

Lycopene β -cyclase plays a critical role in carotenoid biosynthesis during persimmon fruit development and postharvest ripening

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Abstract

The aim of the current study was to characterize the functional role of *DkLCYb* in carotenoid biosynthesis in persimmon fruit. The accumulation of carotenoids and the expression of carotenogenic genes were investigated during persimmon fruit development and postharvest ripening. *DkLCYb* was cloned and its functional role in carotenoid biosynthesis was characterized. The results showed that carotenoids constantly accumulated during persimmon fruit development and postharvest ripening. More carotenoids accumulated during postharvest storage than during fruit development. The carotenoid composition of unsaponified extracts of the persimmon fruit flesh showed that the major carotenoids were lycopene and β -carotene in ‘Huoguan’ and ‘Heishi’, respectively. Consistent with this result, the expression of six carotenogenic genes increased to their highest levels during postharvest storage. The selected carotenoid biosynthesis genes showed an increased expression pattern during fruit development and in the early period of postharvest storage. A significant decrease in carotenogenic genes was found after stage 6 (*DkPSY*, *DkLCYb*, *DkZDS* and *DkBCH*) or stage 5 (*DkLCYe*). Both *DkLCYe* and *DkBCH* showed a steady increase from stage 1 to stage 5; however, dramatically decreased expression of *DkLCYe* and sharply increased expression of *DkBCH* were found at stage 6. Correlation analysis showed that the expression of *DkPSY*, *DkPDS*, *DkZDS*, *DkLCYb* and *DkBCH* was significantly correlated with the accumulation of one or more carotenoid compounds analysed in this study. However, a negative correlation was found between the expression of *DkLCYe* and the accumulation of lycopene, β -cryptoxanthin and zeaxanthin. Specifically, expression of *DkLCYb* showed the highest correlation ($p < 0.01$) with β -

carotene ($r = 0.74$) accumulation. We cloned *DkLCYb* and analysed its functions *in vitro* and *in vivo*. *DkLCYb* contained typical domains that were highly conserved and essential for lcyb protein activity. The cyclization activity of the encoded protein was demonstrated by functional analysis in *E. coli* BL21. Transient overexpression of *DkLCYb* significantly enhanced the accumulation of β -carotene in persimmon fruit. These results indicate that *DkLCYb* is a genuine lycopene cyclase gene and it plays an essential role in β , β -carotenoid (β -carotene and β -cryptoxanthin) biosynthesis in persimmon fruit.