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# Study on Flavonoids in the Caryopsis of Indica Rice *Rdh*

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## Abstract

To detect if there are phenolic flavonoids such as proanthocyanidins in *Rdh*, histochemistry assay toluidine blue O (TBO) staining and vanillin tests were performed. *Rdh* caryopsis transverse sections were stained by metachromatic stain TBO. It showed that there were a great deal of polyphenols in the pericarp and seed-coat tissues, and a little of them was in the cell wall of aleurone layers. The ovaries of *Rdh* and colorless rice were dealt with vanillin. It showed that all fertilized ovaries of *Rdh* were bright red regardless what degree the caryopsis maturity was, and all the ovaries of it before fertilization were not red after vanillin treatment. It suggested that every *Rdh* caryopsis had proanthocyanidins or its precursors, and fertilization was the premise of proanthocyanidins or its precursor synthesis. But colorless rice could not accumulate proanthocyanidins or its precursors. The pericarp, seed-coat and aleurone layer tissues of *Rdh* and colorless rice were anatomized and were dealt with vanillin. It was showed that three layers of *Rdh* were all bright red after treatment, revealing that they all had proanthocyanidins or its precursors, but colorless rice could not. It was suggested that polymerized proanthocyanindins were synthesized and accumulated in the pericarp of *Rdh*, and monomer-proanthocyanidins or leucoanthocyanidins were synthesized and accumulated in the seed-coat and the aleurone layer tissues.

Key words: indica rice, caryopsis, flavonoids, proanthocyanidin, precursor

## INTRODUCTION

Flavonoids are plant secondary metabolites. They have important developmental and physiological functions (Taylor and Grotewold 2005). They are a group of polyphenolic metabolites which are important for plant biology and human nutrition, and play the key roles in a variety of developmental programs, biochemical processes, and environmental responses (Bruce *et al*. 2000). Flavonoids are well known for the red, purple, and brown pigmentation they give to flowers, fruits, and seeds (Nesi *et al*. 2001), and play critical roles in resisting pathogens and invasive predators. And they are necessary for nodule initiation in *Medicago truncatula*, suggesting that they act as auxin transport regulators (Anton *et al*. 2006).

Flavane is the core framework of flavonoids. Its basic structure is three rings represented with A, B and C rings, respectively. Different kinds of flavonoids form lots of flavonoid compounds through further hydroxylation, methylation, acylation, or glycosylation, etc. at the position of $R_1$, $R_2$, $R_3$ and $R_4$ (Fig.1). According to their chemical structures, the main groups are flavonol, flavone, flavonone, catichin, anthocyanidins, isoflanone, dihydroflavonol, proanthocyanidin and chalcone, etc.

Proanthocyanidins (PAs) are a large subclass of the flavonoids (Brenda 2001). Dietary PAs are present in many fruits and plant products like wine, fruit juices,

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and teas, and contribute to their taste and health benefits. PAs can protect plants against herbivores, and act as dietary antioxidants beneficial to human health (Bogs *et al*. 2007). PAs have shown to protect against oxidative stress and tobacco-induced DNA damage, and to exhibit selective cytotoxicity against some human cancers, including breast, lung, prostate, and gastric carcinomas (Bagchi *et al*. 2000; Kim *et al*. 2004; Seeram *et al*. 2004; Vayalil *et al*. 2004). Grape peels, grape seeds, and black raspberries sources that contain high concentrations of PAs, have been demonstrated selective suppression of tumorigenic phenotypes in oral cancers, specifically in oral squamous cell carcinomas (Shirataki *et al*. 2000; Rodrigo *et al*. 2006). PAs are beneficial for human health, including protection against free radical-mediated injury and cardiovascular disease (Middleton *et al*. 2000). They are composed primarily of catechin and epicatechin units in higher plant species (Fig.2, Paolocci *et al*. 2007).

According to the number of the composition elements, PAs can be divided into two parts. One is monoproanthocyanidin, and the other is polyproanthocyanidin. Monoproanthocyanidin is leucoanthocyanidin, it includes flavan-3,4-diols and flavan-3-ols. Flavan-3-ols are the most widely distributed group of flavonoids and can be divided into catechin, epicatechin, afzelechin, etc., according to the different number of hydroxyl groups in B ring and the different structure of 3-hydroxyl groups in C ring (Brenda 2001; Abrahams *et al*. 2002; Bogs *et al*. 2005).

*Rdh* was a diploidization rice being obtained through tissue culture SAR which was a haploid rice. *Rdh* was a diploidized strain that was genetically stable and produced red grains. And the red color of the grians was attributed to the red pericarp. The red phenotype of red grains was determined by a dominant monogene with maternal effect (Han 2006). Generally, to confirm if there are phenolic compounds in red rice *Rdh*, caryopses transverse sections of *Rdh* were stained with metachromatic dye toluidine blue O (TBO). And the presence of phenolic compounds could be confirmed by the colour variation which was observed with the light microscope (Debeaujon *et al*.2003).

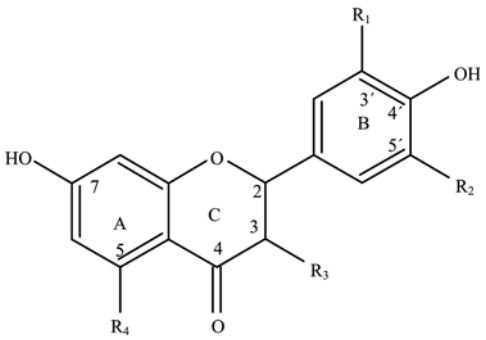


**Fig. 1** Basic structure of flavonoid compounds, $R_1$, $R_2$, $R_3$, and $R_4$ representing groups that can be modified.

And to detect if there are proanthocyanidins in *Rdh*, vanillin tests were performed. According to the color variation of *Rdh* and its dissected tissues, proanthocyanidins and their precursors could be detected . If there are proanthocyanidins in red rice *Rdh*, then the red color in rice is probably caused by proanthocyanidins after vanillin treatment (Maura *et al*. 2002), thus eating red rice will be good for health.

The aim of this work is to confirm if there are phenolic compounds and if the phenolic compounds are proanthocyanidins in red rice *Rdh*. We also want to confirm which kinds the proanthocyanidins are, and which tissues can synthesize them. This research will provide scientific basis for rational utilization of *Rdh* and other red rice.

## MATERIALS AND METHODS

### Materials and field experiments

The material *Rdh* is from the red rice provided by Rice Research Institute of Sichuan Agricultural University, China (RRI, SAU). Plants were grown with normal management at a space of 17 cm × 27 cm in the field of Wenjiang, RRI, SAU, China.

### Polyphenols were evaluated by histochemistry

Histochemistry assay was performed as described by Nesi *et al*. (2001) and Martine *et al*. (1999) with some modifications. Phosphate buffered saline (PBS) were prepared as 130 mM NaCl, 7 mM $Na_2HPO_4$, 3 mM $NaH_2PO_4$, pH 7.2. PBS fixative was prepared as follows: 4% (v/v) formaldehyde was dissolved in PBS, and con-