylene (Abdel-Kader et al., 1968), which is known to stimulate fruit ripening (Brady, 1987; McGlasson, 1985).

In a previous study, we have demonstrated that gamma irradiation strongly and rapidly activated ACC synthesis at the post-transcriptional level, in breaker cherry tomatoes (Larrigaudiere et al., 1990). The present work was undertaken to determine whether the stress ethylene being produced immediately after the treatment could play a role in the onset and advancement of ripening of preclimacteric fruits. The effects of various doses of gamma irradiation on the stimulation of ACC metabolism (ethylene production, ACC and MACC levels, ACC synthase, and EFE activities) on mature-green cherry tomatoes were investigated on a short- and long-term basis.

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Materials and Methods

Plant material and irradiation treatment. 'Sweet 101' cherry tomato plants (F_1 from INRA, France) were grown in a greenhouse (25/18C, day/night) and fruits were harvested at the mature-green stage. They were then selected according to their chromametric value and their ethylene production according to Guclu et al. (1989) with the modifications described below. Fruit color was determined on the calycinal face of the fruit, using a chromameter (CR **200,** Minolta, Japan), by measurement of the 'a' value in the L, a, b system. Previous analysis and correlations with ethylene production, taken as a marker of ripening, have shown that the 'a' value was sufficient to characterize maturity stages; a = – 10, immature; a = - 8.0, mature-green; – 6.0 < a < –2, breaker; a = 14.0, orange stage. Preclimacteric tomato fruits were selected at a fully mature-green stage (a = – 8.0 ± 0.5).

Irradiation treatment was carried out with a ^{60}Co irradiator [Oris, France 30000 Ci (1 Ci = 37 GBq]). All experiments were conducted at 20C at a dose rate of 110 Gy·min-1. Irradiated and control fruits were held in a controlled-temperature room at 20C at 80'% relative humidity.

Determination of the onset of ripening. An individual fruit was considered as having reached the onset of ripening, or breaker stage, when the 'a' was higher than – 6 and the ethylene production rate >0.15 nmo·gl·.h-1. The days to 50% ripening corresponded to the time required for half of the fruits (30 fruits total) to reach the breaker stage.

Ethylene production. Each of the 30 fruits was placed in a 100-ml flask that was sealed with a serum cap. A l-ml gas sample was withdrawn after 1 h incubation and analyzed by a gas chromatography using an activated alumina column and a flame ionization detector.

Determination of ACC and MACC. ACC and MACC were

Abbreviations: EFE, ethylene-forming enzyme.

analyzed on the same crude extract that was prepared for ACC synthase assay. The ACC analysis was carried out on six fruits by oxidative conversion into ethylene (Lizada and Yang, 1979). For the determination of MACC, the sample was first passed through a Dowex 50-W (H^+, 100-200 mesh) column to remove free ACC. MACC in the eluate was then hydrolyzed into ACC and quantified (Liu et al., 1985b).

Enzyme extractions and assays. ACC synthase extraction and activity determination were carried out according to Acaster and Kende (1983) with slight modifications as described by Larrigaudiere et al. (1990). EFE activity was determined in the whole fruit by measuring the conversion of vacuum-infiltrated ACC to ethylene in the presence of cycloheximide (Larrigaudiere et al., 1990). All enzyme activities were determined on three separate fruits.

Results

Radiation-induced changes in ACC metabolism during ripening of cheny tomatoes treated at the mature-green stage. The 3-kGy response is given as an example; similar patterns were obtained for 1 and 5 kGy. Irradiation caused a transient and rapid increase in ethylene production within the first few hours after treatment (Fig. 1A), with a maximum at 4 h. After 9 days, irradiated fruits exhibited a climacteric-like rise in ethylene production that was more pronounced than that of untreated fruits (Fig. 2A). These changes in ethylene production were accompanied by parallel changes in ACC level (Figs. lB and 2B) and by a slow but continuous increase in MACC content of irradiated fruits (data not shown).

