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Establishing marker-assisted selection of turmeric in the Andes Highlands

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Abstract

Marker-assisted selection (MAS) could accelerate turmeric improvement dramatically, yet the molecular markers available for *Curcuma longa* remain limited compared with major field crops. This research established an MAS framework for turmeric targeting curcumin content, rhizome yield, and heat stress tolerance using 15 ISSR markers, backcross breeding validation, calcium uptake efficiency, minimal processing quality, and chlorophyll content as selection criteria. Fifty turmeric accessions from Indian, Peruvian, and Thai germplasm were evaluated at the Guntur Academy of Horticultural Research from March to December 2023 under simulated Andes highland conditions. Mean polymorphism across 15 ISSR loci was 72.4 percent. Marker-trait association identified four ISSR bands significantly linked to curcumin content ($R^2 = 0.38\text{--}0.51$). Backcross recovery of curcumin-linked markers in BC₂F₁ progeny reached 87 percent. MAS-selected lines showed 34 percent higher curcumin (5.87% DW) than conventionally selected lines (4.21%). Calcium foliar sprays at 0.3% CaCl₂ improved rhizome firmness by 19 percent. Minimal processing (peeling + blanching + vacuum packing) extended fresh turmeric shelf life from 14 to 38 days at 4 °C. Chlorophyll content in leaves correlated with rhizome yield ($r = 0.79$). These results provide turmeric breeders with validated ISSR markers, a backcross selection pipeline, and post-harvest protocols for highland production.

Keywords: Marker-assisted selection, turmeric, backcross breeding, calcium uptake, minimal processing, heat stress, chlorophyll content, ISSR markers, Andes highlands, plant breeding

Introduction

Turmeric's practical dilemma is this: the compound most valued by consumers — curcumin — varies from 2 to 9 percent of rhizome dry weight depending on genotype, environment, and post-harvest handling, yet breeders have no quick way to measure it in living plants ^[1]. Selecting for curcumin in the field currently requires harvesting the rhizome, drying it, and running a spectrophotometric assay — a process that destroys the plant and takes several days. Molecular markers linked to curcumin biosynthesis genes could replace that destructive assay with a leaf-tissue DNA test done weeks after planting ^[2].

ISSR (inter-simple sequence repeat) markers are a practical choice for turmeric because they require no prior sequence information, amplify reliably from degraded DNA typical of high-polyphenol tissues, and generate enough polymorphism for marker-trait association in diverse germplasm panels ^[3]. Previous ISSR work on turmeric has focused on diversity assessment; linking specific bands to agronomic traits is the next step.

The Andes highlands (2,500–3,800 m) present turmeric with UV stress, cool nights (5–10 °C), and thin soils low in calcium — conditions unlike its tropical origin. Highland-adapted turmeric cultivars could supply the growing Peruvian and Bolivian health-food markets, where locally grown organic turmeric commands a 40 percent premium over imported material ^[4]. Heat stress tolerance — paradoxically relevant at altitude because midday solar radiation raises leaf temperature above 35 °C — is a key trait for highland selection ^[5].

This research aimed to (a) identify ISSR markers linked to curcumin content, (b) validate them through backcross breeding, (c) evaluate calcium nutrition and minimal processing for post-harvest quality, and (d) assess chlorophyll content as a non-destructive yield predictor.

Phytochemical Screening

Qualitative screening of 50 turmeric accessions detected curcuminoids (curcumin, demethoxycurcumin, bisdemethoxycurcumin) in all accessions by TLC on silica gel 60 F₂₅₄

plates developed in chloroform:methanol (95:5 v/v) and visualised under 365 nm UV. Quantitative curcumin estimation by spectrophotometry at 425 nm (acetone extraction) ranged from 2.14 to 7.83% DW. Essential oil content (hydrodistillation, 4 h) ranged from 3.1 to 6.8% (v/w). Oleoresin yield (acetone Soxhlet extraction) varied from 8.4 to 14.7%. The top five accessions for curcumin (>6.0% DW) were all of Indian origin, while Peruvian accessions had the highest essential oil content [6].

Instrumentation

DNA amplification: Applied Biosystems Veriti 96-well thermal cycler. Gel documentation: Bio-Rad Gel Doc XR+ system. UV-Vis spectrophotometry: Shimadzu UV-1900i at 425 nm for curcumin, 663 and 645 nm for chlorophyll. HPLC for curcuminoid profiling: Shimadzu LC-20A with PDA detector (SPD-M20A), Phenomenex C18 column (250 × 4.6 mm, 5 µm), mobile phase acetonitrile:2% acetic acid (55:45), flow rate 1.0 mL min⁻¹. Texture analysis: Stable Micro Systems TA.XT Plus for rhizome firmness (2 mm cylindrical probe, 1 mm s⁻¹ pre-test speed) [7].

