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Rice protein improvement by genomic and synthetic biology

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Rice grains are rich in starch but low in protein with very low level of both lysine and threonine. Thus, it is important for rice to further improve protein quality and quantity, especially to increase lysine and threonine content in rice grains. In this study, we first genome-widely identified genes that encode proteins with high ratios of lysine and threonine constituents through bioinformatics-based strategies. We have identified several genes up to 30-36% of lysine in the encoded proteins in the soybean genome. These genes were further optimized in their protein coding preference to fit the rice species and were then used to improve rice protein quantity and quality by genetic modification. In the meantime, we artificially synthesized two new genes by fusing endogenous rice genes with lysine (K)/threonine (T) motif (TKTKK) coding sequences. They were designated as *TKTKK1* and *TKTKK2* and their encoded proteins consist of 73.1% and 83.5% of lysine/threonine, respectively. These two genes were under the control of the strong 35S promoter and were independently introduced into the rice genome to generate rice plants. Our data showed that overexpression of *TKTKK1* generated stable proteins with expected molecular weight and the transgenic rice seeds significantly increased lysine, threonine, total amino acids and crude protein content by 33.87%, 21.21%, 19.43% and 20.45%, respectively when compared with wild type control and overexpression of *TKTKK2* also statistically increased by 12.9%, 13.63%, 14.09% and 12.09%, respectively. However, limited improvement in protein quality and quantity was observed in transgenic seeds carrying tandem array of these two new genes under the control of 35S promoter. Our data provides the basis and alternative strategy on further improving protein quality and quantity in other crops or vegetable plants by bioinformatics and synthetic biology.

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