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Isolation of differentially expressed genes in wheat caryopses with contrasting starch granule size

Research Article

Rita Armonienė, Kristina Jonavičienė*, Vytautas Ruzgas, Gintaras Brazauskas

Institute of Agriculture,
Lithuanian Research Centre for Agriculture and Forestry,
LT58344 Akademija, Kedainiai, Lithuania

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Abstract: In order to identify genes responsible for starch granule initiation during early development of wheat caryopsis, nine winter wheat breeding lines were studied. Two breeding lines, which are the most diverse in A–type granule size (26.85 μm *versus* 23.65 μm) were chosen for further differential gene expression analysis in developing caryopses at 10 and 15 days post–anthesis (DPA). cDNA–amplified fragment length polymorphism (cDNA-AFLP) analysis resulted in 384 transcript-derived fragments, out of which 18 were identified as being differentially expressed. Six differentially expressed genes, together with the six well–known starch biosynthesis genes, were chosen for semi-quantitative gene expression analysis in developing wheat caryopses at 10 and 15 DPA. This study provides genomic information on 18 genes differentially expressed at early stages of wheat caryopses development and reports on the identification of genes putatively involved in the production of large A–type granules. These genes are targets for further validation on their role in starch granule synthesis control and provide the basis for the development of DNA marker tools in winter wheat breeding for enhanced starch quality.

Keywords: *cDNA-AFLP • Starch synthesis genes • Triticum aestivum • Endosperm development • Relative gene expression • Starch A–granules*

1. Introduction

Wheat is one of the most important crop species grown worldwide. More than 200 million hectares of wheat are cultivated annually with a total harvest of almost 600 million tonnes of grain (FAO; http://www.fao.org/). The storage starches produced in cereal endosperm account for over 90% of the world market for starch (the major cereals in terms of tonnage of yield are maize and wheat). Most of wheat harvested is consumed by humans for making a wide range of bread items or used for livestock feeding. However, starch being cheap and renewable raw material is used not only in the agri-food sector, but in many non-food industrial applications, such as manufacturing of protein–based films or starch–based plastics [1] and as a source of energy after conversion to ethanol [2,3].

The synthesis of starch in the endosperm occurs within the amyloplasts and is completed by a series of enzymatic reactions catalyzed by several different classes of enzymes [4]. Starch synthases (SS, E.C. 2.4.1.21) are important factors controlling the rate of starch synthesis, while the granule–bound starch synthases (GBSS: EC 2.4.1.21) are related to the synthesis of amylose. Starch branching enzymes (SBEs, E.C. 2.4.1.18) form the branched structure of the amylopectin molecule, while amylopectin is synthesized by the coordinated actions of SS, starch branching enzymes (SBEs, E.C. 2.4.1.18) and starch debranching enzymes (DBEs, E.C. 3.2.1.41 and E.C. 3.2.1.68). Starch consists of two major glucose polymers (amylose and amylopectin) with the ratio of amylose/amylopectin ranging between 25–28 and 72–75%, respectively. The relative amounts of amylose and amylopectin are responsible for starch physical and chemical properties with strong influences on the functional properties of flour and on its specific uses in the food and manufacturing industries.

In wheat, starch granules exhibit a bimodal size distribution, a characteristic unique to members of the grass *Triticeae* family. The starch granules, designated A– and B–type [5], can be distinguished based on

* *E-mail: kristinaja@lzi.lt*

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