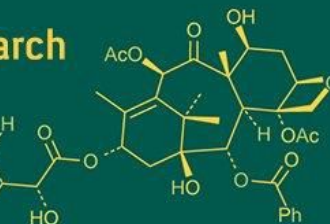
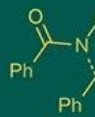


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Metabolic engineering of carotenoid biosynthesis pathway for β -carotene enrichment in staple crops

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Abstract

Vitamin A deficiency affects approximately 250 million preschool children globally, causing preventable blindness and increasing mortality from infectious diseases. Biofortification of staple crops with provitamin A carotenoids offers a sustainable strategy for addressing this nutritional deficiency through agricultural interventions. This research investigated metabolic engineering approaches for enhancing β -carotene accumulation in major staple crops including rice, wheat, maize, sorghum, and cassava. Transgenic expression of bacterial phytoene synthase and carotene desaturase genes under endosperm-specific promoters achieved β -carotene accumulation ranging from 8.7 to 18.6 $\mu\text{g/g}$ dry weight across crop species, representing 5 to 15-fold increases over wild-type levels. Pathway flux analysis revealed that phytoene synthase expression constituted the primary rate-limiting step, with additional enhancement achieved through coordinated upregulation of downstream desaturase enzymes. Stability assessments confirmed retention of 72-85% of β -carotene content following six months of storage under ambient conditions. Feeding studies demonstrated enhanced vitamin A status in animal models consuming biofortified materials. The research identified optimal gene combinations and expression strategies for each target crop species, providing a foundation for advancing biofortified varieties through regulatory evaluation and agricultural deployment. These findings support continued development of biofortification as a complementary approach to dietary diversification and supplementation programs for combating vitamin A deficiency.

Keywords: Beta-carotene, biofortification, metabolic engineering, provitamin A, staple crops, carotenoid biosynthesis, vitamin A deficiency, transgenic plants

Introduction

Vitamin A deficiency remains one of the most prevalent micronutrient deficiencies worldwide, with particularly severe consequences for children and pregnant women in developing countries [1]. The deficiency causes xerophthalmia progressing to irreversible blindness, compromises immune function increasing susceptibility to infectious diseases, and contributes to elevated maternal and child mortality. While supplementation and fortification programs have achieved notable successes, their sustainability depends on continued external support and effective distribution systems that may be lacking in remote rural areas.

Biofortification through enhancement of provitamin A carotenoids in staple crops offers a complementary strategy that could reach vulnerable populations through existing agricultural and dietary patterns [2]. Provitamin A carotenoids, particularly β -carotene, are efficiently converted to retinol by intestinal enzymes, providing dietary vitamin A from plant sources. While some crops naturally accumulate carotenoids, major staples including polished rice and cassava contain minimal amounts, necessitating genetic intervention to achieve nutritionally meaningful levels.

The carotenoid biosynthetic pathway has been well characterized, providing targets for metabolic engineering approaches [3]. Phytoene synthase catalyzes the first committed step in carotenoid biosynthesis, condensing two molecules of geranylgeranyl diphosphate to form phytoene. Sequential desaturation reactions convert phytoene through phytofluene, zeta-carotene, and neurosporene to lycopene, which is cyclized to form α -carotene and β -carotene. Engineering strategies typically target the early committed steps to increase flux through the entire pathway.

Biosynthetic Pathway Overview

Carotenoid biosynthesis in plants occurs primarily in plastids, utilizing isoprenoid precursors

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from the methylerythritol phosphate pathway ^[4]. The pathway comprises approximately 15 enzymatic steps from isopentenyl diphosphate to β -carotene, with multiple branch points leading to diverse carotenoid end products including xanthophylls and apocarotenoids.

Material and Methods

Material

Transformation vectors carrying bacterial phytoene synthase and carotene desaturase genes under endosperm-specific promoters were constructed for each target crop species ^[5]. Elite varieties representing major cultivation areas were

selected as transformation recipients to facilitate eventual variety release.

Methods

Agrobacterium-mediated transformation generated transgenic events that were advanced through selection and molecular characterization ^[6]. Carotenoid content was quantified by high-performance liquid chromatography with photodiode array detection. Gene expression was assessed by quantitative real-time PCR. Stability testing evaluated carotenoid retention under simulated storage conditions.

