Journal of Cereal Science 59 (2014) 48–55

Contents lists available at ScienceDirect

Journal of Cereal Science

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

Chalcone synthase expression and pigments deposition in wheat with purple and blue colored caryopsis

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ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form
8 October 2013
Accepted 15 October 2013

Keywords:
Chalcone synthase
Triticum aestivum L.
Purple pericarp
Blue aleurone

ABSTRACT

Red and white caryopses are typical in common wheat. Genotypes with purple and blue caryopses are also described. This coloring is caused by anthocyanins which deposit in the pericarp (purple) or aleurone layer (blue). The anthocyanins biosynthetic pathway is well described. The key enzyme is chalcone synthase (CHS). It catalyzes the first step. We observed the deposition of anthocyanins in the pericarp and aleurone layer, the expression of a chalcone synthase gene and the amount of two anthocyanins - cyanidin-3-glycoside (pericarp of purple caryopses), and delphinidin-3-glycoside (aleurone layer of blue caryopses) during caryopsis development. Purple pigment deposition was not homogeneous and/or uniform. At first, small isolated spots of purple color were formed and thereafter they expanded. In blue caryopses, however, the coloring process was more homogeneous. The expression of chalcone synthase mRNA occurred five days before pigment deposition and finished earlier than expected. Amounts of cyanidin-3-glycoside and delphinidin-3-glycoside increased in a similar manner. Amounts of these fell at the end of caryopses development probably due to formation of more complex substances.

1. Introduction

The mature caryopsis is classified as a dry indehiscent single-seeded fruit. It develops from an ovary containing one ovule which comprises of two integuments; nucellus and embryonic sac. The diploid embryo and triploid endosperm are products of the double fertilization. These structures occupy the largest part of the caryopsis. The outermost layer of the endosperm is called the aleurone layer. All of the nucellus except for its epidermis is reabsorbed. These parts of the caryopsis are covered by testa (seed coat) fused with pericarp. During maturation the pericarp develops from the carpel. The testa develops from the outer and inner integuments of the ovule. In the mature caryopsis, the outer integument disintegrates and forms the cross layer and tube cells while the inner integument and the nucellar epidermis form the hyaline layer (Fig. 1). Therefore, the outer protective layers of the mature caryopsis are derived from the nucellus, integuments, and carpel, which undergo a combination of wall thickening, cell death, and reabsorption during their development (Esau, 1977). All these structures are of maternal origin and are therefore diploid.

The wheat caryopsis is most often red. The red color is caused by deposition of catechin-tannin derivatives in the pericarp (Miyamoto and Everson, 1958). Genotypes with either purple or blue caryopses also exist. The genes responsible for the production of pigments and their localization on chromosomes have been described and their origin was discussed as well (Zeven, 1991).

The deposition of cyanidin-3-glycoside is predominant in the pericarp of purple caryopses. Delphinidin-3-glycoside is characteristic for the aleurone layer of blue caryopses (Abdel-Aal and Hucl, 2003). These pigments belong to anthocyanins which are a group of flavonoids. The flavonoid biosynthetic pathway is well described and many structural and regulatory genes responsible for the production of multiple compounds including anthocyanins have already been described (Holton and Cornish, 1995; Mol et al., 1998; Nesi et al., 2000; Himi et al., 2011). Alternations of these genes affect the resultant pigment and tissues where it will be deposited (Nesi et al., 2001, 2002). The key enzyme of the flavonoid biosynthetic pathway is chalcone synthase (CHS). It catalyzes the first step of the multi-branched flavonoid pathway. The structure and mechanism of CHS have been examined in detail (Austin and Noel, 2003).

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http://dx.doi.org/10.1016/j.jcs.2013.10.008

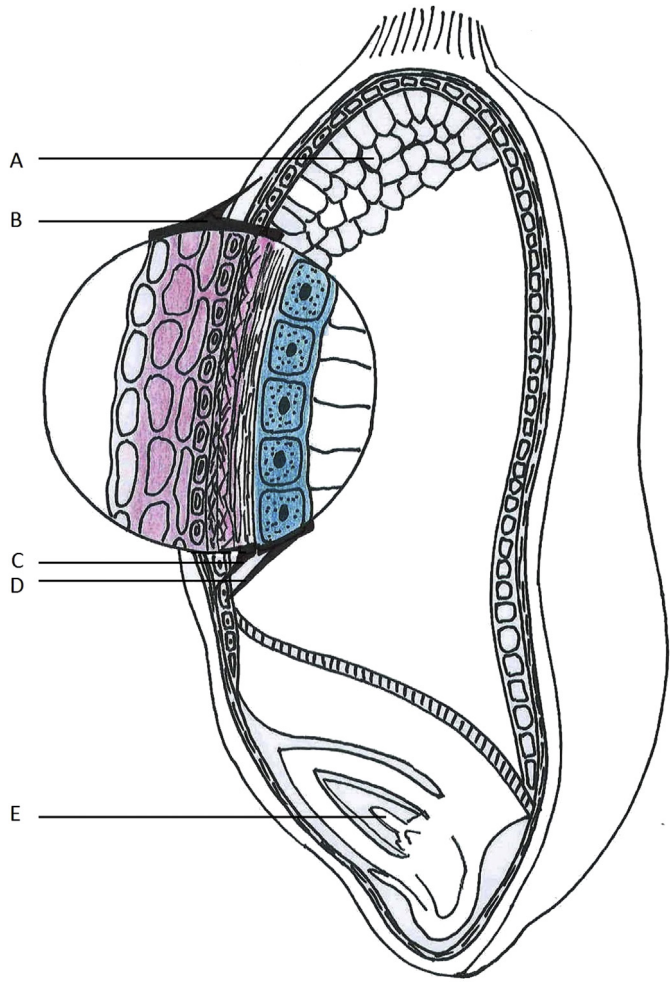

Fig. 1. A caryopsis of wheat cut lengthwise A – endosperm, B – pericarp, C – testa, D – aleurone layer, E – germ.

