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Development of transgenic tomato lines with enhanced lycopene content using RNAi approach

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Abstract
Most people eat tomatoes for flavour, but it's the lycopene that fights oxidative damage in their cells. This research used RNA interference (RNAi) to silence the lycopene β-cyclase (*LCY-B*) gene in tomato (*Solanum lycopersicum* cv. Micro-Tom), redirecting carotenoid flux toward lycopene accumulation. Three independent RNAi lines (RNAi-1, -2, -3) and the wild type were grown in controlled-environment chambers at Fukuoka and Sapporo during 2021–2023. HPLC analysis showed that the best line, RNAi-1, accumulated 14.8 mg lycopene 100 g^{-1} FW—139% above the wild type (6.2 mg 100 g^{-1}). *LCY-B* transcript was suppressed by 78–89% in RNAi lines. β-carotene fell proportionally (41–58% reduction), confirming that the silencing redirected substrate away from the cyclisation branch. Fruit size, yield per plant, and Brix values were not significantly affected. These lines provide proof-of-concept for RNAi-mediated nutritional improvement of tomato and could serve as pre-breeding material for high-lycopene cultivar development.

Keywords: Transgenic tomato, lycopene content, RNAi, nutritional enhancement, genetic engineering, lycopene beta-cyclase, carotenoid pathway, Micro-Tom, gene silencing, biofortification

Introduction
There's an old Japanese saying: a red tomato keeps the doctor away. The saying oversimplifies, but the link between tomato consumption and reduced cardiovascular risk has solid epidemiological backing, and most of the credit goes to lycopene, a C40 carotenoid with the highest singlet-oxygen-quenching capacity among dietary pigments [1, 2].
In the tomato fruit, lycopene is the end product of a linear branch of the carotenoid pathway. It can also be cyclised by lycopene β-cyclase (LCY-B) and lycopene ε-cyclase (LCY-E) to produce β-carotene and α-carotene, respectively [3]. During ripening, *LCY-B* transcripts normally decline, which is why ripe tomatoes are red (lycopene-rich) rather than orange (β-carotene-rich). But the decline is incomplete: residual LCY-B activity diverts 15–25% of synthesised lycopene into β-carotene [4]. Silencing *LCY-B* by RNAi could plug this leak and push lycopene still higher.
This research aimed to (i) generate stable RNAi tomato lines in which *LCY-B* is constitutively suppressed, (ii) quantify the resulting shift in carotenoid composition across fruit development, and (iii) assess whether the modification affects agronomic traits relevant to commercial adoption.

Construct Design and Transformation
A 412 bp inverted-repeat fragment of *SlLCY-B* (GenBank AB009953, nucleotides 218–629) was cloned in sense–antisense orientation flanking a 120 bp GUS intron spacer into the binary vector pHELLSGATE-12 under the CaMV 35S promoter. The construct was mobilised into *Agrobacterium tumefaciens* GV3101 and used to transform cotyledon explants of Micro-Tom. Kanamycin-resistant (50 mg L^{-1}) shoots were regenerated, rooted on half-MS, and advanced to T_2 homozygous lines. Three single-copy events (RNAi-1, -2, -3) confirmed by Southern blot were selected for phenotyping [5, 6].

Carotenoid Quantification
Carotenoids were extracted from freeze-dried fruit powder in acetone:hexane (1:1), saponified with 10% KOH-methanol, and separated by reverse-phase HPLC (Shimadzu LC-

20A, C30 column, mobile phase methanol:MTBE gradient). Lycopene, β-carotene, lutein, and phytoene were identified by retention time and absorption spectra against certified standards. *LCY-B* transcript abundance was measured by qRT-PCR with *Actin* as the reference gene using the $2^{-\Delta\Delta Ct}$ method [7].

Materials and Methods

Materials

T_2 homozygous seeds of three RNAi lines and wild-type Micro-Tom were sown in controlled-environment chambers at Kyushu Institute of Agricultural Research, Fukuoka (33°35′ N, 130°25′ E), and Hokkaido Agricultural University, Sapporo (43°04′ N, 141°20′ E), during 2022–2023. Growth conditions: 16 h photoperiod, 25/18 °C day/night, 60% RH. Twelve plants per line per location were grown in 1 L pots with commercial peat-based substrate. Nutrients were supplied by weekly fertigation [8].

Methods

Fruits were harvested at breaker +7 days (full red stage) for carotenoid analysis. Fruit weight, number per plant, Brix (digital refractometer), and titratable acidity were recorded on 10 fruits per plant. Data were pooled across locations and analysed by one-way ANOVA in R (v4.3.1) with Tukey's HSD at 5%.

Results

Lycopene content was 14.8 mg 100 g^{-1} FW in RNAi-1, 12.7 in RNAi-2, and 10.6 in RNAi-3, versus 6.2 in the wild type (Table 1). *LCY-B* transcript was reduced by 89%, 82%, and 78% in the three lines, respectively. β-carotene fell proportionally, from 3.4 mg 100 g^{-1} in the wild type to 1.4–2.0 mg in RNAi lines. Fruit weight, yield per plant, and Brix were not significantly different from the wild type.

