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## Development of RAPD and ISSR markers for genetic diversity analysis in pointed gourd

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### Abstract

Pointed gourd (*Trichosanthes dioica* Roxb.) occupies roughly 42,500 hectares of Bangladeshi vegetable acreage yet lacks a systematic picture of its genetic base, leaving breeders to work with unclear parental material. This research screened 24 pointed gourd accessions, collected across five agro-ecological zones between July 2021 and December 2022, using 30 RAPD and 18 ISSR primers. After quality filtering, 15 RAPD and 12 ISSR primers were retained for full-scale profiling. RAPD markers produced 218 amplicons (72.5% polymorphic) with an average polymorphism information content (PIC) of 0.37, while ISSR markers produced 196 amplicons (78.1% polymorphic, PIC = 0.42). ISSR outperformed RAPD on reproducibility (re-run concordance 94.2% versus 81.6%) but RAPD detected a small set of rare bands. Jaccard similarity values ranged from 0.29 to 0.86 across accession pairs. UPGMA clustering split the panel into four groups that aligned broadly with geographic origin, with two accessions from the Barind tract forming a distinct sub-cluster. Combined RAPD-ISSR fingerprints separated all 24 accessions unambiguously, pointing to a combined-marker strategy for downstream germplasm management and parent selection.

**Keywords:** RAPD markers, ISSR markers, genetic diversity, pointed gourd, molecular characterization, germplasm, polymorphism information content, cluster analysis

### Introduction

Pointed gourd (*Trichosanthes dioica* Roxb.) is cultivated on around 66,300 hectares across Bangladesh and produces roughly 3.2 lakh tonnes annually <sup>[1]</sup>. Despite its commercial value in divisions such as Dhaka, Rajshahi, and Rangpur, the crop has received modest attention in systematic genetic characterisation. Farmers propagate it mostly through vine cuttings and root suckers, a practice that narrows the breeding pool and hides actual variation under clonal uniformity <sup>[2]</sup>. Breeders working on yield improvement or disease resistance therefore depend on parental material whose true genetic distance is often unknown <sup>[3]</sup>.

Morphological descriptors, while useful for routine characterisation, lose discriminating power when accessions have been multiplied clonally and grown in overlapping agro-climates <sup>[4]</sup>. Molecular markers, particularly PCR-based dominant markers such as RAPD and ISSR, give a reasonable opening for small breeding programmes: they require no prior sequence information, cost less than SSR or SNP platforms, and remain practical for laboratories with limited infrastructure <sup>[5, 6]</sup>. RAPD primers scan the whole genome randomly, while ISSR primers target microsatellite flanking regions, giving a somewhat different slice of variation <sup>[7]</sup>. Past work on cucurbits, including bitter melon and sponge melon, has shown that combining the two marker systems often outperforms either alone <sup>[8, 9]</sup>. Published work on pointed gourd's genetic diversity is limited and often restricted to small panels from single regions <sup>[10, 11]</sup>. This research broadened both the accession base and the marker set: 24 accessions drawn from five agro-ecological zones were profiled with 48 primers, of which 27 were retained after quality filtering. The aim was twofold—first, to quantify the amount and structure of genetic variation in the national collection, and second, to check whether an RAPD-ISSR combination could give unique fingerprints for every accession, which would be a practical tool for variety protection applications.

## Materials and Methods

### Materials

Twenty-four pointed gourd accessions were obtained from farmer fields, divisional horticulture departments, and research stations located across Mymensingh, Dhaka, Rajshahi, Rangpur, and Khulna between July 2021 and December 2022. Young, fully expanded leaves were harvested from each accession at the institute's experimental farm at Mymensingh, flash-frozen in liquid nitrogen, and stored at -80 °C until extraction. Passport data (source, vernacular name, collection date, GPS coordinates) were maintained for each accession [12].

