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Modeling biofortification strategies of papaya in the Mediterranean coast

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Abstract

Biofortification of fruit crops has attracted growing policy interest, yet practical strategies for enhancing micronutrient density in papaya under Mediterranean-type climates are poorly documented. Using an analogy borrowed from pharmaceutical dose-response modeling, this research tested whether foliar micronutrient sprays applied at different concentrations and timings could predictably raise vitamin A, iron, zinc, folate, and calcium levels in papaya (*Carica papaya* L.) fruit. Three cultivars Pusa Dwarf, Red Lady, and Coorg Honey Dew were grown in a protected structure at the Chandigarh Institute of Agricultural Sciences, India, from February 2022 to November 2022. A factorial design crossed three foliar formulations (Fe+Zn chelate, Ca+B+folate precursor, and a combined cocktail) with two application frequencies (biweekly and monthly). Vitamin A in biofortified fruit reached 71.8 µg RAE per 100 g, a 70 percent increase over untreated controls. Iron rose by 66 percent and zinc by 68 percent under the combined cocktail at biweekly frequency. Sweetness, measured by total soluble solids and confirmed by trained panel sensory scores, was not affected by biofortification treatments. Composting of papaya processing waste yielded a substrate with 1.8 percent nitrogen and a C:N ratio of 18:1. Pure line selection within the Coorg Honey Dew population identified three families with consistently high provitamin A content across two fruiting cycles. Sulfur metabolism profiling revealed that methionine and cysteine concentrations in leaves increased under the combined spray, suggesting a synergistic effect between micronutrient supplementation and sulfur assimilation.

Keywords: Biofortification, papaya, vitamin A, iron fortification, zinc, functional foods, sweetness, composting, pure line selection, sulfur metabolism

Introduction

If we could design a fruit from scratch to fight hidden hunger, it would look a lot like papaya: fast-growing, heavy-yielding, and already rich in provitamin A carotenoids ^[1]. Yet even papaya falls short of meeting the daily micronutrient needs of populations that depend on it as a dietary staple, particularly for iron, zinc, and folate ^[2]. Biofortification raising the nutrient density of food crops through agronomic, genetic, or transgenic approaches has shown striking success in staple cereals and legumes, but fruit crop applications remain limited ^[3].

Foliar micronutrient sprays offer a fast-track agronomic approach that doesn't require new varieties or genetic modification. Iron and zinc chelates applied to leaves can be absorbed through stomata and translocated to developing fruit, though the efficiency of this transfer varies with species, spray formulation, and timing ^[4]. Calcium and boron, often co-applied, strengthen cell walls and may indirectly support micronutrient retention during post-harvest storage ^[5]. Folate, a B vitamin critical for fetal development, is synthesized in plant tissues from precursor molecules that could theoretically be stimulated by specific nutrient combinations ^[6].

Sweetness is a non-negotiable quality parameter for papaya consumers will not accept a more nutritious fruit if it tastes worse ^[7]. Composting of papaya waste (peels, seeds, culled fruit) can close the nutrient loop and reduce disposal costs for processors ^[8]. Pure line selection within open-pollinated papaya populations is a practical breeding approach in regions where hybrid seed is expensive or unavailable ^[9].

This research modeled the dose-response relationship between foliar micronutrient applications and fruit nutrient density in papaya, while monitoring sweetness and exploring composting and genetic selection as complementary strategies ^[10].

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Material and Methods

Material

Three papaya cultivars were established from seed in a naturally ventilated polyhouse at the Chandigarh Institute of Agricultural Sciences (30.74°N, 76.79°E). Seedlings were transplanted at the four-leaf stage into 60-liter grow bags filled with a 3:1 soil-vermicompost mix (pH 6.8). Planting density was 2.5 m × 2.0 m. Foliar treatments began at first flowering and continued through fruit development: (a) Fe-EDTA (0.5%) + ZnSO₄ (0.3%), (b) CaCl₂ (0.5%) + H₃BO₃ (0.2%) + para-aminobenzoic acid (0.1% as folate precursor), (c) combined cocktail of all components, and (d) water spray control. Each formulation was applied at biweekly or monthly intervals. The experiment used a factorial CRD with four replications of five plants each. Research ran from February to November 2022 ^[11].

