

## BIOLOGICAL ASSORTMENT OF CURCUMA LONGA L. (TURMERIC) USING RAPID MARKERS

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### ARTICLE INFO

### ABSTRACT

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Turmeric (*Curcuma longa* L.) is presently taken into consideration an ability supply of the latest pills for quite a few sicknesses because of the presence of curcuminoids, the maximum vital being curcumin. The lifestyle of genetic range in turmeric is documented in different international locations however now no longer within side the Philippines, in which turmeric is an added species. This has a look at objectives to decide the feasible lifestyles of genotypic range amongst turmeric accessions in Mindanao, Philippines the use of random amplified polymorphic DNA (RAPD) markers. Ten random primers had been used for RAPD evaluation from which a complete of 209 amplification merchandise had been generated. Almost all of the bands generated had been polymorphic indicating the genetic range of many of the accessions. Six primers (OPD08, OPB07, OPA11, OPA12, OPC05, and OPN16) had polymorphic records content material values of virtually 0.5, thus, had been capable of discriminating among the turmeric genotypes. Effective multiplex ratio and marker index had been additionally fairly excessive for the identical six markers besides for OPA12.<sup>(12)</sup>

### KEYWORDS:

Turmeric; *Curcuma Longa* L; Accession; Biological Assortment;

### I. INTRODUCTION

Turmeric (*Curcuma longa* L.) is a tropical perennial herb belonging to own circle of relatives Zingiberaceae. It turned into at the start valued in particular as a spice for meals and herbal dye for apparel till these days whilst it turned into found as a capability supply of new capsules for a range of diseases. Its significance in remedy began out with the discovery that the dried rhizome of the plant is wealthy in phenolics, diagnosed as curcuminoids, in particular curcumin or diferuloyl methane. Some of the organic sports and healing residences attributed to curcumin had been anti-

inflammatory, anti-oxidant, anti-carcinogenic, wound healing, and anti-viral properties<sup>1</sup>. Turmeric is a cross-pollinated, sterile, triploid species that is clonally propagated the use of its underground rhizomes. Though vegetative propagation is the normal method of reproduction, numerous research have proven the lifestyles of genetic variant within side the species. Nayak et al.<sup>2</sup> turned into capable of stumble on genetic variant amongst 17 promising cultivars of turmeric the use of 4C nuclear DNA content material and random amplified polymorphic DNA (RAPD) evaluation. The good-sized variant in 4C DNA content material recorded turned into hypothesized to be because of

loss or addition of enormously repetitive sequences with inside the genome. RAPD evaluation additionally discovered intra specific polymorphism a few of the 17 cultivars, starting from 35.6 % to 98.6 %. Among the 20 RAPD primers used, primers (OPN06 and OPA04) confirmed sturdy resolving electricity and had been capin a position to distinguish all 17 cultivars. Similarly, Leong-Skornickova et al.<sup>3</sup> found extra than 9 % genome length variant in accessions of *C. longa* having the equal chromosome number.

| Nomenclature |                                  |     |             |
|--------------|----------------------------------|-----|-------------|
| RAPD         | random amplified polymorphic DNA | μl  | microliter  |
| DNA          | deoxyribonucleic acid            | mM  | milli Molar |
| m            | meter                            | V   | Volt        |
| in           | inch (1 inch = 2.54 cm)          | h   | hour        |
| mo           | month                            | min | minute      |
| bp           | base pair                        | U   | unit        |

They counseled that the found intra-precise variant can be associated with the long-time period cultivation and centered choice of suitable genotypes in *C. longa*, which in flip may also have an adaptive fee to the crop. On the different hand, the use of microsatellite markers, a particularly low basic genetic variability turned into found amongst 39 turmeric accessions from a Brazilian germplasm collection<sup>4</sup>. Though genetic variant turned into found inside the states wherein turmeric is cultivated, the general loss of polymorphism turned into attributed to the feasible advent of just a few genotypes in Brazil. In the Philippines, wherein turmeric is reportedly an brought species, small-scale business planting has begun out and numerous groups growing herbal-primarily based totally meals dietary supplements and different neutraceutical merchandise regularly have turmeric as one of the ingredients. In Mindanao, turmeric is processed into tea granules and pills each of which are broadly used via way of means of practitioners of opportunity remedy.

However, in spite of the developing hobby on the many capability fitness advantages that may also be derived from turmeric, no complete observe has been made on Philippine turmeric. Identity of the species that are being cultivated as nicely as the feasible lifestyles of genetic variant because of various introductions that could make a contribution to one-of-a-kind chemical and molecular profiles has now no

longer been verified. This observes consequently objectives to check or examine genetic variant amongst the one-of-a-kind turmeric accessions from Mindanao, Philippines the use of RAPD markers.<sup>(12)</sup>

## MATERIALS AND METHOD

### 2.1. Collection Of Turmeric Rhizomes

Turmeric rhizomes had been acquired from specific localities in seven provinces in Mindanao particularly, Davao del Sur, Davao del Norte, South Cotabato, North Cotabato, Lanao del Sur, Sultan Kudarat and Maguindanao. These had been specific as turmeric accessions (Acc) 1-17 and 19-22. For comparison, turmeric rhizome pattern acquired from Chiang Mai, Thailand become specific as turmeric accession 18. Collected rhizomes of every accession had been washed easy of particles and air-dried for 2 days. Air-dried rhizome bits/finger rhizomes had been then used as planting materials.<sup>(12)</sup>