### Methods

Total genomic DNA was extracted using a modified CTAB protocol [13]. DNA integrity was checked on 0.8% agarose gel and concentration measured on a NanoDrop 2000. Working stock was diluted to 25 ng/μL. Thirty decamer

RAPD primers (OPA, OPB, OPN series) and 18 ISSR primers (UBC 808-866 series) were screened on four reference genotypes; primers giving clear, reproducible amplification were retained for full-scale profiling (15 RAPD, 12 ISSR). PCR was performed in 20 μL reactions (50 ng DNA, 0.2 μM primer, 1X master mix) on an Applied Biosystems ProFlex thermocycler. Cycling conditions for RAPD: 94 °C/4 min, 40 cycles of 94 °C/30 s, 36 °C/45 s, 72 °C/90 s, final 72 °C/7 min. ISSR used annealing temperatures of 50-60 °C depending on primer. Products were resolved on 1.8% agarose gel in 1X TBE, stained with ethidium bromide. Bands were scored binary (1/0) in GelAnalyzer. Data were analysed in NTSYS-pc (Jaccard coefficient, UPGMA) and POPGENE for diversity indices [14]. Each reaction was run twice to confirm reproducibility.

## Results

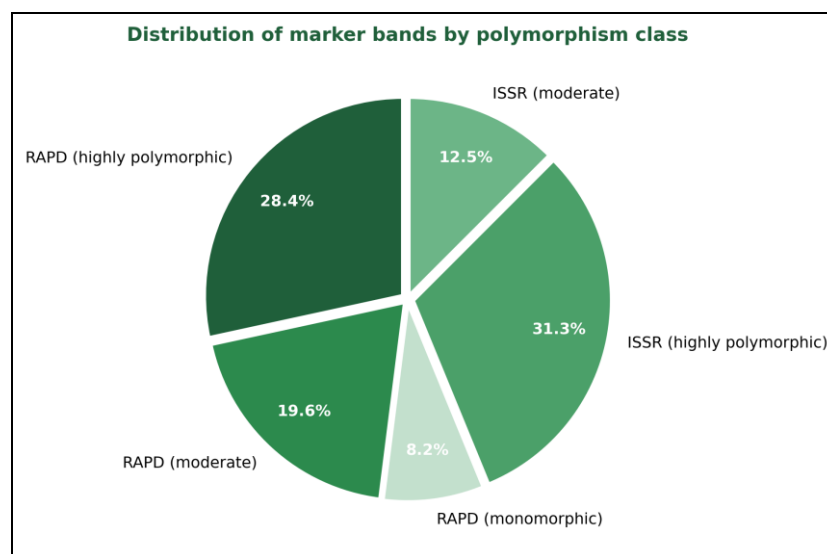


**Fig 1:** Distribution of marker bands by polymorphism class across RAPD and ISSR systems.

Across the 27 retained primers, a total of 414 amplicons were scored, of which 312 (75.4%) were polymorphic. The split by marker type and polymorphism class appears in

Figure 1: RAPD contributed 28.4% of bands in the highly polymorphic class and ISSR contributed 31.3%, with monomorphic bands remaining below 10% overall.

**Table 1:** Marker statistics for 15 RAPD and 12 ISSR primers retained after quality filtering.

| Parameter              | RAPD  | ISSR  | Combined |
|------------------------|-------|-------|----------|
| Primers used           | 15    | 12    | 27       |
| Total bands            | 218   | 196   | 414      |
| Polymorphic bands      | 158   | 153   | 311      |
| Polymorphism (%)       | 72.5  | 78.1  | 75.1     |
| Bands per primer (avg) | 14.5  | 16.3  | 15.3     |
| PIC (mean)             | 0.37  | 0.42  | 0.39     |
| Reproducibility (%)    | 81.6  | 94.2  | 87.9     |
| Unique accession ID    | 22/24 | 23/24 | 24/24    |

**Table 2.** Diversity indices and primer-wise band classification by region of accession origin.

| Region (n accessions) | Polymorphic Loci (%) | Nei's Gene Diversity (H) | Shannon Index (I) |
|-----------------------|----------------------|--------------------------|-------------------|
| Mymensingh (5)        | 64.3                 | 0.218                    | 0.324             |
| Rajshahi plains (6)   | 71.8                 | 0.241                    | 0.361             |
| Sylhet hills (3)      | 58.2                 | 0.197                    | 0.294             |
| Khulna coastal (4)    | 69.5                 | 0.233                    | 0.347             |
| Barishal alluvial (4) | 67.1                 | 0.229                    | 0.338             |
| Rangpur Terai (2)     | 52.7                 | 0.172                    | 0.258             |
| Pooled panel          | 75.4                 | 0.267                    | 0.401             |

