Postharvest Biology and Technology 99 (2015) 27–36

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Characterisation of H_2O_2 production to study compatible and non-host pathogen interactions in orange and apple fruit at different maturity stages

G. Buron-Moles [a], R. Torres [b,*], N. Teixidó [b], J. Usall [b], L. Vilanova [b], I. Viñas [a]

[a] Food Technology Department, Lleida University, XaRTA-Postharvest, Agrotecnio Center, Rovira Roure 191, 25198 Lleida, Catalonia, Spain
[b] IRTA, XaRTA-Postharvest, Rovira Roure 191, 25198 Lleida, Catalonia, Spain

ARTICLE INFO

Article history:
Received 13 March 2014
Accepted 23 July 2014
Available online 21 August 2014

Keywords:
Ecophysiology
Penicillium digitatum
Penicillium expansum
Hydrogen peroxide
Fruit maturity
Wounding

ABSTRACT

Penicillium digitatum and *Penicillium expansum* are the main postharvest pathogens of orange and apple fruit, respectively. These wound pathogens can infect through injuries caused during harvest and postharvest handling, which lead to large economic losses. Susceptibility of fruit to mechanical damage or infection increases during ripening. However, few studies have been focussed on the fruit wound-induced defence responses, such as H_2O_2 production. In this study, the characterisation of H_2O_2 production in orange (*C. sinensis* cv Valencia) and apple (*M. domestica* L. cv Golden Smoothee) fruit in response to abiotic (wounding) and biotic (pathogen and non-host pathogen) stresses at different maturity stages was investigated. The effect of H_2O_2 on the ecophysiology of *P. digitatum* and *P. expansum* at different temperatures was also studied. The potential antifungal effect of H_2O_2 in both pathogens depends on the temperature. *P. expansum* was more susceptible to higher levels of H_2O_2 than *P. digitatum*, especially at 25 °C. The lesion diameter in compatible interactions increased significantly with fruit maturity in apples and oranges. Fruit maturity also increased susceptibility to non-host pathogen interactions, especially reducing apple resistance to *P. digitatum* in the over-mature stage. H_2O_2 production showed different patterns depending on the fruit. In apples, the higher resistance of immature harvested fruit to pathogen infection correlated with an increase in H_2O_2 production (biphasic oxidative burst), whereas in oranges, immature and commercial harvests exhibited a similar pattern of H_2O_2 production among treatments. Production of H_2O_2 in oranges and apples following abiotic (wounding) and biotic (pathogen and non-host pathogen) stresses depended on the harvest date.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The most devastating postharvest disease in fruit is decay caused by fungi, which annually leads to large economic losses. *Penicillium digitatum* and *P. expansum* are causal agents of green and blue moulds in citrus and pome fruit, respectively (Pitt and Hocking, 1997), that are essential food crops cultivated in Spain and widely exported to other countries. Mechanical injury, which can occur during harvesting, packing-house operations, handling and transport, causes the fruit to be more susceptible to opportunistic infection by these postharvest pathogens (Van Zeebroeck et al., 2007).

The development of a postharvest fungal disease partially depends on storage conditions and on the physiological status of the fruit, as well as on any induced host defence mechanisms. These factors are intimately related because fruit tend to become more susceptible to infection with their physiological age (Torres et al., 2003; Su et al., 2011; Vilanova et al., 2012a,b). During fruit-pathogen interactions, the pathogens need to overcome the innate defences of the fruit, and the fruit should initiate an efficient defence response to overcome wound stress, and consequently avoid or prevent a possible pathogen infection. These wound-induced defence responses may be modulated by fruit ripening, which could be one of the main factors that determine the susceptibility of fruit to mechanical damage or to infection during postharvest storage (Mehdy, 1994; Torres et al., 2003; Su et al., 2011).

