Scientia Horticulturae 240 (2018) 288–292

Contents lists available at ScienceDirect

Scientia Horticulturae

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

The elicitor AsES stimulates ethylene synthesis, induce ripening and enhance protection against disease naturally produced in avocado fruit

Silvia M. Perato[a], Martín G. Martínez-Zamora[a], Sergio M. Salazar[b], Juan C. Díaz-Ricci[a,*]

[a] Instituto Superior de Investigaciones Biológicas (INSIBIO), CONICET-UNT, e Instituto de Química Biológica "Dr. Bernabé Bloj", Facultad de Bioquímica, Química y Farmacia, UNT. Chacabuco 461, T4000ILI, San Miguel de Tucumán, Argentina

[b] Estación Experimental Agropecuaria Famaillá, Instituto Nacional de Tecnología Agropecuaria (INTA). Ruta Provincial 301 Km 32, Tucumán, Argentina

ARTICLE INFO

Keywords:
AsES
Elicitor
Avocado
Ethylene
Ripening
1-MCP

ABSTRACT

Acremonium strictum Elicitor Subtilisin (AsES) is a natural elicitor capable of inducing disease resistance in strawberry and *Arabidopsis thaliana* plants. In this paper, the effect of AsES on ripening and defense response in the climacteric fruit, avocado (*Persea americana*) was studied. With this purpose ethylene production, respiration rate, weight loss, firmness and soluble solids content were studied. The effect of AsES on natural infestation with local pathogens was also evaluated to assess its capacity to activate a defense response. Controls consisted in fruits treated with water, or treated with 1-Methylcyclopropane prior to AsES (1-MCP + AsES).

Results showed that AsES treatment increases significantly ethylene production at early stages of ripening (3 days post treatment); while in fruits treated with water or 1-MCP + AsES the maximum production occurs later (6 and 7 days post treatment, respectively). Enhanced respiration rate, weight loss and soluble solids content, accompanied with the decreased in firmness were observed in fruits treated with AsES. Also, fruits treated with AsES halted the growth the opportunistic pathogens, whereas the protection effect was not observed when avocado fruit were pre-treated with 1-MCP, suggesting that the effect is due to the activation of the ET defense signaling pathway. These results uncover a potential use of AsES on the postharvest management of ripening, and open new research lines to study the relationship between fruit quality and induction of disease resistance in AsES-treated fruit.

1. Introduction

Avocado (*Persea americana* Mill.), is an important commercial fruit in which, ripening is accompanied by autocatalytic increases in ethylene production and fruit respiration rate (Biale, 1941; Bower and Cutting, 1988; Abeles et al., 1992). Ethylene diffuses freely from cell to cell through membranes and integrates the ripening process throughout the fruit. Fruit ripening is a highly coordinated genetic program that involves a series of physiological, biochemical, and organoleptic changes that leads to fruit softening, color change, aroma development, sugar accumulation and reduction in acidity (Saltveit, 1999). Ethylene gas is used as a phytohormone in horticulture due to its beneficial effects of plant growth and quality. These uses include ripening triggering, color enhancement, de-greening of citrus fruit, flowering promotion in pineapple, and inhibition of stem elongation, among others (Prasanna et al., 2007). Exogenous application of ethylene can also induce these irreversible events (Yang, 1987). There are a variety of techniques currently applied to induce or accelerate the ripening of climacteric fruits, thus reducing the costs of handling and storage and achieving a homogeneous ripening. One of the most used practices is the application of exogenous ethylene. Ho et al. (2011) obtained capsules of ethylene powder to replace the use of ethylene gas. However, ethylene postharvest applications are mainly using compressed gas cylinders that are diluted with air and since ethylene is explosive, safety is an important issue in these practices (Blankenship and Sisler, 1991). Other techniques applied are the use of ethylene release chemicals (e.g. ethephon) and the catalytic production of ethylene from ethanol, propylene and acetylene that can cause the same physiological effects of ethylene, but often require very high concentrations (Abeles et al., 1992).

