Role of ethylene on various ripening pathways and on the development of sensory quality of Charentais cantaloupe melons

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Abstract

Charentais melons (*Cucumis melo* L., var *cantalupensis* Naud.) in which ethylene biosynthesis has been suppressed by an antisense ACC oxidase gene have been used to better understand the role of ethylene in the regulation of the ripening process of climacteric fruit and on the development of sensory qualities. We have shown that a number of biochemical and molecular processes associated with the ripening of climacteric fruit are ethylene-independent. In some cases, such as softening of the flesh, the same pathway comprises both ethylene-dependent and -independent components. The various ethylene-dependent events exhibit differential sensitivity to ethylene. The threshold level for degreening of the rind is 1 ppm, while 2.5 ppm are required to trigger the ethylene-dependent component of the softening process. The saturating level of ethylene for all these events is less than 5 ppm, which is by far lower than the internal ethylene concentrations found in the fruit at the climacteric peak (around 100 ppm). Detachment of the fruit influences the development of respiratory climacteric. Fruit remaining attached to the vine, although producing higher levels of ethylene, exhibit a reduced climacteric rise in respiration as compared to detached fruit. The response of antisense ACO fruit to exogenous ethylene in terms of respiration is higher in detached than in attached fruit. Ethylene-suppressed melons show a severe reduction of aroma volatiles production, particularly in ester production. In the biosynthetic pathway of aliphatic esters, the dehydrogenation of fatty acids and aldehydes appears to be ethylene-dependent. In contrast, alcohol acetylation comprises ethylene-dependent and ethylene-independent components, probably corresponding to differentially regulated alcohol acetyl transferases. In terms of sensory quality, these data show that the extension of shelf-life through the inhibition of ethylene production has some beneficial effects on texture and sugar accumulation but is detrimental for the generation of aroma.

INTRODUCTION

The ripening of fleshy fruit involves a series of organoleptic changes in texture, aroma and colour that make fruit attractive to the consumer. These changes correspond to biochemical and physiological events that are genetically programmed and that involve the regulated expression of specific genes. Although these processes vary from one type of fruit to the next, fruit can be divided into two broad groups: climacteric and non-climacteric. The classification into one group or the other depends on whether or not a fruit exhibits a peak in respiration and ethylene production during ripening. Autocatalytic ethylene production is a major feature of climacteric fruit (McMurchie et al., 1972) as compared to non-climacteric types, where ethylene is not considered as essential. Among melon cultivars, some are climacteric others are non-climacteric (Kendall and Ng, 1988). The traditional Charentais cantaloupe melon has a typical climacteric behavior with a sharp respiratory peak during ripening and a short shelf life. It exhibits good sensory qualities, especially in terms of aroma volatiles production.

In the last ten years, the function of a number of ripening-related genes has been elucidated by altering their expression in transgenic plants (Giovanonni, 2001). However, most of these studies have been carried out using tomato as a model fruit. The value of melon as an alternative model system to study fruit ripening has been demonstrated in the studies of ethylene biosynthesis (Ayub et al., 1996) and action (Sato-Nara et al., 1999), the mechanisms of cell wall disassembly (Rose et al., 1998) and the search for novel ripening-regulated genes (Hadfield et al., 2000). A review on melon biotechnology was recently published (Guis et al., 1998). Our group has generated antisense ACC oxidase Charentais melons that exhibit almost complete inhibition of ethylene production (Ayub et al., 1996). Using this transgenic material, we have been able to show that melon fruit ripening involves ethylene-dependent and -independent pathways (Guis et al., 1997). In this paper, we will review some of our recent data on the sensitivity to ethylene of various ripening pathways, the effects of detachement on the respiratory climacteric and the biosynthesis of aliphatic esters.

THE VARIOUS RIPENING PATHWAYS OF MELON EXHIBIT DIFFERENTIAL SENSITIVITY TO ETHYLENE

The use of ethylene-suppressed, antisense ACC oxidase melons (AS) has shown that the ripening of climacteric fruit has both ethylene-dependent and -independent pathways (Guis et al., 1997). More recently, Flores et al. (2001a) demonstrated that certain individual events of the ripening process, such as softening and membrane deterioration, also have ethylene-independent and -dependent components. In contrast, degreening of the rind and cell separation in the peduncular abscission zone are totally dependent upon ethylene.