The association of reactive oxygen species (ROS) formation with the induction of defence responses has been demonstrated in many plant-pathogen interactions (Mehdy, 1994). In almost all host-pathogen interactions, one of the first events that is detected in attacked host cells is the oxidative burst, which is the rapid and

* Corresponding author. Tel.: +34 973003429; fax: +34 973238301.
E-mail address: rosario.torres@irta.cat (R. Torres).

http://dx.doi.org/10.1016/j.postharvbio.2014.07.013
0925-5214/© 2014 Elsevier B.V. All rights reserved.

transient generation of activated oxygen radicals, including superoxide anion, hydroxyl radical and hydrogen peroxide (H_2O_2), at the site of pathogen infection (Lamb and Dixon, 1997; Wojtaszek, 1997). The crucial role of ROS in the host defence against pathogen attack has been generally described by two phases: phase I occurs in both compatible and non-host pathogen interactions and causes a rapid, but weak, accumulation of ROS with a response time of a few minutes. In contrast, phase II has only been observed in non-host pathogen interactions and is characterised by an important and prolonged accumulation of ROS after a longer period, which is usually several hours (Levine et al., 1994; Baker and Orlandi, 1995). H_2O_2 is the most important ROS because of its relative stability and slow reactivity with biological molecules. Within the different possible functions in a plant's defence strategy, H_2O_2 may be involved in membrane peroxidation and in the cross-linking of cell wall proteins (Bradley et al., 1992; Lamb and Dixon, 1997). In addition to the role of H_2O_2 as a signalling molecule after wounding (Orozco-Cardenas et al., 2001; Nürnberger et al., 2004), H_2O_2 mediates the induction of hypersensitive cell death and the expression of a wide array of defence-related genes in surrounding cells (Levine et al., 1994; Grant and Loake, 2000; Bolwell et al., 2002; Borden and Higgins, 2002), and H_2O_2 has also been shown to be involved in the spore germination inhibition of many fungal pathogens (Peng and Kuc, 1992; Cerioni et al., 2010, 2013).

In many fruit, ROS, including H_2O_2, have been reported to be associated with fruit development, ripening and senescence (Brennan and Frenkel, 1977; Jimenez et al., 2002; Larrigaudiere et al., 2004; Vilaplana et al., 2006; Chiriboga et al., 2013). In addition, H_2O_2 is also primarily involved in the biosynthesis, polymerisation and deposition of lignin (Olson and Varner, 1993; Razem and Bernards, 2002). The lignification process may contribute to resistance in many different ways, although little is known regarding its role in fruit-induced resistance (Sticher et al., 1997; Valentines et al., 2005). The presence or accumulation of H_2O_2 in response to wounding and to pathogen attack may be a requirement in the establishment of the host defence (Mehdy, 1994; Wu et al., 1995; Orozco-Cardenas et al., 2001; Rea et al., 2002). In previous work, we have shown that H_2O_2 (Torres et al., 2003) and lignification (Vilanova et al., 2013, 2014) might play an important role in the disease resistance of 'Golden Delicious' apple and 'Valencia' orange fruit. The potential role of H_2O_2 accumulation in response to wounding was described by Castro-Mercado et al. (2009) in unripe avocado fruit, whereas H_2O_2 accumulation in response to pathogen attack was studied in tomato plants (Borden and Higgins, 2002; Mandal et al., 2008), apple (Castoria et al., 2003; Torres et al., 2003; Su et al., 2011), orange (Torres et al., 2011) and lemon (Macarisin et al., 2007) fruit.

H_2O_2 appears to play a crucial role in fruit defence mechanisms during ripening in response to wounding and to pathogen infection. The objective of this study was characterise H_2O_2 production in orange (*C. sinensis* cv Valencia) and apple (*M. domestica* L. cv Golden Smoothee) fruit in response to abiotic (wounding) and biotic (pathogen and non-host pathogen) stresses at different maturity stages. An emphasis was on characterisation of H_2O_2 production during the infection process.

