AcERF1B and AcERF073 positively regulate IAA degradation by activating *AcGH3.1* transcription during postharvest kiwifruit ripening

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Abstract

Although IAA and ethylene are both important for postharvest kiwifruit ripening, the interaction between the two remains unclear. In the present study, we found that ethylene could promote the conjugation of IAA and amino acid, and accelerate the degradation of free IAA. We also found that *AcGH3.1* promoter activity is positively regulated by ethylene. Meanwhile, AcERF1B and AcERF073 were found directly bound to the promoter of *AcGH3.1* gene and enhance its expression. Our results show that ERFs regulate IAA degradation during postharvest ripening process of kiwifruit by targeting *AcGH3.1* promoter.

Introduction

Recent studies have found that IAA is also crucial for the ripening of climacteric fruits [1]. The endogenous IAA content in kiwifruit showed a decreasing trend with fruit ripening and the peak of the ethylene jump began to appear as IAA content decreased [2]. GH3 protein controls the development and maturation of fruit by decreasing the level of free IAA in the fruit [3]. It is apparent that ethylene plays an important role in the process of climacteric fruit ripening. Ethylene response factor (ERF), a subfamily of the plant-specific AP2/ERF superfamily, is a core transcription factor in the ethylene signal transduction pathway and its role provides additional insights into fruit ripening [4]. Our previous study found that the IAA degradation-related gene AcGH3.1 plays an important role in postharvest kiwifruit ripening and is stimulated by ethylene 5; however, the molecular mechanism by which ethylene regulates IAA degradation is unclear.

Materials and Methods

❖**Materials:**.'Hongyang' kiwifruit (*Actinidia chinensis*) were harvested at the commercial mature stage, Fruit samples that were of similar size and free from physical injuries were divided into three groups: the first group was treated with 1-MCP (5 μL/L, fumigation for 24 h, 20 ° C); the second group was treated with ethephon (50 mg/kg, soak for 5 min, 20 ° C); and the third group was treated with air as a control.

❖**IAA content Analysis:** The phytohormone was quantitated using a high-performance liquid chromatography-mass spectrometer (LCMS-8040, Shimadzu) equipped with a C18 column (4.8 × 150 mm) in an ES (+) model.

❖**qRT-PCR:** qRT-PCR analysis was conducted with a CFX96 Touch Real-time PCR instrument (Bio-Rad, Hercules, CA, USA).

❖**AcERF1B/073 directly binds to the AcGH3.1 :**Yeast one-hybrid assay and dual-luciferase reporter assay.

❖**AcERF1B interacts with AcERF073:** Yeast two-hybrid assay and bimolecular fluorescence complementation (BiFC) assay.

Results

1. Ethylene can accelerate IAA degradation during postharvest kiwifruit ripening (Figure 1).

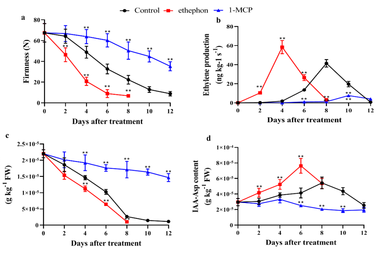

Figure 1. Changes of firmness (a), ethylene production (b), free indole-3-acetic acid (IAA) content (c), and IAA-aspartic acid (Asp) content (d) by ethephon and 1-MCP in kiwifruit.

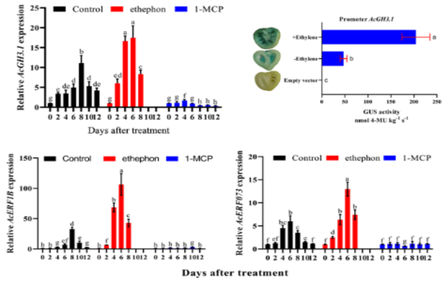

Figure 2. Analysis of the expression of the *AcGH3.1*, *AcERF1B* and *AcERF073*.

2. Ethylene can promote the activity of AcGH3.1 promoter and the expression of AcGH3.1, AcERF1B and AcERF073 (Figure 2).

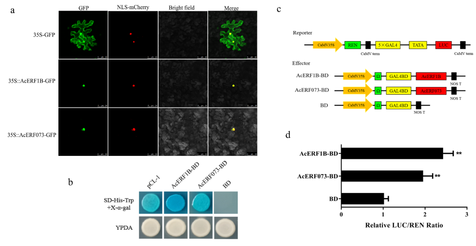

Figure 3. The transcription activity of AcERF1B and AcERF073.