The sensitivity to exogenous applications of ethylene of individual components of the ripening process has been evaluated (Table 1). Ethylene treatment of AS fruit induced colour changes in the rind with no lag phase at very low levels of the hormone (<1 ppm). The rate of degreening of the rind was quantitatively related to the level of applied ethylene up to a saturating dose of 5 ppm at which the wild type (WT) phenotype was completely restored within 5 days. However, a threshold level of 2.5 ppm ethylene was required to stimulate changes in flesh firmness, membrane deterioration and cell separation in the peduncular abscission zone. The saturating level of ethylene was at 2.5 ppm for flesh softening and 5 ppm for membrane deterioration and cell separation in the peduncular abscision zone. The cessation of the ethylene treatment resulted in a complete arrest of the fully ethylene-dependent processes (degreening of the rind and cell separation) and a slow-down of the processes that are only partly ethylene-dependent (softening and membrane deterioration). These results indicate that: (i) a hierarchy of sensitivity to ethylene exists between the various physiological pathways of the ripening process; (ii) some individual pathways have both an ethylene-independent and -dependent components; and (iii), the internal ethylene concentration found in WT fruit (over 100 ppm) is more than the saturating level for all of these physiological and biochemical changes.

THE SENSITIVITY TO ETHYLENE OF THE CLIMACTERIC RESPIRATION OF MELON IS AFFECTED BY DETACHMENT OF THE FRUIT

A recent study with Charentais melons found that the ethylene climacteric was not accompanied by a corresponding rise in respiration when fruit remain attached to the plant during ripening (Shellie and Saltveit, 1993). However, Hadfield et al. (1995) found that both ethylene and respiratory climacterics occur as melons ripen on the plant, although ripening is delayed relative to harvested fruit of the same physiological age. To clarify the relationship between attachment and the respiratory climacteric, ethylene production and O_2 consumption were monitored during the ripening melon fruits grown in France and Australia (Bower et al., 2001). All fruit detached from the vine produced both respiratory and ethylene climacterics (Fig. 1). However, although ethylene production increased in attached fruit, the concurrent rise in respiration was either eliminated or reduced in magnitude. When the data from the Australian trial was analysed, the stimulation of respiration for a given rate of ethylene production was less in attached than detached fruit (Bower et al., 2001). The application of exogenous ethylene to AS melons stimulated O_2 consumption only if they were detached from the vine (Fig. 2). These data demonstrate that attachment to the plant inhibits the effects of ethylene on respiration. The inhibition may be mediated by changes in gas permeance, turgor, or the supply of plant factors from the phloem. Its magnitude is affected by environmental factors influencing plant growth and development. The data also support the hypothesis that the respiratory climacteric is not an essential part of ripening, but an artefact caused by stress or detachment.

THE BIOSYNTHESIS OF ALIPHATIC ESTERS IS UNDER PARTIAL CONTROL OF ETHYLENE

Compared to other melon types, cantaloupe Charentais melons are highly aromatic with a major contribution to the aroma being made by aliphatic and branched esters. We have undertaken to determine the steps of the aliphatic ester pathway at which ethylene exerts its regulatory role by comparing AS and WT fruit (Flores et al., 2001b). Our data show that the production of aliphatic esters, such as hexyl and butyl acetate, was blocked in AS fruit and could be reversed by ethylene. Using fruit disks incubated in the presence of various precursors, we have demonstrated that ester formation was inhibited due to a lack of dehydrogenation of fatty acids and aldehydes, the last step of the pathway, acetyl transfer to alcohols, being unaffected. However, treating AS fruit with the ethylene antagonist, 1-methylcyclopropene, resulted in about 50% inhibition of acetyl transfer activity, indicating that this portion of activity was ethylene dependent and was supported by the low, residual ethylene concentration of AS fruit disks (around 2 ppm). In conclusion, the dehydrogenation of fatty acids and aldehydes appears to be essentially ethylene-dependent, while the last step of alcohol acetylation comprises ethylene-dependent and ethylene-independent components, probably corresponding to differentially regulated alcohol acetyl transferases.