Plants are in contact with a myriad of microorganisms found in the environment; however, in order for the disease to develop the host must be susceptible to a virulent pathogen and the environment must be conducive to the infection (Ferreira et al., 2006). Although disease

Abbreviations: AsES, *Acremonium strictum* Elicitor Subtilisin; 1-MCP, 1-Methylcyclopropane; dpt, dayspost treatment; SSC, soluble solids content

* Corresponding author.
E-mail addresses: marisaperato815@hotmail.com (S.M. Perato), mgmartinezzamora@fbqf.unt.edu.ar (M.G. Martínez-Zamora), salazar_unt@yahoo.com.ar (S.M. Salazar), juan@fbqf.unt.edu.ar (J.C. Díaz-Ricci).

https://doi.org/10.1016/j.scienta.2018.06.030
Received 17 January 2018; Received in revised form 11 June 2018; Accepted 13 June 2018

response processes in plants have been extensively studied, in the case of postharvest fruits many of them are still unknown, and also the results are different for each pathosystem. The role of ethylene in pathogen-infected plants have been reviewed by Hoffman et al. (1999) who studied resistance to various pathogens in ethylene-insensitive mutants of soybean. The novel elicitor AsES (Patent EPC N∘12.720.221.6-1410) is an extracellular protein produced by the avirulent fungal pathogen *Acremonium strictum* and has been characterized as an effective activator of systemic acquired resistance (SAR) in strawberry and *A. thaliana* plants against *Colletotrichum acutatum* and *Botrytis cinerea*, respectively (Chalfoun et al., 2013; Hael-Conrad et al., 2015). Recently, by using *A. thaliana* knockout mutants, Hael Conrad et al. (2015) observed that AsES induces a defense response by activating the three main phytohormones signaling pathways (e.g. salicylic acid, jasmonic acid and ethylene).

The objective of this work was to evaluate the action of the defense elicitor AsES on key parameters of the ripening process, the protection against natural pathogen in avocado fruit, and elucidate the participation of the ethylene in this effect.

2. Materials and methods

2.1. AsES purification and solution

AsES elicitor protein was purified as previously described by Chalfoun et al. (2013). Briefly, the supernatant of a 21 days static liquid culture of *A. strictum* SS71 was centrifuged, filtrated through 0.2 µm Millipore membrane, ultrafiltrated (cut-off 30 kDa), and chromatographed first by anionic exchange (Q-Sepharose, pH 7.5), and then by hydrophobic interaction (phenyl-Sepharose). AsES purity was confirmed by 2D-PAGE. Finally, the purified protein was lyophilized and kept at 4 °C until use, then it was dissolved at a concentration of 60 nM in distilled sterile water and kept at −20 °C.

2.2. Fruit material

Fruits of avocado (*Persea Americana* Mill.) cv. Torres were harvested at the mature green stage, selected by shape, size, lack of physical injuries, and disease evidence. The fruits were packaged in cardboard boxes, transported to the laboratory, gently washed with distilled sterile water and air dried until treatments.

2.3. Sampling and treatments

Selected avocado fruits were randomly divided into three groups of 50 fruits, and subjected to following treatments: group 1 with water, and group 2 with AsES. Fruit were sprayed with 1 cm^3 of water or 60 nM AsES per fruit, respectively. The group 3 was treated with 1-MCP prior to AsES treatment (1-MCP + AsES). Fruits were placed in 18 L plastic containers, treated with 1-MCP (0.5 $\mu L.L^{-1}$) by using a commercial formulation (Ethyblock® Floralife, Burr Ridge, IL) and sealed. After 24 h, fruits were sprayed with 1 cm^3 of 60 nM AsES per fruit. After the three treatments, fruits were stored at 25 °C, 55–60 % RH. The assay was repeated three independent times.