ACC synthase activity of gamma-irradiated tomatoes increased sharply within the first few hours following irradiation (Fig. IC). EFE activity was stable during the same period (data not shown) but was also stimulated within 2 days (Fig. 2D). Thereafter, both ACC synthase and EFE activities remained higher than in control fruits (Fig. 2 C and D, respectively). EFE activity was not limiting for ethylene production in treated or control fruits. At their lowest points, for instance, EFE activity was 1.20 and 0.5 nmol·g-1 -h 1 while ethylene production was only 0.7 and 0.2 nmo·g^{-1}·h^{-1} in treated and control fruits, respectively.

The metabolic flow through the ACC pathway was analyzed as a function of the irradiation dose for 15, days after treatment (Fig. 3). The total ACC produced during this period was calculated from ethylene production integrated during the period and from ACC and MACC levels at the end of the period. ACC flow increased with doses up to 3 kGy and ACC conversion into ethylene was prominent, being 90%, 80%, and 70% of total ACC at 1,3, and 5 kGy, respectively. The amount of ACC converted into MACC was low.

Ripening as a function of stress ethylene production and radiation dose. Fruits irradiated at doses lower than 1 kGy exhibited stress ethylene production that was almost linearly correlated to the dose (Fig. 4). The time required for 50% of the fruits to ripen was negatively correlated with stress ethylene production (Fig. 4).

When higher doses of radiation (up to 5 kGy) were applied (Fig. 5), stress ethylene production further increased almost linearly. However, the time for 50% ripening stopped declining beyond 1 kGy (stress ethylene production: 2 nmoI/g fresh weight per 24 h) and increased to be almost equivalent to untreated fruits for 5 kGy (stress ethylene production: 6 nmol/g fresh weight per 24 h).

Synchronization of ripening. The time course for ripening, as estimated by the percentage of fruits reaching the breaker stage, was studied in a population of 30 mature-green tomatoes treated with 1, 3, or 5 kGy and compared with untreated fruits. Figure 6 clearly shows that the 3- and 5-kGy treatments induced a more synchronous ripening, with >50% of fruit ripening between days 9 and 12 than 0 or 1 kGy. In control or 1 kGy-treated fruits, the ripening period was longer, although some fruits started to ripen earlier when treated with 0 than with 1 kGy.

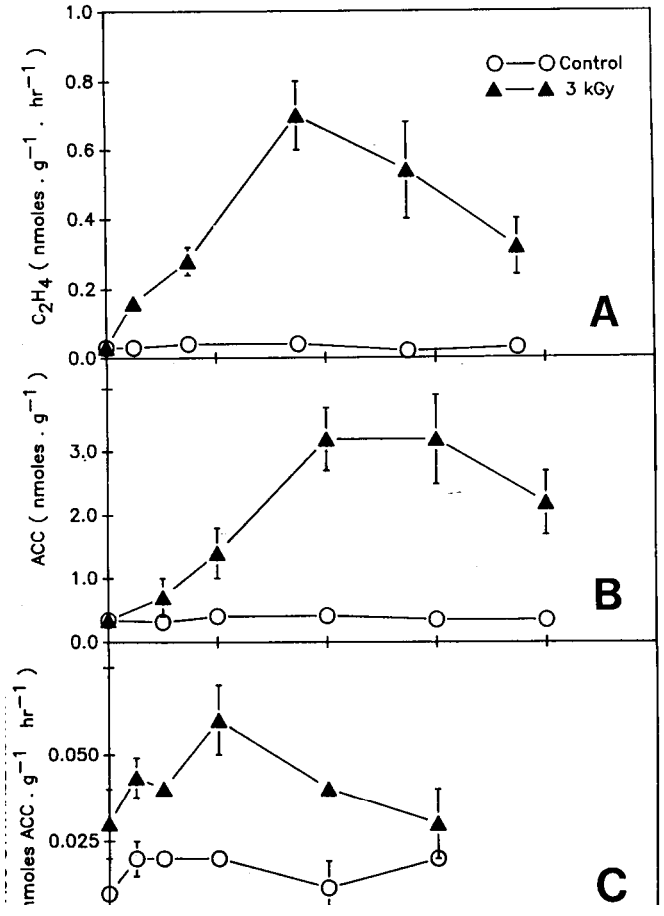

Fig. 1. Short-term evolution of C_2H_4 production **(A),** ACC levels (B), and ACC synthase activity (C)in mature-green cherry tomatoes exposed to O (control, O) or 3 kGy of irradiation (A). Fruits were kept at 20C and 80% RH. For ethylene production **(A),** values represent means ±SD of 30 fruits. Other results are mean ±SD of six and three replicates for ACC levels (B) and ACC synthase activity (C), respectively. Vertical bars represent SD; when omitted, **SD** is lower than symbol size.

