Plant Physiology and Biochemistry 70 (2013) 81–92

Contents lists available at SciVerse ScienceDirect

Plant Physiology and Biochemistry

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

Research article

Isolation and characterization of ethylene response factor family genes during development, ethylene regulation and stress treatments in papaya fruit

Xueping Li*,[1], Xiaoyang Zhu [1], Jia Mao, Yuan Zou, Danwen Fu, Weixin Chen, Wangjin Lu

State Key Laboratory for Conservation and Utilization of Subtropical Agro-Bioresources/Guangdong Provincial Key Laboratory for Postharvest Science and Technology of Fruits and Vegetables, College of Horticulture, South China Agricultural University, Guangzhou 510642, PR China

ARTICLE INFO

Article history:
Received 8 March 2013
Accepted 13 May 2013
Available online 25 May 2013

Keywords:
Ethylene response factors (ERFs)
Biotic and abiotic stress
Ethylene regulation
Gene expression
Papaya

ABSTRACT

Ethylene response factors (ERFs) play important roles in fruit development, ripening, defense responses and stress signaling pathways. After harvest, climacteric fruit such as papaya are subject to a range of problems associated with postharvest handling and storage treatments. There have been few attempts to evaluate the role of *ERFs* in fruit's responses to environmental stimuli. To investigate the transcriptional mechanisms underlying fruit developmental, ripening and stresses, we cloned four ERFs from papaya. The deduced amino acid sequence of CpERFs contained the conserved apetalous (AP2)/ERF domain, which shared high similarity with other reported AP2/ERF domains. The phylogeny, gene structures, and putatively conserved motifs in papaya ERF proteins were analyzed, and compared with those of *Arabidopsis*. Expression patterns of *CpERFs* were examined during fruit development, under 1-MCP treatment, ethephon treatment, biotic stress (temperature stress) and pathogen stress. *CpERFs* displayed differential expression patterns and expression levels under different experimental conditions. *CpERF2* and *CpERF3* showed a close association with fruit ripening and *CpERFs* had a high expression level in the earlier stages during the fruit development period. The expression of *CpERFs* strongly associated with stress response. These results support the role for papaya *ERFs* in transcriptional regulation of ripening-related or stress-respond genes and thus, in the regulation of papaya fruit-ripening processes and stress responses.

1. Introduction

Fruit are frequently subjected to continuous exposure to various biotic and abiotic natural environmental stresses during their development, and to those from man-made environments during postharvest storage as well. Fruit ripening is a complex and genetically programmed process that results in marked changes in color, aroma, flavor, texture, and nutritional content of the flesh [1]. These changes are the results of the coordinated activation of multiple genetic and biochemical pathways, which are influenced by both internal and external incentives, including regulation by many critical transcription factors (TFs) [2]. Numerous plant TFs, such as apetalous (AP2), basic leucine zipper domain (bZIP), myelocytomatosis oncogene (MYC), myeloblastosis oncogene (MYB), WRKY and so on, have been identified and characterized according to their DNA-binding motifs [3]. The TFs, AP2/ethylene-responsive factors, are one of the most important families that are involved in plant response to biotic and abiotic stresses, and in the regulation of metabolism and development processes in various plant species [4]. The AP2/ERF superfamily is defined by the AP2/ERF domain, which consists of about 60–70 amino acids and is involved in DNA binding. It could be divided into three subfamilies including the AP2 family, ERF family and the RAV family. Proteins of the AP2 family contain two repeated AP2/ERF domains; the proteins of ERF family contain a single AP2/ERF domain, and the proteins of RAV family contain a B3 domain and a single AP2/ERF domain [5,6]. The family of ERFs is a unique family in the plant kingdom and a part of the AP2/EREBP-type of TFs, which function as trans-acting factors at the last step of ethylene signal transduction [7]. ERFs have been shown to play a critical role during plant development. They exhibit varied expression patterns (either induction or repression) during plant growth and development in response to external or internal ethylene and trans-activation of other TFs, which enable plant to fight against ambiances including stress-related stimuli, biotic or abiotic stress conditions [8,9]. Over-expression of some *ERF* genes has been found to confer enhanced resistance to biotic [8] or abiotic stresses [10].

* Corresponding author. Tel.: +86 20 38294892; fax: +86 20 85288280.
E-mail address: lxp280477@yahoo.cn (X. Li).
[1] These authors contributed equally to this work.

0981-9428/$ – see front matter © 2013 Published by Elsevier Masson SAS.
http://dx.doi.org/10.1016/j.plaphy.2013.05.020

Several proteins in the ERF family have been identified. For instance, 122 and 139 genes of ERF family have been identified from *Arabidopsis* and rice (*Oryza sativa*), respectively, by a comprehensive computational analysis [5]. In *Arabidopsis*, several members of *AtERF* genes are stress-responsive, including DREB2 subgroup that is drought- and heat stress-responsive [11], C-repeat-binding factor/DRE-binding factor 1 (CBF/DREB1) subgroup that are cold responsive [12], *AtERF1* and *AtERF14* [13] that are related to pathogen tolerance and *HRE1* and *HRE2* [14] that are related to anaerobic response. *ERF* genes have been studied only in a few fruit species, including grape [15], plum [16], tomato [6], kiwifruit [17], and apple [18]. However, the characterization of *ERF* genes in harvested papaya fruit has not been reported.