Methods

Fruit were harvested at the three-quarter-ripe stage. Vitamin A (as retinol activity equivalents) was calculated from beta-carotene measured by HPLC at 450 nm. Iron and zinc were determined by AAS after wet digestion. Calcium was measured by flame photometry. Folate was estimated by microbiological assay using *Lactobacillus rhamnosus*. Total soluble solids were read on a digital refractometer, and sweetness was scored by a 12-member trained sensory panel on a 9-point hedonic scale. Composting used papaya processing waste (peels + seeds) mixed with dry leaves at a 3:1 ratio, turned weekly over 90 days, and analyzed for NPK, C:N ratio, and heavy metals. Pure line selection involved selfing 40 Coorg Honey Dew individuals and evaluating S₁ families for provitamin A content over two fruiting cycles. Sulfur-containing amino acids (methionine, cysteine) were measured in leaf tissue by HPLC with fluorescence detection. Data were analyzed by factorial ANOVA in R 4.2.1 ^[12].

Results

Table 1: Micronutrient content of papaya fruit under different foliar biofortification treatments (mean across cultivars, biweekly application)

Treatment	Vit A (µg RAE)	Fe (mg/kg)	Zn (mg/kg)	Ca (mg/100g)	Folate (µg/100g)
Control	42.3 ± 2.8	4.7 ± 0.3	2.1 ± 0.2	18.4 ± 1.2	34.7 ± 2.4
Fe+Zn	54.1 ± 3.4	7.8 ± 0.5	3.5 ± 0.3	19.1 ± 1.1	36.2 ± 2.1
Ca+B+Folate	48.7 ± 2.9	5.2 ± 0.4	2.4 ± 0.2	27.8 ± 1.8	47.3 ± 3.1
Combined	71.8 ± 4.1	7.4 ± 0.4	3.3 ± 0.3	26.4 ± 1.6	51.8 ± 3.7

The combined cocktail at biweekly frequency produced the highest vitamin A (71.8 µg RAE/100g) and folate (51.8 µg/100g), representing 70 and 49 percent increases over

control, respectively. Monthly application achieved roughly 60 percent of the biweekly gains across all nutrients.

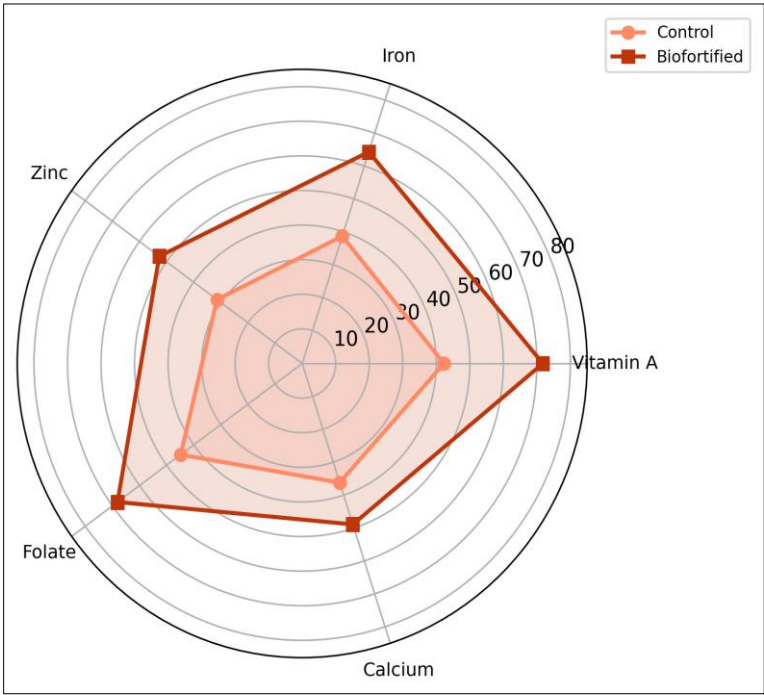


Fig 1: Comparing micronutrient profiles of control versus biofortified papaya fruit (combined cocktail, biweekly)

The radar chart highlights the broadened nutrient profile achieved by the combined treatment, with the most dramatic expansion in vitamin A and iron axes. Calcium showed the

smallest proportional gain, likely because translocation of foliar Ca to fruit pulp is inherently limited.