Discussion

Ethylene production of mature-green cherry tomatoes exposed to gamma irradiation increased sharply during the first 24 h. This stimulation was dose-dependent and was associated with an increase in ACC synthase activity. Our previous data had shown that, in the case of cherry tomatoes at the breaker stage of ripeness, a post-transcriptional stimulation of ACC sythase activity occurred that was responsible for the rapid increase in ethylene production during the first 15 min after irradiation (Larrigaudiere et al., 1990). Such fast stimulation was not observed in mature-green tomatoes (Fig. 1), suggesting

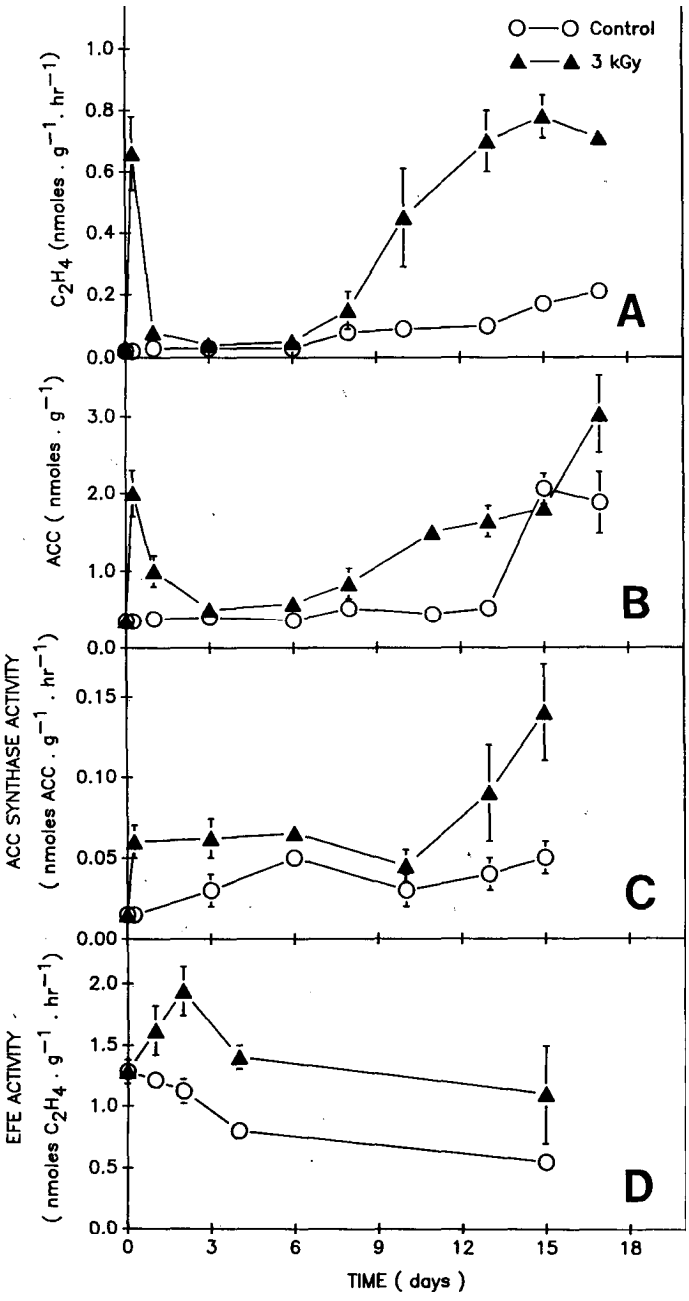

Fig. 2. Evolution of C^2H, production (A), ACC levels (B), ACC synthase (C), and EFE (D) activities during ripening of mature-green cherry tomatoes exposed at time zero to 3 kGy irradiation (A) or untreated (0). Conditions for fruit storage and statistical analysis are the same as in Fig. 1; results for EFE activity (D) represent mean ± SD of three replicates.