Papaya is widely cultivated and consumed for its agreeable flavor, nutritional benefits as well as various pharmacological properties such as laxative, anti-fertility agent, and meat tenderizer [19]. As a typical climacteric fruit, papaya fruit undergo massive problems such as rapid ripening and susceptible to biotic or abiotic stresses, which usually result in a high percentage of production loss [20,21]. A better understanding of the postharvest physiology and molecular biology of papaya fruit is helpful for us to overcome these problems. In the present study, we isolated four novel *ERF* genes from *Carica papaya*, namely *CpERF1–4*, characterized their molecular and biochemical properties, and predicted the subcellular localization and three-dimensional structures of their proteins. In additional, we investigated the expression patterns of *CpERF1–4* in papaya fruit after treatments with hormone (ethephon), 1-MCP and stress treatments, as well as their expression patterns during the development of fruit. Our results revealed that *CpERF* genes showed different expression patterns under different experimental conditions and suggested that they may play different roles in papaya during fruit development, ripening and stress responses.

2. Results

2.1. Fruit evaluation under different experiment conditions

As shown in Fig. 1, 1-MCP treatment significantly delayed the fruit color change (Fig. 1A) and had a restraint effect on the firmness decreasing in the later storage time (Fig. 1C). The firmness of control fruit decreased slightly during the first 9 days (Fig. 1C) but decreased sharply at 10 d, but the 1-MCP-treated fruit kept a large hardness until 12 d and drop to a low level. However, ethephon treatment accelerated the fruit color change and decreased firmness (Fig. 1A, C). High temperature (35 °C) also accelerated the fruit color change but low temperature (7 °C) completely blocked the

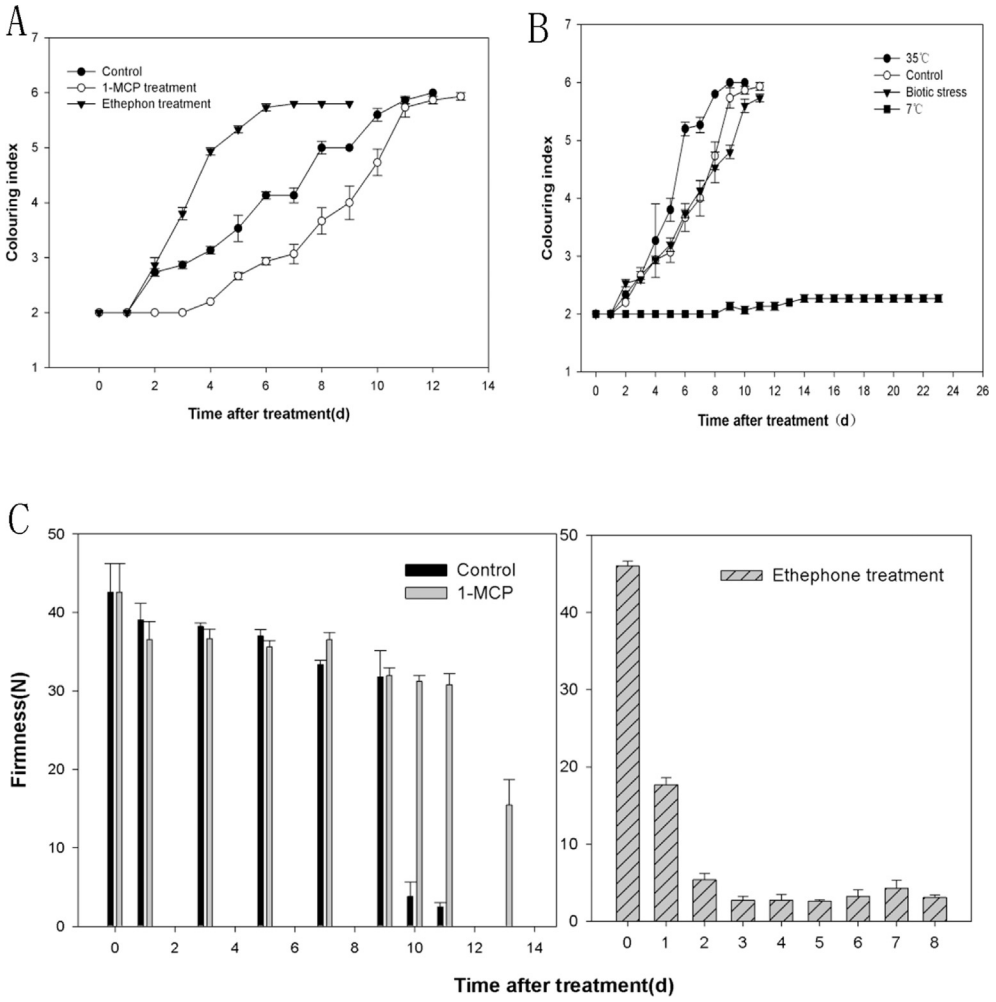

Fig. 1. The effects of 1-MCP, ethephon treatment, pathogen stress and different temperatures on the coloring index and firmness of papaya fruits. A and B coloring index changed with the storage time under different conditions; C fruit firmness changes after ethylene regulation treatments. The means are generated from three independent measurements and the bars indicated the standard errors (mean ± SE). In Fig. 1B, the data for 35 °C and 7 °C treatments have been published in Ref. [22]. They were integrated here for a complete information and comparison with the permission conferred by Food Research International.