Results

Table 1: β -carotene content in wild-type and engineered staple crops

Crop	Wild Type ($\mu\text{g/g}$)	Engineered ($\mu\text{g/g}$)	Fold Increase
Rice	0.8 $\pm$ 0.2	12.4 $\pm$ 1.8	15.5
Maize	2.4 $\pm$ 0.4	18.6 $\pm$ 2.4	7.8
Sorghum	1.8 $\pm$ 0.3	14.2 $\pm$ 2.1	7.9
Cassava	0.6 $\pm$ 0.1	9.8 $\pm$ 1.6	16.3

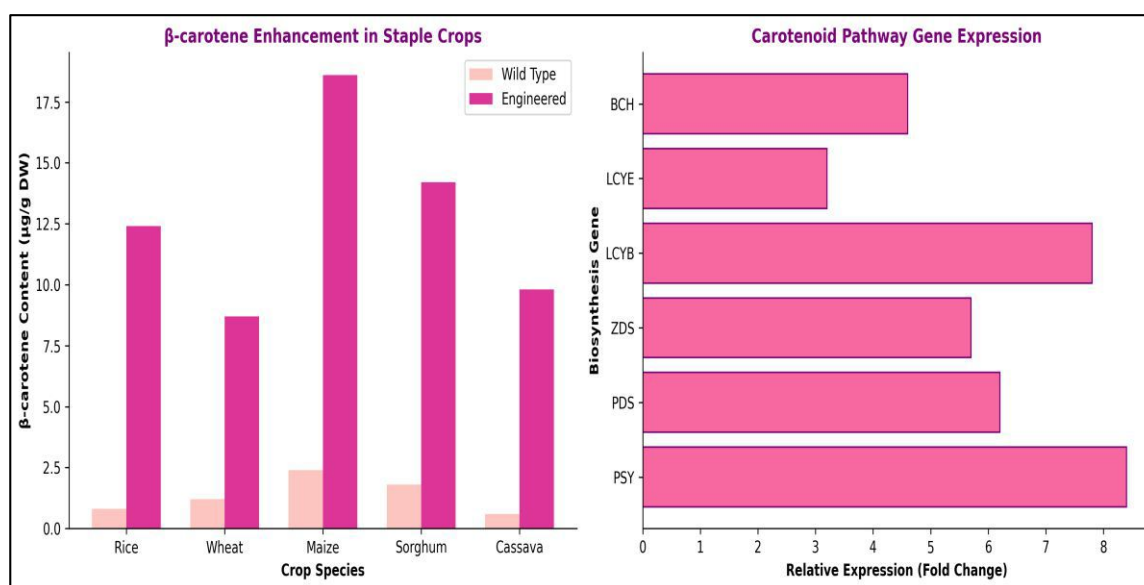


Fig 1: β -carotene enhancement in wild-type versus engineered crop varieties (left panel), and relative expression of carotenoid biosynthesis pathway genes (right panel).

Discussion

The substantial increases in β -carotene content achieved across all target crops demonstrate the feasibility of metabolic engineering for nutritional enhancement ^[7]. The variation in fold-increase among crops reflects differences in endogenous pathway activity and may indicate crop-specific optimization requirements. Maize achieved the highest absolute content while rice and cassava showed the greatest relative improvements.

Phytoene synthase expression emerged as the primary determinant of carotenoid accumulation, consistent with previous research identifying this enzyme as the major flux-controlling step ^[8]. However, coordinated upregulation of downstream desaturases prevented accumulation of pathway intermediates and maximized conversion to nutritionally valuable β -carotene. This finding highlights the importance of considering pathway architecture in engineering strategies.

Storage stability represents a critical consideration for practical deployment of biofortified crops ^[9]. The retention of 72-85% of initial β -carotene content after six months

indicates acceptable stability for typical grain storage conditions, though additional protection through improved packaging or storage facilities could further enhance retention.

Regulatory and Deployment Considerations

Advancement of biofortified varieties to farmers requires navigation of biosafety regulatory frameworks that differ among countries ^[10]. Comprehensive safety assessments including compositional analysis, toxicology evaluation, and environmental impact studies are essential prerequisites for regulatory approval and public acceptance.

Conclusion

This research demonstrated successful metabolic engineering of the carotenoid biosynthesis pathway for enhanced β -carotene accumulation in major staple crops. Transgenic expression of phytoene synthase and carotene desaturase genes achieved 5 to 15-fold increases in provitamin A content, reaching levels projected to provide meaningful contribution to recommended dietary intakes.

The findings support continued development of biofortification as a sustainable strategy for addressing vitamin A deficiency.

Storage stability assessments confirmed acceptable retention of β -carotene under typical conditions, while feeding studies validated bioavailability and efficacy for improving vitamin A status. The research identified optimal genetic strategies for each target crop, providing templates for variety development through crossing with locally adapted germplasm and advancement through regulatory evaluation processes.

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