CONCLUSIONS

The ripening of melon fruit is a complex developmental process in which ethylene plays a major role. We have used transgenic melons in which ethylene suppression was so high that fruit ripening was greatly affected both on the vine and after detachment. Lowering ethylene production by 70% or 90% was not enough to profoundly modify ripening of tomatoes, especially when kept on the vine (Klee, 1993; Murray et al., 1993) or melons (Clendennen et al., 1999). Residual ethylene was enough to stimulate some ripening events such as polygalacturonase gene expression that were thought to be ethylene-independent (Sitrit and Bennett, 1998). The highly suppressed line of melon that we have used has allowed us to show that truly ethylene-independent pathways are involved in the ripening of climacteric fruit, reminiscent of a non-climacteric regulation. The threshold level of ethylene capable of inducing changes of the ethylene-regulated pathways is variable from 1 to 2.5 ppm and the saturating levels of ethylene capable of producing maximum effect vary from 2.5 to 5 ppm. These levels are by far lower than the ethylene concentration in the fruit at the climacteric peak (around 100 ppm). Fruit attached to the vine exhibit lower sensitivity to ethylene in terms of respiration than detached fruit. The presence of ethylene is essential for the generation of aroma volatiles. In ethylene-suppressed melon, the steps of reduction of acids into aldehydes and aldehydes into alcohols in the aliphatic esters biosynthetic pathway are blocked, while the step of ester formation is reduced by around 50%. These data show that inhibition of ethylene synthesis results in beneficial effects such as a prolonged storage life, a preservation of the flesh texture and an increased sugar content associated with the inhibition of peduncular abscission. However, ethylene suppression is detrimental to the production of aroma volatiles. Due to the high level of inhibition of ethylene in our transgenic line and clearly defined events during fruit ripening (such as a sharp climacteric and production of intense aroma volatiles), Charentais melons appears to be a good alternative to tomatos for elucidating the molecular mechanisms of ripening and development of sensory quality of fruit in general.

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Tables

Table:1 Ethylene dependence of several ripening pathways and characterisitics of ethylene action.

Ripening parameter	Presence of an ethylene-independent component	Characteristics of the ethylene-dependent component	
		Threshold (ppm)	Saturation (ppm)
Flesh softening	Yes	2.5	2.5
Membrane deterioration	Yes	2.5	2.5
Abscission zone detachment	No	2.5	5.0
Rind colour	No	1.0	5.0

Table 2: Production of hexyl and butyl acetate by whole fruit and bioconversion of Na-hexanoate and hexanol by melon disks of WT and AS fruit treated or not with 1-MCP. The biosynthetic route leading to hexyl and butyl acetates involves the following steps : hexanoate → hexanal → hexanol→ hexyl acetate. The conversion of hexanoate into butyrate by beta oxidation leads to butyl acetate through the following pathway : butyrate → butanal→ butanol → butyl acetate.

	Production by whole fruits	Bioconversion of substrates by discs of tissues	
	Hexyl + butyl acetates (µmoles kg^{-1} FW)	Na-Hexanoate → Esters (µmoles h^{-1} g^{-1} FW)	Hexanol → Hexylacetate (µmoles h^{-1} g^{-1} FW)
WT	6.0 ± 2.2	33.0 ± 3.0	31.0 ± 4.0
WT + MCP	nd	0.5 ± 0.05	19.0 ± 2.0
AS	tr	1.5 ± 0.5	34.0 ± 5.0
AS + MCP	nd	1.0 ± 0.01	17.0 ± 1.5
AS + C2H4	8.2 ± 3.0	nd	nd