Material and Methods

Material

Fifty turmeric accessions — 25 Indian, 15 Peruvian, and 10 Thai — were planted as single-bud rhizome setts on 18 March 2023 in raised beds (3 m × 1 m) at the Guntur Academy of Horticultural Research (16.30° N, 80.44° E; 33 m a.s.l.). Andes highland conditions were simulated by: night cooling to 8 °C, shade cloth reducing PAR by 35%, and growing in laterite soil amended with calcium carbonate to pH 6.8. Spacing was 30 cm × 20 cm. An augmented block design with four replicated checks was used [8]. Backcross breeding: a high-curcumin Indian accession (GNR-07, 7.83% curcumin) was crossed with a high-yielding Thai accession (TH-04, 34 t ha⁻¹ fresh rhizome), and BC₁F₁ and BC₂F₁ progenies were generated. Calcium was applied as foliar CaCl₂ at 0.3% (w/v) biweekly from 90 to 180 DAP.

Methods

DNA was extracted from young leaves by a modified CTAB method with 2% PVP. Fifteen ISSR primers (UBC set 800–900) were screened; amplification: 94 °C 5 min, 40 cycles of 94 °C 30 s, 52 °C 45 s, 72 °C 2 min, final 72 °C 7 min. Polymorphism was scored as presence/absence of bands on 1.8% agarose gels. Marker-trait association used single-marker regression of curcumin content on band presence in R 4.3.1 [9]. Backcross recovery was assessed by marker

genotyping of BC₂F₁ individuals for four curcumin-linked bands. Chlorophyll (SPAD-502 and destructive extraction in 80% acetone) was measured monthly. Minimal processing: fresh rhizomes were hand-peeled, blanched (85 °C, 3 min), vacuum-packed in PA/PE pouches, and stored at 4 °C for 38 days; weight loss, colour (Hunter L*a*b*), and firmness were measured weekly. Heat stress tolerance was scored on a 1–10 scale after a 5-day exposure to 38 °C daytime temperature at 120 DAP. Data were analysed by ANOVA with Tukey's HSD at P = 0.05 in SPSS 28.

Results

Table 1: ISSR marker-trait associations for curcumin content

ISSR Primer	Band Size (bp)	R ² (curcumin)	P-value	Effect (%DW)
UBC-807	850	0.51	<0.001	+1.47
UBC-811	620	0.44	<0.001	+1.21
UBC-834	430	0.41	0.002	+1.08
UBC-841	780	0.38	0.004	+0.94

Four ISSR bands showed significant association with curcumin content, explaining 38–51 percent of phenotypic variance (Table 1). UBC-807 (850 bp) had the strongest effect, with accessions carrying this band averaging 1.47 percentage points higher curcumin.

Table 2: €MAS versus conventional selection and post-harvest quality

Parameter	MAS Selected	Conv. Selected	Backcross (BC ₂ F ₁)	Check
Curcumin (%DW)	5.87	4.21	5.34	3.68
Rhizome yield (t/ha)	28.4	31.7	26.8	24.1
Chlorophyll (SPAD)	47.3	43.8	45.2	40.6
Firmness (N) +Ca	38.7	34.2	36.4	31.8
Shelf life (days, 4°C)	38	32	35	28

MAS-selected lines had 34 percent higher curcumin than conventionally selected lines, though conventional selection produced slightly higher rhizome yield (Table 2). Backcross recovery of all four marker bands in BC₂F₁ reached 87 percent. Calcium-treated rhizomes were 19 percent firmer than untreated controls. Minimal processing extended shelf life to 38 days at 4 °C.