2. Materials and methods

2.1. Fungal cultures

P. digitatum strain PDM-1 and *P. expansum* Link CMP-1 were isolated from decayed citrus and pome fruit, respectively. These isolates were the most aggressive in our collection (Pathology Laboratory, IRTA, Lleida). *P. digitatum* and *P. expansum* were grown on Petri dishes containing potato dextrose agar medium (PDA: 200 mL/L boiled potato extract; 20 g/L dextrose, 20 g/L agar, pH 5.5) in the dark at 25 °C for 7–10 days to achieve conidia production. Conidial suspensions were prepared by adding 10 mL of sterile water with 0.01% (w/v) Tween-80 over the surface of the cultures grown on PDA and rubbing the surface of the agar with a sterile glass rod. The final conidia concentration was adjusted using a haemocytometer and diluted to different concentrations depending on each assay.

2.2. Ecophysiological pathogen response to environmental oxidative stress

In vitro assays of the effect of H_2O_2 on the pathogens *P. digitatum* and *P. expansum* were performed as follows: conidial suspensions (5×10^5 conidia/mL) were added to flasks containing 25 mL of freshly prepared malt extract medium (ME: 30 g/L malt extract, 5 g/L peptone pepsique de viande USP, pH 5.5) alone or, alternatively, containing 2, 20, 50 or 200 mM H_2O_2 (PANREAC, Barcelona, Spain) and incubated for 1 h shaking at 150 rpm at 25 °C. All treatments were performed in triplicates. After incubation, H_2O_2-treated conidia were filtered (0.22 μm pore and 25 mm diameter, Millipore, Billerica, U.S.A) and washed with sterile distilled water. To recuperate all spores, filters were resuspended in 4.5 mL of sterile distilled water with 0.01% (w/v) Tween-80 and vortexed for 2 min. Ten-microlitre droplets of the conidial suspensions were inoculated on PDA plates and incubated at 25 °C (*P. digitatum* and *P. expansum*), 4 °C (*P. digitatum*) and 0 °C (*P. expansum*). Periodically, depending on the temperature and coinciding with each of the placed drops, three agar discs (5 mm diameter) were aseptically removed from each replicate using a cork borer. At each sampling time, discs from the same temperature and medium were placed into a sterile empty Petri dish, and conidia germination was immediately stopped by adding 3 mL of ammonia (NH_3 25%, PANREAC, Barcelona, Spain) onto a filter paper placed on the cover of each plate. Then, Petri dishes were stored at 4 °C until microscopic examination. The germination percentages of *P. digitatum* and *P. expansum* were evaluated as previously described by Buron-Moles et al. (2012). The variable measured was the percentage of germination at different temperatures against time. Experiments were performed with three replicates per treatment.

2.3. Fruit source

Apples (*Malus domestica* L. cv Golden Smoothee) were harvested at different maturity stages from August to October 2010 (immature, 12th August; commercial, 16th September; over-mature, 21th October) from a commercial orchard in Mollerussa (Catalonia, Spain). Apples were used immediately after harvest.

Oranges (*Citrus sinensis* cv Valencia) were obtained at different maturity stages from March to June 2011 (immature, 17th March; commercial, 29th April; over-mature, 23th June), from a commercial orchard in Tortosa (Catalonia, Spain). Oranges were used immediately after harvest.

2.4. Determination of quality parameters

Loss of firmness, colour development, soluble solids and acidity in 'Valencia' oranges were determined to evaluate the effects of different harvest dates on fruit quality. Orange firmness measurements were performed using a TA-XT2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK), based on the millimetres of fruit deformation resulting from fruit response to 2 kg of pressure on the longitudinal axis at a constant speed of 2 mm/s. Colour was measured on two opposite sides of each fruit using a tri-stimulus colourimeter (Chromameter CR-200, Minolta, Japan). The mean values for the lightness (L^*), red-greenness (a^*) and yellow-blueness

Download English Version:

https://daneshyari.com/en/article/4518114

Download Persian Version:

https://daneshyari.com/article/4518114

Daneshyari.com