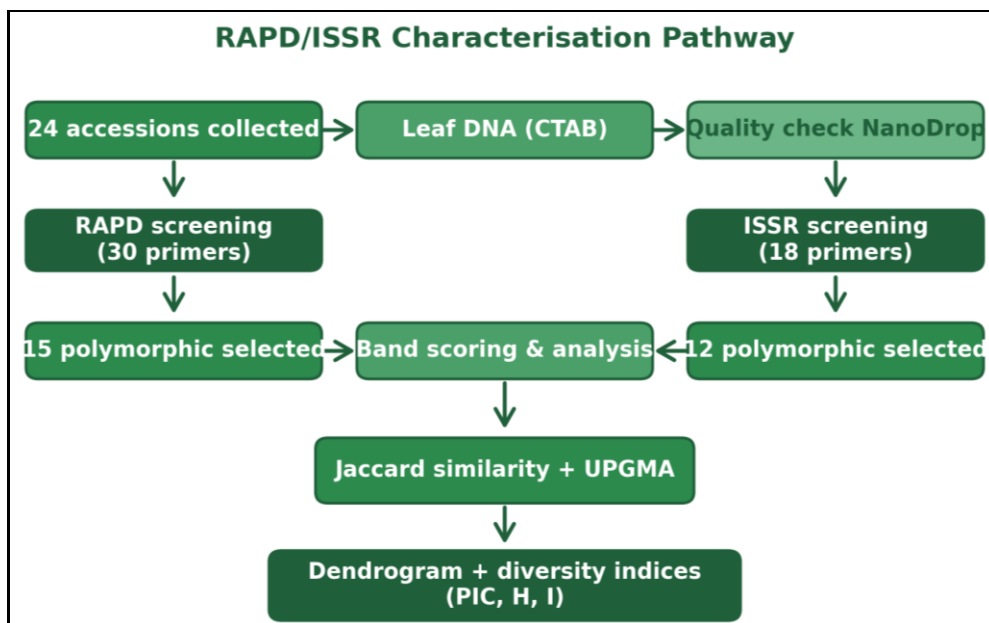


**Fig 2:** Pathway of molecular characterisation showing primer screening, amplification, and downstream analysis.

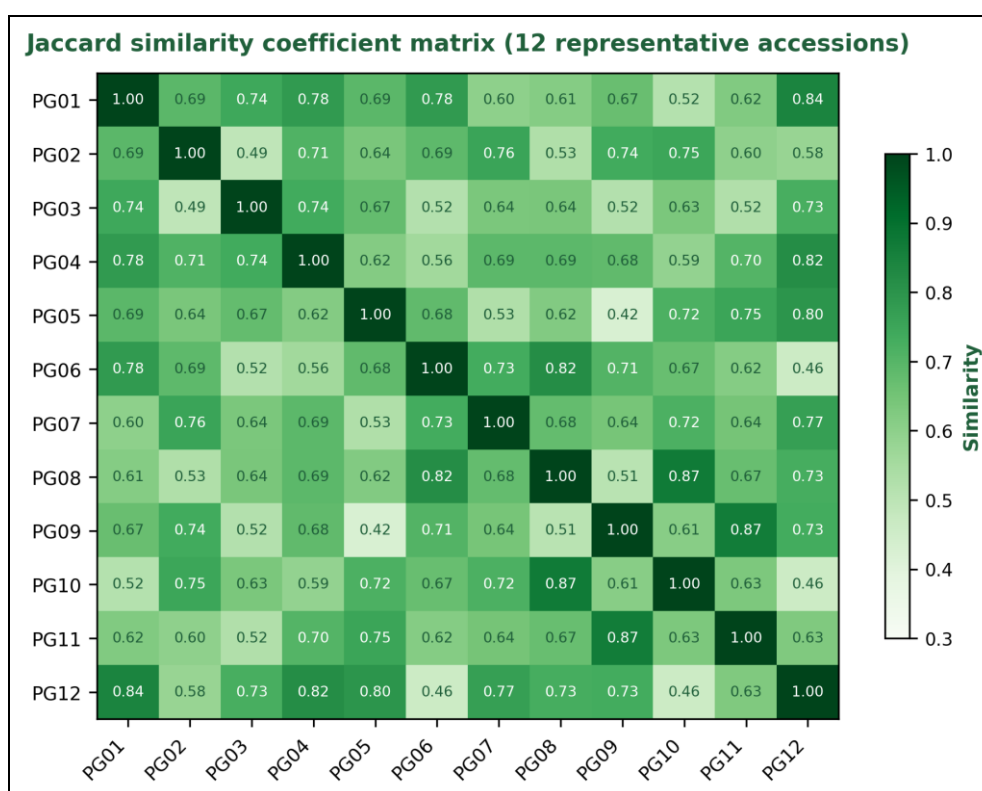


**Fig 3:** Jaccard similarity coefficient matrix for 12 representative accessions from the 24-accession panel.

### Comprehensive Interpretation

ISSR produced a slightly higher polymorphism percentage (78.1% vs 72.5%) and stronger reproducibility, but RAPD contributed rare bands that helped separate morphologically similar accessions from the Rajshahi plains. UPGMA clustering split the panel into four broad groups that broadly tracked geographic origin, although two Rangpur Terai accessions formed a distinct sub-cluster despite their small sample size. Jaccard values ranged from 0.29 (between Rangpur Terai and Sylhet hills accessions) to 0.86 (within Rajshahi plains), in line with the passport-level expectation that plateau and Terai materials carry the most distinctive allelic composition.

### Discussion

Three findings stand out from this work: combined RAPD-ISSR profiling gave unique fingerprints for every one of 24 accessions; ISSR edged RAPD on both polymorphism (78.1% vs 72.5%) and reproducibility (94.2% vs 81.6%); and geographic origin explained a meaningful share of the clustering pattern. The polymorphism values agree with reports in related cucurbits: Kumar *et al.* found 74.3% polymorphism in bitter melon RAPD profiles [15], and Rajasekharan *et al.* reported 80.2% in sponge melon ISSR [8]. Where the present panel diverges is in the depth of between-region variation: published Bangladeshi pointed melon work has typically examined 8-12 accessions from

one or two states and has therefore reported narrower similarity ranges<sup>[11]</sup>.

