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Commentary

Multimoics Reveals the Fatty Acid Desaturase 2 (FAD2) Mutation Induced Expression Network from Gene Atlas to Metabolic in Peanut

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COMMENTORY

Peanut (*Arachis hypogaea* L.) is an allotetraploid oil-seed crop, which originates from abiogenetic event of diploid ancestors *Arachis* duranensis (AA) cross-fertilized with *Arachis* ipaensis (BB) in South American. At present, peanut has been cultivated in more than 115 countries, covers 26 million hectares, and yields 40 million tons worldwide. As the rapid increase in vegetable oil consumption in the developing countries, peanut provides major element in the edible oil for many countries. Recently, diet with monounsaturated oleic acid (OA, C18:1) shows a positive effects on human health, including lowering cholesterol levels and decreasing risks of coronary heart diseases. Current evidence supporting the positive link between the OA and health management of cardiovascular disease, as well diversity beneficial effects of the high OA diet was observed on the improvement of long-term complications in type 2 diabetes. Additionally, Due to the presence of natural antioxidants protecting against harmful substances, peanut oil with high OA contains a well-balanced FAs composition and exhibits an excellent stability of extending oil storage time. Therefore, future breeding objective is to breed market-oriented peanut variety with high OA.

Based on the principle of the OA biosynthesis, high OA peanut materials are usually obtained by screening the FAD2 mutant. AhFAD2 encodes the fatty acid desaturase to control the OA conversion into linoleic acid, which recessive allele mutant exhibits defect in this conversion, thereby inducing OA accumulation. Interestingly, a systemic understanding of the dynamic change of gene and metabolism expression profile in high OA peanut seed have been explored by multiple omics technology. The comparative transcriptomics was conducted to analyze global gene expression profile in high oleic acid peanut variety. Transcriptomic analysis identified 74 differentially expressed genes (DEGs) involved in lipid metabolic, of which five DEGs encoded the fatty acid desaturase, the Stearoyl-ACP Desaturase 2 (SAD2) that converted the C18:0 into C18:1, which expression modulated by FAD2 mutation. Subcellular localization indicated that FAD2 located at endoplasmic reticulum (ER) and SAD2 targeted to the plastid, fad2 mutant probably increased the ROS concentrate that as signaling to connect the feedback regulation between the FAD2 and upstream SAD2. This transcriptome provide a potential peanut breeding strategy based on identified candidate genes to constantly improve the content of oleic acid.

Furthermore, comparative proteome analysis based on iTRAQ was performed to identify the critical candidate factors involved in OA formation. A total of 389 differentially expressed proteins (DEPs) were identified between high-oleate and low-oleate cultivars. In addition, these DEPs were categorized into biosynthesis pathways of unsaturated FAs at the early stage, and several DEPs involved in lipid oxidation pathway were found at the stage of seed maturation. 28 DEPs were sporadically distributed in distinct stages of seed formation, and their molecular functions were directly correlated to FA biosynthesis and degradation. Fortunately, the expression of FAB2 (stearoyl-acyl carrier protein desaturase), the rate-limiting enzyme in the upstream biosynthesis process of OA, was significantly increased at the early stage and then decreased at the late stage of seed development in the high-oleate cultivar. Taken together, comparative proteome analysis provided valuable insights into the molecular dynamics of OA accumulation during peanut seed development.

Even though FAD2 mutation is capable of producing benefit high oleic acid. But the dynamic changes of the lipidome regarding of fad2 remains elusive in peanut seed. Lipidomics identified 733 lipid features in high-oleic peanut seed. The fad2-induced differently expressed lipids (DELs) were polar distributed at

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early and maturation stages during high OA seed development. Integration of previously published proteomic data and lipidomic data revealed that 21 proteins and 191 DELs were annotated into the triacylglycerol assembly map, of which 10 enzymes and 75 lipid species shared similar variation tendency. Collectively, the understanding of the lipid composition correlated with fad2 established a foundation for future high OA peanut breeding based on lipidomic data.

Next, the mission is creating more fad2 mutant for peanut breeding. The clustered regularly interspaced short palindromic repeat (CRISPR/Cas) system is a powerful technology that allows for the development of FAD2 mutant breeding, able to mimic/create various genetic mutations. CRISPR/Cas9 has been widely utilized in the construction of knock-out FAD2 mutant plant in cotton. Similar systems have also been implemented, substituting Cas9 in an attempt to reduce the off-target effects of the technique; the CRISPR assisted protein, Cpf1 and Cas13 proteins have all been used for this purpose. The new ease of plant genomic editing has ushered in a new era of research, however, CRISPR/Cas9 mediated mutagenesis of allopolyploid species remains restricted by the techniques of genetic mutation. Currently, TALEN-mediated FAD2 mutants enhance the accumulation of oleic acid in peanut, suggesting that genome editing process could be tremendously accelerated in peanut, genome editing tool will makes profound effect on the understanding of FAD2 function.

Moreover, FAD2 mutant activated gene expression network correlated with negative plant growth phenotype. While the current methods of physiology and molecular biology research have provided great insights into FAD2 mutant, they have failed to capture the heterogeneity of function that individual cells exhibit in mutant seed. Single cell RNA-seq presented widespread heterogeneous and monoallelic transcriptome landscape, which differed greatly when compared to the results of bulk RNA-seq. While the use of scRNA-seq on none-model plant cells is limited due to absence of information on cellular compositions and a lack of suitable techniques. We believe this technology will have a profound effect on the functional study of FAD2 mutant cell in the allotetraploid peanut.