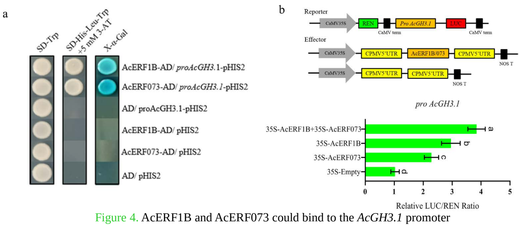

Figure 4. AcERF1B and AcERF073 could bind to the *AcGH3.1* promoter

3. AcERF1B/073 may act as transcriptional activators and enhanced AcGH3.1 transcription by directly interacting with its promoter (Figure 3-5)

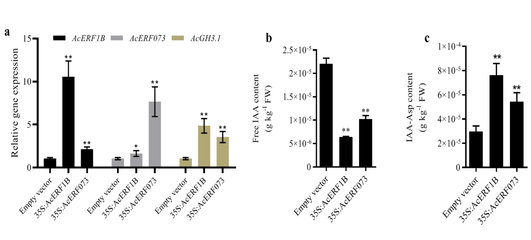

Figure 5. Transient overexpression of *AcERF1B* and *AcERF073* in kiwifruit.

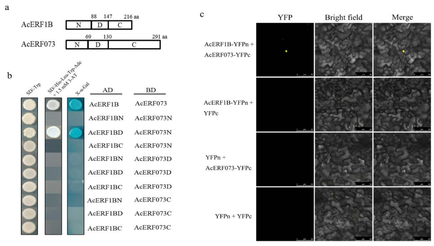

Figure 6. AcERF1B and AcERF073 protein interaction analysis.

4. The ERF domain of AcERF1B (AcERF1BD) interacted with the N terminus of AcERF073 (AcERF073N) (Figure 6)

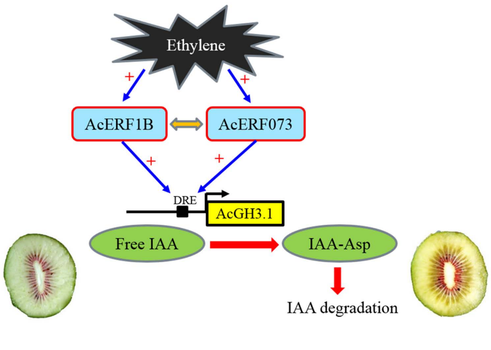

Figure 7. A model of how ethylene regulates indole-3-acetic acid (IAA) degradation during postharvest kiwifruit ripening..

Conclusion: Taken together, our data indicate that ethylene promotes IAA degradation during postharvest kiwifruit ripening through the following mechanism: (i) AcERF1B and AcERF073 can respond to ethylene, directly bind to the *AcGH3.1* promoter, and promote its expression; and (ii) AcERF1B and AcERF073 directly interact, enhancing the activation of the *AcGH3.1* promoter by either AcERF1B or AcERF073 and resulting in an increase in *AcGH3.1* transcription (Figure 7).

References:

1. El-Sharkawy, I., Kim, W.S., Jayasankar, S., Svircev, A.M. & Brown, D.C.W. Differential regulation of four members of the ACC synthase gene family in plum. J Exp Bot 59, (2008).
2. Bregoli, A.M., Fabbroni, C., Costa, F., Raimondi, V. & Costa, G. Auxin and ethylene interaction during fruit growth and ripening of *Actinidia deliciosa*. Adv Plant Eth Res (2007).
3. Boettcher, C., Keyzers, R.A., Boss, P.K. & Davies, C. Sequestration of auxin by the indole-3-acetic acid-amido synthetase GH3-1 in grape berry (*Vitis vinifera* L.) and the proposed role of auxin conjugation during ripening. J Exp Bot 61, 3615-3625 (2010).
4. Yin, X., Allan, A.C., Chen, K. & Ferguson, I.B. Kiwifruit EIL and ERF genes involved in regulating fruit ripening. Plant Physiol 153, 1280-1292 (2010).
5. Gan, Z., Fei, L., Shan, N., Fu, Y. & Chen, J. Identification and expression analysis of *Gretchen Hagen 3 (GH3)* in kiwifruit (*Actinidia chinensis*) during postharvest process. Plants-Basel 8, 473 (2019).