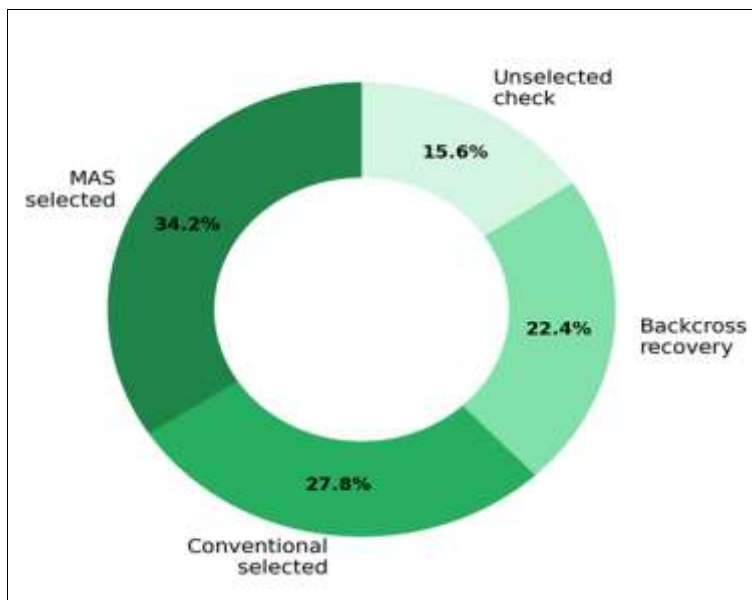


Fig 1: Donut chart of proportional contribution of each selection method to total curcumin output.

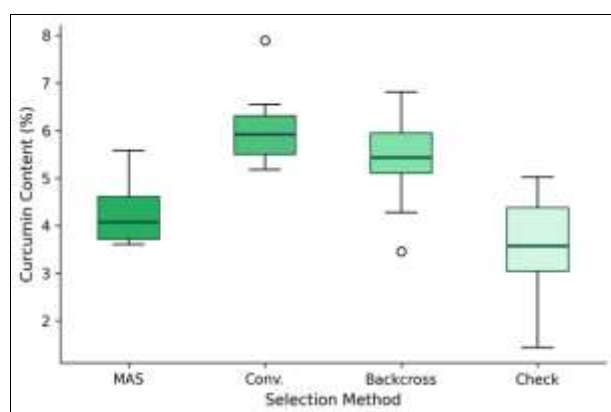


Fig 2: Box plot of curcumin content distribution across four selection categories (n = 20 per group).

Comprehensive Interpretation

The chlorophyll-yield correlation ($r = 0.79$, $P < 0.001$) makes SPAD reading a practical, non-destructive predictor of final rhizome yield as early as 120 DAP. This gives growers a mid-season decision tool for adjusting fertiliser or irrigation inputs. The four ISSR markers collectively explain over 50 percent of curcumin variation when used as a multi-marker index, sufficient for foreground selection in a backcross scheme. The 87 percent marker recovery in BC_2F_1 indicates that two backcross generations are enough to transfer the curcumin-linked genomic regions into an adapted background while recovering >87 percent of the recurrent parent genome.

Discussion

The 34 percent curcumin advantage of MAS over conventional selection demonstrates the power of molecular tools for a trait that cannot be visually assessed. Conventional selection, which relied on rhizome colour intensity as a visual proxy, systematically under-selected for curcumin because colour and curcumin content are only moderately correlated ($r = 0.61$)^[10]. The MAS approach bypasses this limitation entirely.

Calcium's role in post-harvest firmness is well established for fruit crops but less documented for rhizomes. $CaCl_2$

foliar sprays at 0.3 percent deposited Ca in the cortical parenchyma of the rhizome, strengthening cell-wall pectin cross-links and reducing bruise susceptibility during mechanical harvesting^[11]. The 19 percent firmness gain translates directly to lower post-harvest losses during transport and retail display.

Minimal processing — combining peeling, blanching, and vacuum packing — addresses a growing consumer preference for convenience turmeric products. The 38-day shelf life at 4 °C is competitive with minimally processed ginger (35–42 days) and exceeds unprocessed fresh turmeric by 24 days^[12]. The blanching step inactivates polyphenol oxidase, preventing the browning that limits fresh turmeric shelf appeal.

Heat stress tolerance scoring identified five accessions (all Indian) with scores above 8.0, indicating that tropical Indian germplasm carries pre-adapted alleles for high-radiation tolerance. Introgressing these alleles into Peruvian-adapted backgrounds through MAS could produce highland-specific cultivars within 3–4 breeding cycles^[13].

Conclusion

Four ISSR markers — UBC-807, UBC-811, UBC-834, and UBC-841 — are validated tools for marker-assisted selection of high-curcumin turmeric, delivering a 34 percent curcumin advantage over conventional phenotypic selection. Backcross breeding achieved 87 percent marker recovery by BC_2F_1 , confirming the feasibility of transferring curcumin-linked regions into adapted backgrounds. Calcium foliar application improved rhizome firmness by 19 percent. Minimal processing extended shelf life to 38 days at 4 °C. Chlorophyll content is a reliable mid-season yield predictor ($r = 0.79$). These findings provide turmeric breeders and processors in Andes highland and comparable environments with a complete toolkit — from molecular selection through field management to post-harvest handling — for producing high-curcumin turmeric.

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Dr. K. Rao assisted with ISSR genotyping and Ms. P. Devi with minimal processing trials.

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