tr: levels below 0.3 µmole kg^{-1}; nd: not determined

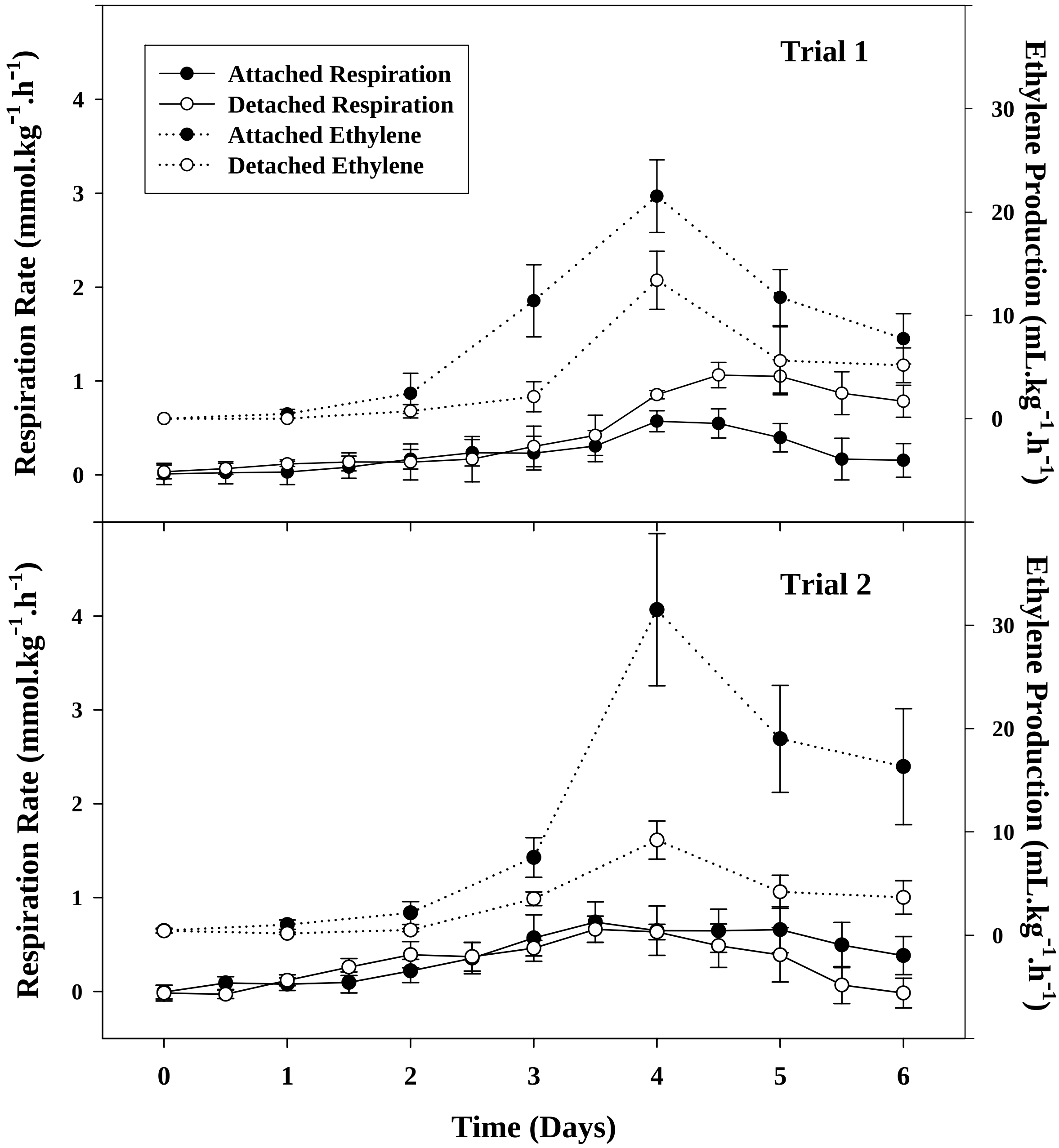

Fig. 1. Respiration rates and ethylene production of Charentais melons during ripening either attached to the vine or removed from the plant. The plants were grown at Toulouse, France (Trial 1) and Richmond, Australia (Trial 2). Each data point represents the mean of three fruit and the error bars are the standard errors of the means.

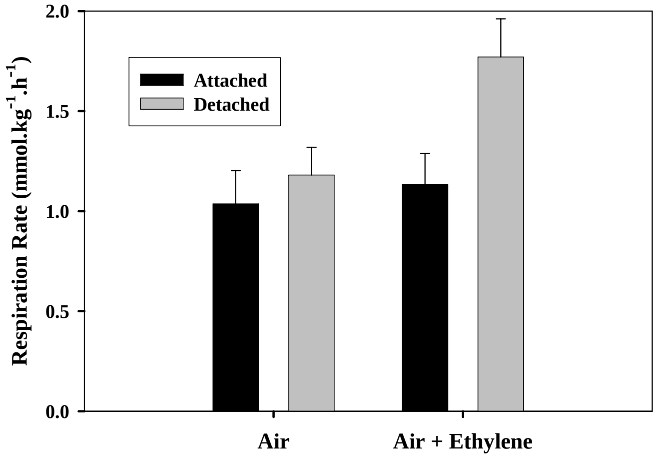

Fig. 2. The effects of an exogenous application (200 ppm) of ethylene on the respiration rate of AS melon fruit either attachment to, or detachment from the vine.