Abstract

Eukaryotic cells store neutral lipid in lipid droplet (LD), which is enclosed by a phospholipid monolayer having several types of embedded proteins. These LDs and LD-associated proteins play physiological functions in lipid and energy homeostasis, and have several practical and biotechnological applications. In plant system, oleosin has been recognized as a crucial LD-associated protein, and several oleosins from different plant species have been characterized. Although rice (*Oryza sativa* L.) genome contains six oleosin genes, none of them has been thoroughly investigated. In this study, the smallest (16 kDa) seed-specific oleosin isoform of rice, designated as OsOle1 was first characterized *in silico* for its physico-biochemical properties, and subsequently characterized *in vivo* for physiological functions through heterologous expression in yeast *Saccharomyces cerevisiae* and seed-specific expression in rice. Bioinformatics analyses revealed that the OsOle1 has seed-specific highest relative expression, contains a central hydrophobic region with characteristic proline knot motif connecting two transmembrane helices and a C-terminal acyltransferase motif. Molecular docking unveiled stable interactions of OsOle1 with various lipid ligands, including glycerol tripalmitate having the highest binding affinity of − 11.44 kcal/mol. Inducible expression of *OsOle1* with C-terminally fused *mCherry* reporter gene under GAL1 promoter resulted in targeted localization of fusion proteins in LDs of transformed *S. cerevisiae* INVSc1 cells. The OsOle1 transformed yeast cells exhibited doubling of cellular lipid content and showed early development of LDs. Moreover, OsOle1 expression promotes formation of larger LDs in INVSc1 as well as in *S. cerevisiae* Pet10Δ strain (defective in LD biogenesis), implying its role in LD formation and enlargement of LD. When *OsOle1* gene was ectopically expressed in rice under seed bran layer-specific promoter, two stably inherited transgenic lines showed reduced seed length (38-39.5%), breadth (30-32%) and weight (40%). Late arrival of inflorescence and a slow development were also noticed for the transgenic rice lines. Microscopic observation of the isolated LDs upon staining with florescence dye revealed larger LDs in the transgenic seeds as compared to the untransformed control. Quantitative estimation of three major macromolecules disclosed ~31% reduction in starch content, while there was an increase in crude protein and lipid contents ~ 49% and 60%, respectively in the transgenic seeds compared to the control. Together, the present study demonstrates OsOle1 as a positive regulator of lipid accumulation, LD formation and LD enlargement; and this gene could be an attractive candidate for engineering rice plant towards increasing the seed-oil content.

Keywords: Lipid content; Lipid droplet enlargement; Lipid droplet protein; Seed-specific oleosin, Transgenic rice, Transformed yeast