2.4. Respiration rate and ethylene production

Respiration rate and ethylene production were measured daily for the different treatments (water, AsES or 1-MCP + AsES). Fruits were randomly picked and individually placed once a day in 2 L plastic jars, sealed with a rubber stopper, and held at 25 °C for 1 h. Ethylene concentration was measured by taking a 1 cm^3 gas sample from the headspace of jars using a gastight syringe. Then the sample was injected in a AGILENT 6890 N Gas Chromatograph equipped with a 30 m x 0.53 mm alumina column; running conditions: 120/80/240 °C for the injector/column/FID temperatures, respectively, and 0.50 $cm^3 s^{-1}$ carrier gas (N_2) flow rate. CO_2 production was measured by taking a 100 cm^3 gas sample from each bottle and injected in an Oxygen/Carbon Dioxide Headspace Analyzer equipped with an infrared detection cell (Servomex 01514/701 infrared transducer; Servomex PLC, Crowborough, East Sussex, UK). Measurements were repeated every day over 8 days. Ten fruits were evaluated by treatment (n = 10), and the assay was repeated three independent times.

2.5. Fruit quality measurements

Firmness (N) of whole, unpeeled fruits was determined using a penetrometer (IRC Force Gage®, Norfolk, VA, USA.), with a 1 mm diameter plunger tip, at two equidistant points on the equatorial region of each fruit. Soluble solids content (SSC) (%) was determined from avocado juice using a refractometer (Arcano REF103) and three readings were taken for each fruit. Both parameters were determined at 2, 4, 6 and 8 days post treatment (dpt). Fruit weight loss was recorded using a scale with an accuracy of 0.01 g and expressed as the percentage of initial weight. Ten fruits were evaluated by treatment and by time (n = 10), and the assay was repeated three independent times.

2.6. Disease incidence

The influence of AsES on the occurrence of latent natural infection was evaluated. After being treated with water, AsES or 1-MCP + AsES, fruit were placed in trays, covered with plastic film to maintain a high relative humidity (RH: 95%), and incubated at 25 °C. Symptoms were evaluated at 12 dpt by measuring lesion size (cm^2) using the ImageJ Software. Ten fruits were evaluated by treatment (n = 10), and the assay was repeated three independent times.

2.7. Statistical analysis

The statistical analysis was carried out using the software InfoStat 2013 version (http://www.infostat.com.ar). ANOVA analysis was performed to detect significant variances among treatments and was followed by a Fisher test with a 99% confidence level.

3. Results and discussion

The relationship between fruit ripening and ethylene/respiration pattern allows the classification of fruit as climacteric or non-climacteric. In climacteric fruit, ethylene biosynthesis increases and shows a peak that coincides with respiration pattern, while in non-climacteric fruit the ethylene production declines with fruit ripening and senescence (Abeles et al., 1992). In this paper, various physiological parameters were measured to obtain a representative data set describing fruit development, climacteric ripening, and postharvest storage. Immediately after treatment of fruit, ethylene production and respiration rates were measured together with SSC and firmness, two important fruit quality parameters. Typical climacteric changes in respiration rate and ethylene production were observed in both AsES- and water-treated (control) fruit, while a minor change was observed in fruit pre-treated with 1-MCP. The increase in ethylene production occurred three days earlier, and was 49.6% higher, in fruit treated with AsES compared to water-treated, reaching a peak of 726 $pmol.kg^{-1}.s^{-1}$ at 3 dpt (Fig. 1A). Furthermore, AsES treatment enhanced the respiration rate of fruit during the storage being 50.8% higher than the water-treated fruits, reaching a peak of 5.9 $mmol.kg^{-1}.s^{-1}$ at 3 dpt (Fig. 1B). Bower and Cutting (1998) observed an increase of ethylene production and an acceleration of ripening after the application of isopentenyl adenosine. When fruit were pre-treated with 1-MCP and then with AsES, ethylene production and respiration peaks were delayed and less pronounced as compared to fruit treated with AsES alone, with peaks of 120 $pmol.kg^{-1}.s^{-1}$ and 3.6 $mmol.kg^{-1}.s^{-1}$ at 7 dpt respectively (Fig. 1A and 1B). Similar effects have been observed in avocado fruit treated