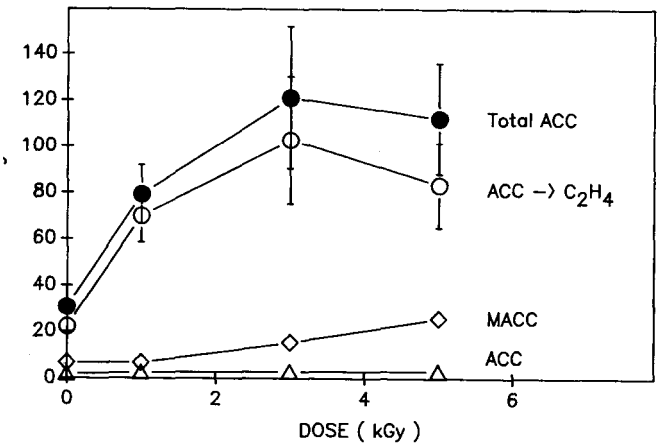

Fig. 3. Dose response of the metabolic flow through the ACC pathway following radiation treatment. Total ACC being produced within 15 days by the fruit **(0);** ACC converted into ethylene (0), ACC (A) and MACC (O) levels in the tissue at the end of the period. Conditions for fruit storage and statistical analysis are the same as in Fig. 1.

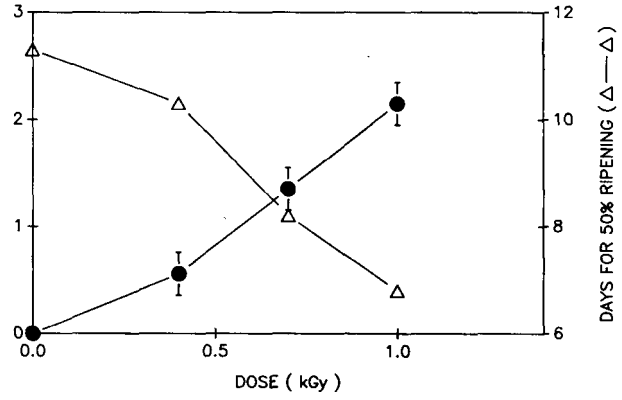

Fig. 4. Dose dependence (<1 kGy) for stress ethylene production and its relationship to ripening. Stress-ethylene was determined by measuring ethylene production during 24 h following irradiation and by correction from the production of unirradiated fruits. The day for 50% ripening was calculated for each dose when 50% of fruits had reached the breaker stage ('a' value higher than – 6), i.e., the onset of climacteric ethylene production (>0.15 mmol·g-). Values of stress ethylene production represent means ±SD of six replicates, from a fruit population of 30 fruits used to determine ripening behavior. For conditions of storage, see Fig. 1.

that the corresponding preformed RNAs were not yet present at this stage. The stress ethylene production observed in the present work during the first 24 h therefore probably resulted from transcriptional activation of ACC synthase.

Ripening induction by ethylene is a well-known process (Brady, 1987; McGlasson 1985) that leads to the production of "autocatalytic ethylene" via the stimulation of ACC synthase and EFE activity (Bufler, 1986; Liu et al., 1985a). Irradiated mature-green tomato fruits exhibited accelerated ripening. This phenomenon has already been described but not explained in preclimacteric tomatoes (Lee et al., 1968). Therefore, it seems that the effect of radiation-induced stress ethylene triggers autocatalytic ethylene production. Indeed, the ACC metabolism of irradiated fruits remains very active after the stress response (Fig. 2). ACC is mainly converted to ethylene, probably as a result of the high EFE activity, and only at a low level of MACC (Fig. 3). Further, this hypothesis is substantiated by the quantitative relationship obtained between the rate of ripening and the amount of stress ethylene being produced during the first 24 h for fruits irradiated below 1 kGy (Fig. 4). At higher radiation doses (3 and 5 kGy), in spite of the continuous enhancement of stress ethylene production, ripening was not accelerated (Fig. 5). This result may be caused by the well-known action of gamma rays on DNA structures and by its consequences on protein synthesis and overall metabolism of the cell (Casarett, 1968; Errera and Forssberg, 1961). However, damage maybe repaired (McGlasson and Lee, 1971; Romani et al., 1968). After recovery from radiation damage, a synchronization of fruit ripening occurred (Fig. 6), probably due to a stimulation by enhanced stress ethylene.