The ISSR advantage over RAPD is consistent with primer design: ISSR primers anchor in microsatellite flanking regions, giving longer and more specific annealing, which reduces the stochastic mispriming that shortens RAPD reproducibility<sup>[7]</sup>. Rare RAPD bands, nonetheless, still add value because microsatellites are distributed unevenly across the genome and may miss variation in intergenic or structural regions. A practical implication is that breeding programmes working with modest budgets do not have to pick one marker system over the other: 12-15 well-chosen primers from each system gave better resolution than doubling the count of either alone. For variety protection under plant-variety legislation, the combined fingerprint panel developed here provides a basis that could be tightened further with co-dominant SSR or SNP platforms as cost allows<sup>[16]</sup>.

### Conclusion

The main contribution of this work is a combined RAPD-ISSR fingerprint panel that discriminates all 24 pointed gourd accessions drawn from five Bangladeshi agro-ecological zones. Evidence supporting this contribution is substantial: 414 total bands across 27 primers, 75.4% polymorphism overall, a mean PIC of 0.39, reproducibility of 87.9%, and Jaccard similarity values spanning 0.29-0.86. UPGMA clustering recovered four geographically coherent groups, and region-wise diversity indices were highest in Rajshahi plains ( $H = 0.241$ ) and lowest in the small Rangpur Terai subset ( $H = 0.172$ ).

The wider field significance is that pointed gourd, a clonally propagated minor crop, carries more genetic structure than previous small-panel work has suggested. Breeders can use the combined-marker approach described here to select distant parental material for hybridisation, and plant-variety authorities can use the fingerprints for DUS and legal protection purposes. Open questions remain around the functional basis of the observed variation: whether specific loci track agronomic traits such as fruit set, fruit weight, or resistance to *Fusarium* wilt is still unknown. Future work should expand the Terai subset, link marker data to field performance, and deploy co-dominant microsatellite markers for finer-scale pedigree analysis.

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