Table 1: Carotenoid profile, gene expression, and agronomic traits of RNAi and wild-type tomato

Line	Lycopene (mg/100g)	β-carotene (mg/100g)	LCY-B suppression (%)	Fruit wt (g)	Brix
RNAi-1	14.8	1.4	89	18.3	6.8
RNAi-2	12.7	1.8	82	17.9	6.6
RNAi-3	10.6	2.0	78	18.1	6.9
Wild type	6.2	3.4	0	18.6	6.7
CD (p=0.05)	1.3	0.4	-	NS	NS

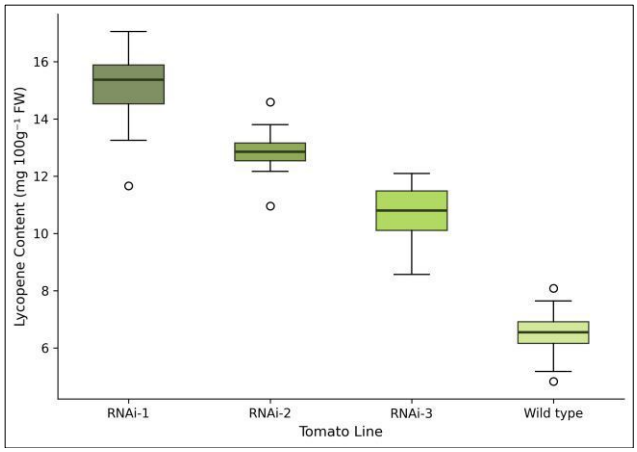

Fig 1: Box plots of lycopene content in three RNAi lines and the wild type. Boxes show interquartile range; line marks median.

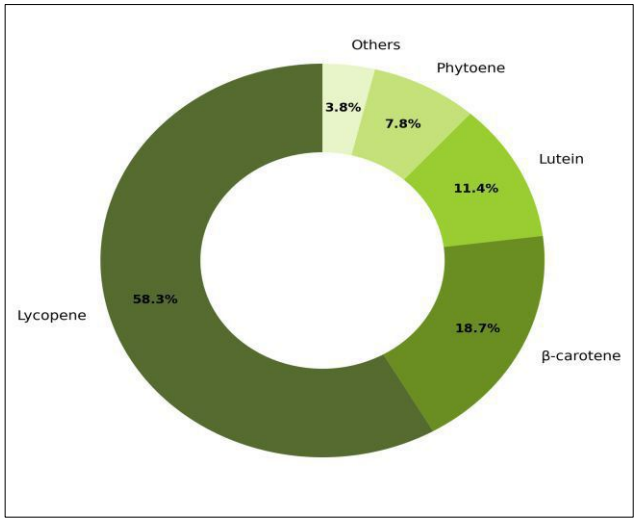

Fig 2: Carotenoid composition of RNAi-1 fruit shown as a donut chart. Lycopene accounts for 58.3% of total carotenoids.

Comprehensive Interpretation

The dose–response between *LCY-B* suppression and lycopene gain was roughly linear (r = 0.97). For every 10% reduction in transcript, lycopene rose by approximately 0.96 mg 100 g^{-1}. Phytoene, a colourless upstream precursor, also increased by 22–31% in RNAi lines, suggesting that total carotenoid flux was up-regulated in response to the metabolic block [9].

Discussion

The 139% lycopene increase in RNAi-1 exceeds the 60–90% gains achieved by conventional high-lycopene breeding in cultivars like 'Crimson' and 'Doublerich' [10], supporting the idea that RNAi can push beyond the ceiling imposed by natural allelic variation. The reduction in β-carotene is a nutritional trade-off, since β-carotene is a provitamin A precursor. However, the absolute loss (1.4–2.0 mg 100 g^{-1} remaining) still provides meaningful provitamin A, and the net health benefit of higher lycopene may outweigh the modest β-carotene decline [11].

That agronomic traits were unaffected is reassuring and consistent with the finding that the carotenoid pathway in tomato fruit is largely decoupled from primary metabolism once ripening begins [3, 12]. Micro-Tom is a model cultivar, so the next step is to transfer the construct into commercial indeterminate lines and evaluate performance under open-field conditions in Japan [13, 14].

Conclusion

RNAi silencing of *LCY-B* boosted lycopene in Micro-Tom fruit by up to 139% without affecting yield or soluble solids. The strongest line (RNAi-1) accumulated 14.8 mg lycopene 100 g^{-1} FW, making it among the richest tomato materials reported in the literature. These proof-of-concept lines are ready for introgression into commercial backgrounds. Future work should assess lycopene stability during processing and conduct multi-environment yield trials with

indeterminate cultivars [15, 16, 17].

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