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Association Mapping and Marker Development of Genes for Starch Lysophospholipid Synthesis in Rice

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Abstract: Phospholipids are a major kind of lipids in rice grains and have fundamental nutritional and functional benefits to the plant. Their lyso forms (lysophospholipids, LPLs) often form inclusion complexes with amylose or independently influence the physicochemical and functional properties of rice starch. However, the genetic basis for LPL synthesis in rice endosperm is largely unknown. Here, we performed a preliminary association test of 13 LPL compositions among 20 rice accessions, and identified 22 putative main-effect quantitative trait loci responsible for all LPLs except for LPC14:0 and LPE14:0. Five derived cleaved amplified polymorphic sequences and one insertion/deletion marker for three LPL-synthesis-related candidate genes were developed. Association analysis revealed two markers significantly associated with starch LPL traits. These results provide an insight into the genetic basis of phospholipid biosynthesis in rice and may contribute to the rice quality breeding programs using functional markers.

Key words: rice; starch lysophospholipid; phospholipid biosynthesis; grain quality; QTL; molecular marker; association mapping

Rice (*Oryza sativa* L.) has long been cultivated and consumed worldwide as a vital nutritional source. It satisfies over 21% of the daily calorie needs of the world's population (Zhan et al, 2014). According to the reported world rice statistics from the International Rice Research Institute (http://www.irri.org), the global paddy rice and milled rice productions were 700 and 565 million tons in 2015, respectively. Besides the sustained challenge of rice yield, with the increasing of living standards, rice quality improvement has also been a new mission for meeting the consumer's demands (Ren et al, 2015).

To date, functional lipids in rice grains have greatly attracted considerable attentions owing to their nutritional and healthy benefits. As a major class of complex lipids in cereal grains, phospholipids (PLs) serve as one of the necessary bioactive components of cell membranes by forming lipid bilayers. Increasingly emerging evidences have shown that PLs play a pivotal biological role in clathrin-mediated endocytosis, phagocytosis and macropinocytosis (Bohdanowicz and Grinstein, 2013). According to different involved hydrophilic heads, such as choline, ethanolamine, inositol and serine, the PLs in organisms are mainly comprised of phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol, phosphatidylserine and the corresponding lyso forms of different PLs (Choi et al, 2005; Liu et al, 2013). In contrast to other classes of lipids, PLs in rice grains only account for a relatively minor proportion, however, they make a great contribution to the physicochemical and nutritional properties of grains via combining with

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starch to form complexes in endosperm (Maniñgat and Juliano, 1980; Putseys et al, 2010; Tong et al, 2015). For example, Tong et al (2015) found that lysophospholipids (LPLs) have significant correlations with pasting properties, the important traits of eating quality of rice, such as cool paste viscosity, breakdown and consistency. Although a number of experiments investigating the components, fractions, contents, structural and distribution features of rice PLs have been reported (Choi et al, 2005; Yoshida et al, 2011), the broadly diverse results are possibly owing to the different genotypes and environmental effects (Choi et al, 2005; Liu et al, 2014; Tong et al, 2014).

The results obtained when PLs begin to accumulate in plants are controversial. For example, Shewry et al (1973) found that PL synthesis occurred as early as seed germination imbibition, whereas others indicated it took place during the stage of rice seed ripening and was unchanged during storage (Nakamura et al, 1958; Perry and Harwood, 1993). However, the importance to uncover the biosynthesis of PL compositions in plants cannot be overstressed. Hence, a lot of efforts were made to illuminate the underlying genetic network. Several PL-synthesis-related enzymes and biosynthetic genes have been reported. For instance, *acetyl-CoA carboxylase* (*Acc1*) influences the length distribution of acyl-chain by regulating the relative proportion of C16 versus C18 fatty acids during lipid synthesis (Hofbauer et al, 2014). *INO1*, a structure gene encoding inositol-3-phosphate synthase of PL biosynthesis, has been found in yeast (Gaspar et al, 2011). Three major genes *cdsA*, *pgsA* and *pgpP* participate in the PL biosynthetic steps of cytidine triphosphate to cytidine diphosphate-diacylglycerol, cytidine diphosphate-diacylglycerol to phosphate-dylclycerolphosphate, and phosphatidylclycerolphosphate to phosphatidyldlycerol, respectively (Martin et al, 1999; Kuhn et al, 2015). Two Mg^{2+}-dependent phosphatidic acid phosphohydrolases (PAH1 and PAH2) have been characterized in *Arabidopsis*, and they catalyze the first committed step of choline synthesis and define a special PC biosynthetic pathway at endoplasmic reticulum (Eastmond et al, 2010; Farquharson, 2010). Fatty acid desaturase 2 is negatively correlated with oleic acid composition while positively correlated with linoleic acid composition in maize (Li et al, 2013). The seed gene *phospholipase D* (*PLD*) controlling the degradation of PL membranes of oil bodies has also been mapped in rice (Suzuki, 2011a, b).

At present, since the enzymes participating in starch PL biosynthesis in rice have just been scarcely identified, the specific biosynthetic pathway of starch PLs in rice is still unclear. A possible and modified biosynthetic network of PLs in rice is outlined based on the studies on animals and yeasts (Fig. 1) (Kinney, 1993; Liu et al, 2013). Cytidinediphosphate-diacyglycerol and 1,2-diacylglycerol are both originated from phosphatidic acid after the acylation by cytidine-diphosphate-diacylglycerol synthase and phosphatidate-phosphohydrolase. Their main synthetic pathways have been reported (Fig. 1) (Liu et al, 2013). Especially, PE maybe produced from the reactions of three enzymes, phosphatidylserine decarboxylase, aminoalcoholphosphotransferase (AAPT) and ethanolaminephosphotransferase, while PC is probably produced by displacement through phospholipid N-methyltransferase, AAPT and cholinephosphotransferase. Interestingly, AAPT is a common enzyme mediating the biosyntheses of PC and PE. LPLs, such as lysophosphatidic acid, are possibly produced from the hydrolysis of diacylphospholipids by phospholipase A_2 (Fig. 1).

Quantitative trait loci (QTLs) responsible for lipid synthesis in rice have been reported (Liu et al, 2009; Qin et al, 2010; Shen et al, 2012; Ying et al, 2012). Ying et al (2012) identified 29 QTLs associated with fatty acid composition and oil concentration, and some of them are strongly associated with the rice ortholog genes *acyl-CoA:diacylglycerol acyltransferase* (*DGAT*) and *acyl-ACP thioesterase* (*FatB*). Shen et al (2012) identified three fat-content-related QTLs that are stably expressed in different environments and populations. Similarly, 7 and 14 QTLs for rice lipids were identified via different doubled haploid populations under diverse environments, respectively (Liu et al, 2009; Qin et al, 2010). These QTLs may contribute to improving rice nutritional quality via marker-assisted breeding. However, QTL analysis of rice starch lipids has not been reported.

The objectives of this study were to identify the QTLs or genetic loci for rice starch LPL content and composition across different environments, identify LPL-synthesis-related candidate genes and develop molecular markers for these candidate genes, and confirm whether these molecular markers are associated with LPL traits.

MATERIALS AND METHODS

Rice materials

Two sets of rice accessions were used. Set 1 planted at