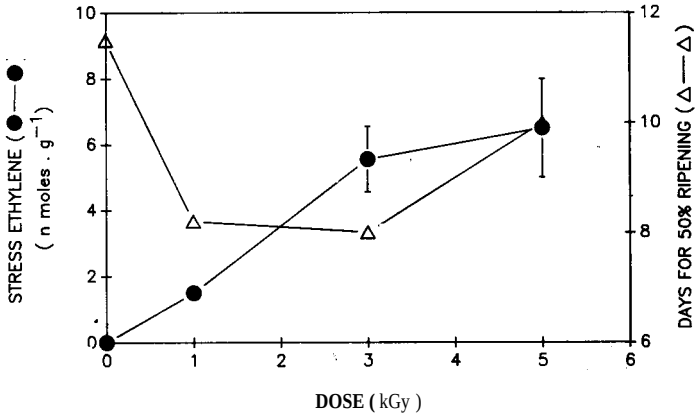

Fig. 5. Dose dependence (up to 5 kGy) for stress ethylene production and relationship with ripening. Conditions for fruit storage and statistical analysis are the same as in Fig. 1.

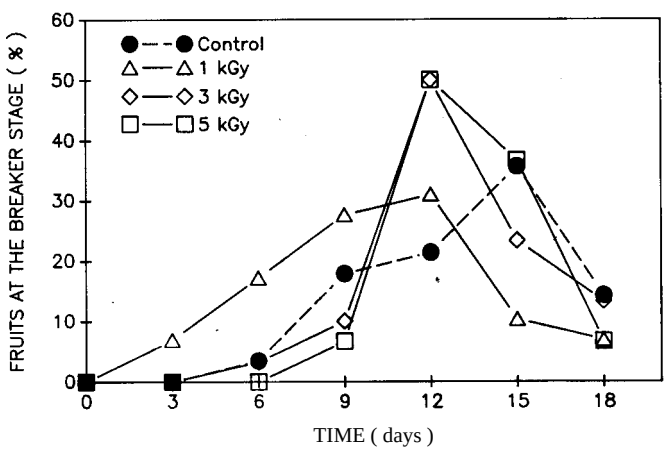

Fig. 6. Time course of ripening of mature-green cherry tomatoes, untreated (.) or irradiated at 1 kGy (A), 3 kGy (O), or 5 kGy (u). - The onset of ripening was characterized for each fruit when the color 'a' value was higher than – 6.0 (breaker stage). For conditions of storage, see Fig. 1; analysis on populations of 30 fruits.

These data, together with our recent work on the short-term effects of gamma irradiation on cherry tomatoes at the breaker stage of ripeness (Larrigaudiere et al., 1990), give a metabolic explanation of the earlier observation that gamma rays stimulate ethylene production in climacteric tomato fruits (Abdel-Kader et al., 1968; Lee et al., 1968). Our results demonstrate that this stimulation occurs within a short time via the translation and/or transcriptional stimulation of ACC metabolism, depending on the stage of fruit development. We further demonstrated that ACC metabolism was also stimulated over a long period, and, as a consequence, fruit ripening was accelerated. At doses <1 kGy, the advance of ripening was correlated with radiation dose and intensity of stress ethylene production. At higher doses, further enhancement of stress ethylene production occurred, which resulted in. a better synchronization of fruit ripening but not in additional acceleration. We conclude that the early stress response induced during the first 24 h by gamma rays is responsible for the advance and/or synchronization of ripening via the stimulation of autocatalytic ethylene production.

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