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ScienceDirect
Agricultural Sciences in China
2009, 8(6): 746-752
June 2009

Effect of Light on Flavonoids Biosynthesis in Red Rice *Rdh*

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Abstract

The effect of light on flavonoids biosynthesis in red rice *Rdh* was studied. The panicles of red rice *Rdh* produced colorless caryopses after darkness treatment; and these colorless caryopses displayed bright-red after vanillin treatment, but did not display red color after light inducing for 15 days, suggesting that red rice *Rdh* could produce leucoanthocyanidin, but could not produce polyproanthocyanidins in darkness. Histological study revealed that the aleurone layers of *Rdh* colorless caryopses displayed bright-red after vanillin assay, but the pericarp and seed coat layers did not display color change, which indicated that the aleurone layers could accumulate precursors of polyproanthocyanidins in darkness, but the pericarp and seed coat could not. Additionally, color of *Rdh* caryopses changed from green in immaturity to red in maturity, and the green caryopses changed color from green to red gradually indoor for 7 days after harvest, suggesting that leucoanthocyanidins could synthesize polyproanthocyanidins. It was concluded that light was necessary for red pigment biosynthesis in red rice *Rdh*, leucoanthocyanidins biosyntheses in the aleurone layers did not need light, leucoanthocyanidins biosynthesis in pericarp and seed coat needed light inducing, the effect of leucoanthocyanidin biosynthesis in *Rdh* to light had tissue specificity.

Key words: red rice, flavonoid, light

INTRODUCTION

Bioflavonoids are polyphenols in nature, and they are the secondary metabolites in plants. So far, more than 5000 kinds of flavonoids have been found (Gattuso *et al*. 2007). The red pigment in rice grains is proanthocyanidin, also called condensed tannins (Sweeney 2006). Proanthocyanidins are colorless polymers of flavan-3-ols. They are polymers of flavan-3-ol subunits such as epicatechin and catechin (Pourcel *et al*. 2005). The enzyme leucoanthocyanidin reductase (LAR) produced catechin, a precursor of proanthocyanidin (PA) (Abrahams *et al*. 2003). Polyproanthocyanidins, leucoanthocyanidins (flavon-3, 4-diol), epicatichins and catechins (flavon-3-ols) are all flavonoids. In acidic condition, leucoanthocyanidins, catechin or epicatechin, which are present either as monomers or as terminal subunits of polyproanthocyanidins, and polyproan-thocyanidins condensed from them all could combine with vanillin. Vanillin could turn red upon binding to flavan-3,4-diols and flavan-3-ols. Condensed reactions were found between proanthocyanidins and vanillin, and cherish red matter formed (Papi *et al*. 2002; Cook and Sarnman 1996; Devic 1999; Debeaujon *et al*. 2003). Therefore, in the current study, proanthocyanidins in red rice *Rdh* were detected using vanillin assay. The flavonoid biosynthesis was mostly light-induced (Liao *et al*. 2001), because CHS (chalcone synthase) and other enzymes

Received 24 August, 2008 Accepted 16 January, 2009
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doi:10.1016/S1671-2927(08)60274-6

involved in flavonoid biosynthesis were studied in details for a better understanding of molecular aspects of gene regulation by light (Buchholz *et al*. 1995).

Whether flavonoid biosynthesis in *Rdh* is induced by light has not been reported yet at home and abroad. To ascertain the response of flavonoids biosynthesis to light, caryopses of *Rdh* during developmental stage were treated in darkness. Polyproanthocyanidins and their precursors in rice *Rdh* were identified. It was cleared that how the flavonoids in *Rdh* were biosynthesized and influenced in darkness.

MATERIALS AND METHODS

Materials

One material, *Rdh*, was an indica rice with red pericarp seed provided by Rice Research Institute of Sichuan Agricultural University (RRI, SAU), China. It was obtained through tissue culture of a haploid rice SAR (Sichuan apomixis rice). SAR could diploidize after tissue culture. *Rdh* was a diploidized strain that was genetically stable, and produced red grains. The red color was caused by red pericarp. The red phenotype of red grain was determined by a dominant monogene with maternal effect (Han *et al*. 2006). Plants were grown with normal management at a space of 17 cm × 27 cm in the field of of RRI, SAU in Wenjiang City. The other material Shuhui 527 was common colorless rice provided by RRI, SAU.

Methods

Darkness treatment Darkness treatment was performed as described by Dooner and Ralston (1996) with some modifications. Rice panicles in the full-bloom stage were chosen. The fertilized florets were cut off with the surgical scissors. And the panicles with residual unfertilized florets were wrapped with aluminum foils. Thus, the unfertilized florets wrapped in aluminum foils were in total darkness. After pollination the seeds wrapped in aluminum foils grew in total darkness, too. The unwrapped panicles in the same plant were taken as the control.

The seeds in darkness were harvested 25 days later. The lemma and palea were taken away with sharp forceps to expose the caryopses. The color of caryopses was observed. Caryopses were scanned with 300 mega pixel and real color using Zhongjing ScanMaker 4850 (Zhongjing Technology Limited Company, Shanghai). The pictures were processed with software ACDSee ver. 3.1.

Vanillin treatment The vanillin test was performed as described by Papi *et al*. (2002), Cook and Sarnman (1996), and Devic *et al*. (1999) with some modifications. A fresh solution of saturated vanillin (4-hydroxy-3-methoxybenzaldehyde) was prepared in 6 N HCl at room temperature. Every nearly mature caryopsis in darkness was cut into two halves after being harvested. One half was directly incubated in saturated vanillin solution for 5 min, the other half was used as the material in light exposure test. The half after vanillin treatment was taken out of the solution and was dried by filter paper. Every caryopsis of the control was aslo treated in the same way. The color changes of the them before and after vanillin treatment were observed in the following way: A slide glass was put on the glass plate of the scanner Zhongjing ScanMaker 4850, then the dissected caryopses were put on the slide glass and were scanned. The photos processing was as mentioned above.

Histology test The pericarp and seed coat were separated from the half caryopsis after vanillin treatment using dissecting needle and sharp forceps. The residual parts such as aleurone layers and endosperm were observed as a whole. The caryopses of the control panicles were separated too. The color of every part was observed as follows: A slide glass was put on the glass plate of Zhongjing ScanMaker 4850, then the separated pericarp and seed coat, endosperm and aleurone layer were respectively put on the slide glass and were scanned. The photos processing was as mentioned above.

Exposure to light The half colorless caryopsis for light exposure test was put into a culture dish with wet filter paper, as well as the caryopsis of the control panicle. Then they were incubated for 15 days with illumination time for 12 h every day in the incubator at 30°C to show if they could continue to accumulate red color at light condition. The wet filter paper was changed every day. Then they were scanned and their photo pro-