Journal of Plant Physiology 224–225 (2018) 1–10

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Transcript analyses of ethylene pathway genes during ripening of Chinese jujube fruit

Zhong Zhang[a,b,c], Jian Huang[a,b,c], Xingang Li[a,b,c,*]

[a] College of Forestry, Northwest A&F University, Yangling 712100, Shaanxi, China
[b] Research Center for Jujube Engineering and Technology of State Forestry Administration, Northwest A&F University, Yangling 712100, Shaanxi, China
[c] Key Comprehensive Laboratory of Forestry of Shaanxi Province, Northwest A&F University, Yangling 712100, Shaanxi, China

ARTICLE INFO

Keywords:
Chinese jujube (*Ziziphus jujuba* Mill.)
Ethylene
Fruit ripening
Non-climacteric
1-MCP

ABSTRACT

The fruit of Chinese jujube (*Ziziphus jujuba* Mill.) is immensely popular worldwide, while its fleshy fruit has a very short shelf life and suffers serious postharvest damage. The fruit has been controversially classified as non-climacteric, though the mechanisms underlying its ripening behavior, particularly the role of ethylene, have remained unclear. In this study, low and stable ethylene production was detected during ripening of *Z. jujuba* 'Dongzao' fruit, with production increasing at the full maturity stage. To determine potential ripening behavior, the fruit of five cultivars were harvested at the white mature stage, and all exhibited a first decreasing and then moderately increasing respiration rate without concomitant climacteric-like ethylene production during shelf storage. Treatment with 1.0 μL L^{-1} 1-methylcyclopropene (1-MCP) inhibited respiration and ethylene production in white mature fruit, though the effects of 100 μL L^{-1} exogenous ethylene were not significant. The transcript levels of genes involved in ethylene biosynthesis, perception, and signal transduction were not elevated during fruit-ripening onset but substantially increased at the full-red ripening stage. Moreover, expression of genes controlling ethylene biosynthesis and perception mainly occurred in an auto-inhibited System-1-like manner, but signaling pathway genes were minimally affected by exogenous ethylene or 1-MCP. These results show that the ripening of Chinese jujube is non-climacteric. The basal level of ethylene likely plays a minor role in ripening regulation but is necessary to maintain normal ripening. This study elucidates the effects of ethylene on jujube fruit ripening, characterizing the ripening of this fruit as non-climacteric, and also provides strategies for the improvement and maintenance of fruit quality and the extension of shelf life during postharvest storage.

1. Introduction

Chinese jujube (*Ziziphus jujuba* Mill.), an economically important fruit crop, has a long history of cultivation in China and is distributed worldwide (Huang et al., 2016). The fleshy fruit is popular due to its unique flavor and nutrient enrichment (Gao et al., 2013). However, harvested Chinese jujube fruit has a short shelf life owing to several physiological characteristics, such as high respiration, rapid dehydration, and serious deterioration (Zhu et al., 2010). These problems cause serious losses during postharvest storage and represent the major limiting factors of the development of the jujube industry. Therefore, understanding the ripening behavior and physiological changes in harvested fruit would help improve storage and extend shelf life. However, because some reports have described climacteric-like respiration and ethylene production during postharvest storage (Zhu et al., 2010; Zhang et al., 2012), classification of this fruit as non-climacteric is controversial. Climacteric ripening behavior may be cultivar-dependent (Jiang et al., 2003; Barry and Giovannoni, 2007), even though selection of the proper fruit maturity stage for sampling was not considered in most previous studies (Supplementary File S1). Overall, molecular evidence for the ripening behavior, particularly the role of ethylene, of jujube fruit is lacking. All of these factors suggest that the ripening behavior of Chinese jujube is worthy of study.