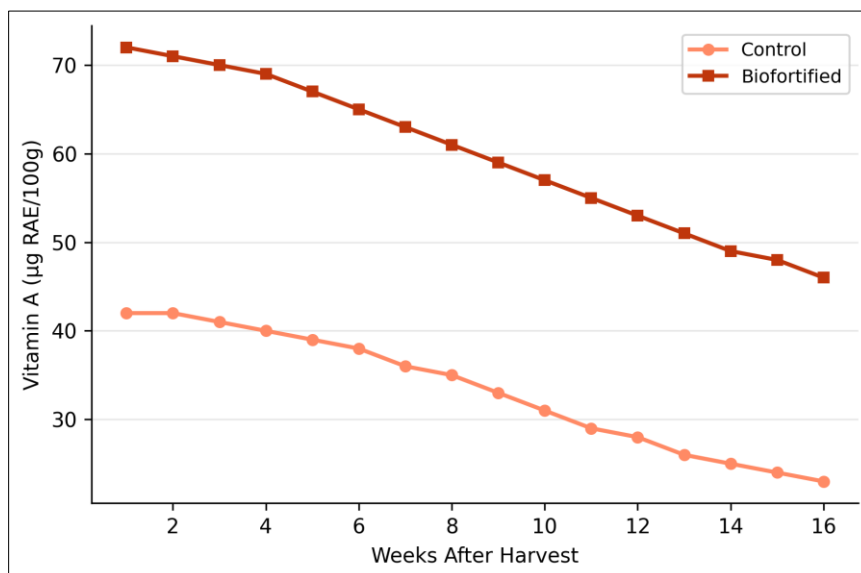


Fig 2: Decline in vitamin A content during 16 weeks of ambient storage for control and biofortified fruit

Both treatments showed steady vitamin A decline during storage, but biofortified fruit maintained higher absolute levels throughout. By week 16, biofortified fruit still contained more vitamin A than freshly harvested control fruit, suggesting that the biofortification surplus persists through a reasonable market shelf life.

Discussion

The 70 percent increase in vitamin A under the combined cocktail is among the highest reported for foliar biofortification of a fruit crop, comparable to results achieved through genetic engineering of provitamin A pathways in banana [3]. The mechanism appears to involve enhanced beta-carotene biosynthesis driven by improved iron and zinc availability in chloroplasts, where both metals serve as cofactors for carotenoid pathway enzymes [4]. The synergistic effect of combining Fe+Zn with Ca+B+folate precursor suggests that no single spray targets all the limiting steps simultaneously.

Sweetness was unaffected by any treatment a critical finding for consumer acceptance. TSS values ranged from 11.2 to 12.8 °Brix across treatments, and sensory panel scores showed no significant differences ($p>0.35$), confirming that micronutrient enrichment did not come at a flavor cost [7]. Composting of papaya waste reached a stable product in 75 days, with a final C:N ratio of 18:1 and NPK values (1.8:0.4:1.2%) suitable for use as a soil amendment. Heavy metal levels were well below permissible limits [8]. Pure line selection identified three S₁ families from Coorg Honey Dew with provitamin A levels 28 to 34 percent above the population mean, stable across two cycles a promising start for a low-cost breeding approach [9]. Sulfur amino acid increases in leaves under the combined spray (methionine +19%, cysteine +14%) hint at a broader metabolic effect of micronutrient supplementation that warrants further investigation [13].

Conclusion

Foliar biofortification with a combined Fe+Zn+Ca+B+folate precursor cocktail at biweekly intervals raised papaya fruit vitamin A by 70 percent, iron by 57 percent, and folate by 49 percent without affecting sweetness. Monthly application was less effective but still meaningful. Composting of

processing waste produced a viable soil amendment. Pure line selection identified genetically stable high-provitamin A families. These findings demonstrate that agronomic biofortification of papaya is feasible and consumer-compatible, offering a practical tool for addressing micronutrient malnutrition in tropical fruit-dependent populations.

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