CHS genes have been cloned and expressed in a number of plants and it was found that many plant species contain multiple copies of *CHS* genes (c.f. Yang and Gu, 2006). In the GenBank there are more than 650 *CHS* and *CHS*-like gene sequences (Austin and Noel, 2003).

The aim of this study was to find still lacking knowledge of the correlation between chalcone synthase expression, the development of coloring of whole caryopsis and pericarp and aleurone layer, resp., and deposition of cyanidin-3-glycoside and delphinidin-3-glycoside by the wheat with purple and blue colored caryopsis.

2. Experimental

2.1. Plant material

The spring form of common wheat (*Triticum aestivum* L.) genotypes with white caryopsis, i.e. Novosibirskaya 67 (N67) as a control, purple caryopsis, i.e. ANK-28B (ANK) and Abyssinskaya Arraseita (AA), and blue caryopsis, i.e. UC66049 (UC) and Tschermaks Blaukörniger Sommerweizen (TBS), were used in the experiments. The seed material was obtained from the Agricultural Research Institute Kroměříž, Ltd., Czech Republic. It was sown in spring 2011 in the Botanical Garden and Arboretum, Mendel University in Brno, Czech Republic. Anthesis of the experimental material was in the period 13–16 June 2011. Anthesis corresponded with 65 BBCH-scale (Biologische Bundesanstalt, Bundessortenamt and Chemical Industry/): 13 June UC, 15 June ANK and TBS, and 16 June AA and N67. The developing caryopses were sampled 10; 15; 20; 25; 30; 35 and 40 days post anthesis (dpa) in a manner similar to Knievel et al. (2009).

On each sampling date, at least 100 mg of caryopses were sampled from the central part of the spike. Thereafter, samples were stored for subsequent studies at −70 °C. Two spikes of each genotype were used. Photographs of intact developing caryopses were taken on each sampling date to monitor the development of caryopsis color.

2.2. Anatomical study

For the observation of anthocyanin deposition in individual caryopses layers during development, sections of caryopses were prepared from one genotype with purple pericarp (ANK) and one genotype with blue aleurone layer (TBS). The CRYO-CUT (American optical corporation, USA) was used. The cross-sections (0.04 mm) were prepared from the central part of developing caryopses and observed with an Olympus Provis light microscope. Photographs were taken using an Olympus SP-350 digital camera with QuickPHOTO Micro 2.3 (PROMICRA, Czech Republic) software.

2.3. Genomic and gene expression analyses

Total RNA was isolated: (i) from the whole caryopses; (ii) separately from testa together with pericarp (testa and pericarp are fused, the fused structure is called a seed coat for simplicity when it is not necessary to distinguish these two layers) and (iii) from endosperm with aleurone layer by the phenol-chloroform method using RNA Blue (Top Bio, Czech Republic). The concentration and purity of the isolated RNA was measured by spectrophotometer Picopet (Picodrop, UK). RNA samples were purified with Turbo DNA Free kit (Ambion, USA). The two-step reverse transcription of RNA into cDNA was performed using the Enhanced Avian HS RT PCR kit (Sigma Aldrich, USA). The success of transcription into cDNA was examined by PCR with a housekeeping gene (*GAPDH* - glyceraldehyde-3-phosphate dehydrogenase, Wang et al., 2003). From the National Centre for Biotechnological Information (NCBI) database, sequences TaCHS_I 434 bp (AB187025.1), TaCHS_II 1279 bp (AY286095.1) and HvCHS 1509 bp (EU921436.1) were selected and compared using the Basic Local Alignment Search Tool (BLAST). Selected sequences were used for primer design by Primer3 software (Rozen and Skaletsky, 2000). The cDNA served as a template for gradient PCR to test the optimal temperature of annealing, cloning, sequence analysis, and optimization for the qPCR method. Reaction conditions were as follows: 95 °C/30s; 57 °C/30s; 72 °C/60s (35 cycles). The final extension at 72 °C lasted for 10 min. PCR products were cloned using pGEM®-T Vector System (Promega, USA). Recombinant pGEM-T plasmids (after ligation with purified PCR products) were transferred into competent cells of *E. coli* bacteria via electroporation. Petri dishes with solid lysogeny broth (LB) medium and selective antibiotic carbenicillin (100 mg/L) and components for blue/white selection (X-gal – 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside and IPTG – isopropyl β-D-1-thiogalactopyranoside) were used for the selection of *E. coli* cells. After blue-white screening, clones with recombinant plasmids were transferred into 1.5 mL of liquid LB medium and cultivated overnight at 37 °C. Plasmid DNA was isolated by GeneJET Plasmid Miniprep Kit (Fermentas, USA) and sequence analyses (four colonies of each genotype in three repetitions, twelve different colonies totally for every genotype) were performed by the company