Ethylene plays critical roles in triggering ripening onset and in regulating several physiological and biochemical changes in climacteric fruit (Bapat et al., 2010). Although non-climacteric fruit can also release high levels of ethylene, the phenomenon is not related to the ripening period (Pareek, 2016), such as in grape at veraison (Chervin et al., 2004), citrus at the young immature stage (Katz et al., 2004), and strawberry at the red-ripe stage (Lannetta et al., 2006; Sun et al., 2013). These findings suggest that ethylene has some role in non-climacteric ripening and indicate the complex regulation by ethylene-dependent and ethylene-independent pathways (Hiwasa and Ezura, 2014). Exogenous ethylene and 1-methylcyclopropene (1-MCP) have been widely

* Corresponding author at: College of Forestry, Northwest A&F University, No. 3, Taicheng Road, Yangling 712100, Shaanxi, China.
E-mail address: xingangle@nwsuaf.edu.cn (X. Li).

https://doi.org/10.1016/j.jplph.2018.03.004
Received 18 December 2017; Received in revised form 9 March 2018; Accepted 9 March 2018
Available online 16 March 2018
0176-1617/

used to explore the role of ethylene during ripening. However, jujube fruit exhibit varying sensitivities to ethylene and 1-MCP, in association with treatment time, reagent concentration, and fruit maturity (Li, 2003; Jiang et al., 2003; Almansa et al., 2016). Moreover, molecular analysis of the effects on ethylene pathways has not yet been performed.

Ethylene biosynthesis, perception, and signal transduction pathways have been well characterized (Boller et al., 1979; Yang and Hoffman, 1984; Klee and Giovannoni, 2011); the genes involved have been cloned, and their expression has been investigated (Giovannoni, 2007; Seymour et al., 2013). In the climacteric category, System-2-associated gene expression, which is responsible for climacteric ethylene production, is auto-induced and positively regulated by exogenous ethylene (Giovannoni, 2004). Conversely, in non-climacteric production, only constant basal ethylene levels are produced by auto-inhibited System-1 processes, and System-2-associated gene expression has been suggested to be absent (Aizat et al., 2013; Alos et al., 2017). Basal ethylene perception is needed for various ripening processes and for maintaining expression of several genes (Chervin et al., 2004; Trainotti et al., 2005; Sun et al., 2013). Hence, elucidation of the specific gene transcript patterns involved would help in distinguishing whether the ripening behavior of Chinese jujube is climacteric or non-climacteric and would also provide insight into the role of ethylene regulation during fruit ripening.

In this study, we investigated the Chinese jujube fruit respiration rate and ethylene production during postharvest storage and examined the effects of treatment with exogenous ethylene or 1-MCP. Genes involved in ethylene biosynthesis, perception, and signal transduction pathways were identified, and transcript levels during ripening stages and in response to exogenous ethylene and 1-MCP were analyzed. The aim of the present study was to determine whether Chinese jujube fruit displays climacteric or non-climacteric ripening behavior and to explore the effects of ethylene during jujube fruit ripening. The study would provide important strategies for the improvement and maintenance of fruit quality during postharvest storage and the extension of shelf life.

2. Material and methods

2.1. Plant materials, treatment, and storage

Six jujube cultivars, *Z. jujuba* 'Dongzao', 'Qiyuexian', 'Fengmiguan', 'Xiangfenyuanzao', 'Jinsixiaozao', and 'Luzao No.5', were used. All fruit samples were collected in three biological replicates from the Jujube Experimental Station of Northwest A&F University (Qingjian, Shaanxi, China; N 37.13, E 110.09) in 2016. 'Dongzao' was selected for gene transcription analyses and determination of sugar content and ethylene production. Fruit at different developmental stages [young (YF), enlarging (EF), white mature (WM), beginning red (BR), half-red (HR), and fully red (FR) fruit] were cut into pieces and immediately frozen in liquid nitrogen. The samples were then stored in a freezer at −80 °C until analysis.

For determination of respiration rate and ethylene production during postharvest storage, fleshy fruit of five cultivars at the WM stage were carefully harvested, avoiding any mechanical injury, and stored at 15 °C until transfer to the lab within 6 h. The fruit were cleaned in water and dried for 30 min at room temperature. Fruit of each cultivar (approximately 3 kg) was placed in a 10-L uncovered plastic container and stored in darkness at 20 °C and 80% relative humidity (RH).

