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Lysine biofortification in rice by modulating feedback inhibition of aspartate kinase and dihydrodipicolinate synthase

发布日期: 2020-09-18 浏览次数: 26

Plant Biotechnology Journal, n/a(n/a). doi:10.1111/pbi.13478

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Abstract

Lysine is the main limiting essential amino acid (EAA) in the rice seeds, which is a major energy and nutrition source for humans and livestock. In higher plants, the rate-limiting steps in lysine biosynthesis pathway are catalysed by two key enzymes, aspartate kinase (AK) and dihydrodipicolinate synthase (DHDPS), both are extremely sensitive to feedback inhibition by lysine. In this study, two rice AK mutants (AK1 and AK2) and five DHDPS mutants (DHDPS1–DHDPS5), all single amino acid substitution, were constructed. Their protein sequences passed an allergic sequence-based homology alignment. Mutant proteins were recombinantly expressed in *Escherichia coli*, and all were insensitive to the lysine analog S-(2-aminoethyl)-L -cysteine (AEC) at concentrations up to 12 mM. The AK and DHDPS mutants were transformed into rice and free lysine was elevated in mature seeds of transgenic plants, especially those expressing *AK2* or *DHDPS1*, 6.6-fold and 21.7-fold higher than the wild-type (WT) rice, respectively. We then engineered 35A2D1L plants by simultaneously expressing modified *AK2* and *DHDPS1*, and inhibiting rice *LKR/SDH* (lysine ketoglutaric acid reductase/saccharopine dehydrogenase). Free lysine levels in two 35A2D1L transgenic lines were 58.5-fold and 39.2-fold higher than in WT and transgenic rice containing native AK and DHDPS, respectively. Total free amino acid and total protein content were also elevated in 35A2D1L transgenic rice. Additionally, agronomic performance analysis indicated that transgenic lines exhibited normal plant growth, development and seed appearance comparable to WT plants. Thus, AK and DHDPS mutants may be used to improve the nutritional quality of rice and other cereal grains.

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<https://onlinelibrary.wiley.com/doi/10.1111/pbi.13478>