In addition, 'Dongzao' fruit at the WM stage was treated with either 100 μL L^{-1} ethylene or 1.0 μL L^{-1} 1-MCP (FreshDoctor ™, Xianyangxiqin Inc., China) for 16 h according to the description of Aizat et al. (2013). After treatment, each group of fruit samples in a 10-L uncovered plastic container, with 100 g potassium permanganate and 100 g calcium hydroxide placed in the bottom, was stored at 20 °C and 80% RH (Scott et al., 1970; Aizat et al., 2013). Fruit at 1, 3, and 5 d after treatment (DAT) were cut into pieces, frozen in liquid nitrogen, and prepared for gene expression analyses.

2.2. Determination of soluble sugar content during fruit development and ripening

Methods of soluble sugar extraction and determination were modified from the description of Gao et al. (2012). Briefly, fruit samples of approximately 0.5 g were homogenized in 5 mL water and extracted at 45 °C for 30 min assisted by ultrasonication. The homogenates were centrifuged at 12,000*g* for 10 min, and the supernatant was concentrated to 2 mL using a rotary evaporator. Samples were filtered through a 0.45-μm syringe filter and prepared for liquid chromatography analysis. A high-performance liquid chromatography (HPLC) system (L2000, Merck-Hitachi, Tokyo, Japan) with a refractive index detector (L2490, Merck-Hitachi, Tokyo, Japan) was used for glucose, fructose, and sucrose content determination. Separation was carried out on a Cosmosil NH_2 packed column (4.6 × 150 mm, 5 μm; Nacalai Tesque Inc., Kyoto, Japan) at 35 °C with a flow rate of 1.0 mL min^{-1}. The mobile phase was 80% acetonitrile and 20% water. The sample injection volume was 10 μL.

2.3. Determination of respiration rates and ethylene production

Fruit respiration rate and ethylene production in harvested or treated fruit during shelf-life storage were measured. The respiration rate was measured as carbon dioxide (CO_2) production using LI-6400XT Photosynthesis System (Lincoln, NE, USA), which was attached to a 0.45-L cylindrical chamber with an air flow of 700 μmol s^{-1}. The measurement protocol was modified from the description of Fugate et al. (2010). Fruit samples of approximately 100 g were assessed in triplicate at room temperature at 14:00 every day. For each measurement, the stable variation in ΔCO_2 was used to calculate the respiration rate.

Ethylene production was determined by sealing approximately 100 g fruit in a 0.5-L glass bottle for 2 h at room temperature and drawing 5-mL headspace gases into a 20-mL penicillin bottle filled with water. For each developmental stage, fruit were separated from the tree and immediately sealed in a jar for 2 h. The remaining steps were the same as those in the collection of ethylene produced by harvested fruit. Gas samples were prepared and analyzed together. One milliliter of the gas was injected into a gas chromatograph (Trace GC ULTRA2010, Thermo, America) fitted with a flame ionization detector and a Porapak 80-100 packed column (200 × 3 mm). The oven, injector, and detector temperatures were 70, 70, and 150 °C, respectively. The carrier gas (N_2, H_2, and air) flow rates were 35, 35, and 350 mL min^{-1}, respectively. Three independent biological replicates were prepared for each sample.

2.4. Measurement of jujube fruit peel colorimetric changes

Fruit peel color was assessed using color space parameters L*, a*, and b* with a CR400 colorimeter (Konica Minolta, Osaka, Japan). Defined by the Commission Internationale de L'Eclairage (CIElab), L* indicates lightness, and a* and b* indicate the color opponents of green-red and blue-yellow, respectively (Mcguire, 1992). At each stage, fruit color was determined using five fruits. For each fruit, color was measured on the peel at five sites of the equatorial zone (Wang et al., 2012).

2.5. Gene identification and phylogenetic analyses

Based on the jujube gene model dataset (https://doi.org/10.5061/dryad.83fr7) (Huang et al., 2016), ethylene pathway genes were identified and confirmed by BLASTN and SMART analyses (Letunic et al., 2015). Detailed sequence information of candidate ethylene pathway genes is listed in Supplementary File S2. Predicted proteins were aligned with those corresponding to known protein sequences in tomato