### 2.2.Maintenance Of Turmeric Accessions Withinside The Area (Field)

The specific turmeric accessions had been planted in a 500 m<sup>2</sup> area. Rhizome bits of every accession had been to start with planted in large plastic pots (10 rhizome bits in keeping with bag) containing lawn soil mix. Germinated buds had been for my part replanted in small plastic baggage containing the equal lawn soil mix. Ten flowers of every accession had been replanted in large plastic baggage (24 in × 36 in) containing bagging media composed of 70 % coconut coir and 30 % soil, after which randomly laid out with inside the area. All flowers had been watered every day besides whilst heavy rain came about the preceding night time or at some stage in the day. Monthly fertilization the usage of entire fertilizer or ammonium sulfate becomes carried out. Vermicast included with *Trichoderma* become delivered a month after replanting in large plastic baggage. Spraying of insecticide (Decis) become carried out on every occasion insect infestation becomes observed. Rhizomes of every accession had been harvested whilst the decreased leaves of the flowers began out to wither, approximately 7

month to eight month after planting, relying at the accession.

(12)

### 2.3. Preparation Of Leaf Samples

Rhizome's bits harvested from tagged flowers of the 22 accessions had been planted in plastic pots containing lawn soil mix. Tender, younger leaves from flowers that advanced and grew had been acquired and served as supply of DNA for every accession. Two extra accessions belonging to Family Zingiberaceae had been protected for functions of comparison. These accessions specific as Acc 23 and 24, which had been acquired from Iligan and Zamboanga del Norte, respectively, had been known as turmeric via way of means of the locals however their morphology become notably specific from the relaxation of the accessions and did now no longer healthy the morphological description of *C. longa*. Fresh leaves of every accession had been cautiously washed with cleaning soap and water, blotted dry with absorbent paper then positioned in for my part categorized plastic baggage. All accumulated leaves had been positioned in ice chest with ice percent at some stage in shipping to the laboratory for genomic DNA extraction. (12)

### 2.4. Genomic Dna Extraction And Rapd Evaluation

Genomic DNA extraction becomes carried out following the manner defined previously<sup>5</sup>. The extracted DNA pellet becomes re-suspended in 50 µl to a hundred µl Tris-EDTA buffers and saved at -20°C previous to RAPD evaluation. Ten arbitrary decamer primers, particularly OPA11, OPA12, OPA18, OPB07, OPC02, OPC05, OPN04, OPN06, OPN16 and OPD08 (AITbiotech Singapore), had been used for RAPD evaluation. These primers, that have been utilized in in advance studies, confirmed robust resolving strength and had been capin a position to differentiate turmeric cultivars. RAPD evaluation become accomplished following the manner described<sup>6</sup>. Each amplification of 25 µl response extent contained five µl of five × assay buffer, one. five µl of 25 mM MgCl<sub>2</sub>, 0.2 µl of five U · µl<sup>-1</sup> DNA Taq Polymerase (PromegaGoTaq® Green Promega Corporation, USA), zero. five µl of 10 mM dNTP mix (Invitrogen), two. Five µl of 10 PM particular primer, 1 µl DNA template and 14.3 µl DEPC water (Invitrogen). The amplification response becomes performed the usage of a thermal cycler

(Veriti ninety-six properly Thermal Cycler, Applied Bio systems, USA). Amplification becomes accomplished in 3 steps. First, the pattern becomes maintained at 94 °C for five min for entire de-naturation of template DNA. The 2nd step consisted of forty-two cycles, every cycle with 3 temperature regimes particularly, 92 °C for 1 min for denaturation of template DNA, 37 °C for 1 min for primer annealing and seventy-two °C for two min for primer extension, accompanied via way of means of entire polymerization at seventy-two °C for 7 min. Polymerase chain response (PCR) ended with an indefinite preserve at a soaking temperature of four °C. The PCR merchandise had been then saved at -20 °C previous to gel electrophoresis. (12)

### 2.5. Agarose Gel Electrophoresis

Four microliters of every amplified PCR product had been separated on 15% agarose gel in 1 × Tris-acetate-EDTA buffer for three.five h at a steady voltage of seventy five V, stained with Gel Red nucleic acid stain, visualized and photographed the usage of a gel documentation gadget (Bio Rad Gel Doc California USA). The amplicon length becomes decided via way of means of evaluating with a 1 kb Plus DNA ladder (Invitrogen). (12)

### 2.6. Data Evaluation

The banding sample acquired for every accession generated from electrophoresis of the amplified segments of DNA had been represented in a binary shape and scored as “1” Or band presence and “0” for absence for every primer genotype mixture. The polymorphic statistics content material (PIC) which degree the distinguishability of genotypes for every primer mixture changed into computed the use of the formula:

$$PIC^1 = 1 - \sum p_{ij}^2 \quad (1)$$

In which  $p_{ij}$  represents the  $j$ th allele for primer 1 and the summation extends for  $n$  variety of alleles.

Cluster evaluation changed into executed the use of the Un weighted Pair Group Mean Algorithm (UPGMA) thru the

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Power Marker ver. 3.25 software<sup>7</sup> the use of the similarity index as defined previously<sup>8, (12)</sup>

### 3. RESULTS AND DISCUSSION

To decide the lifestyles of genetic variety amongst the one-of-a-kind turmeric accessions from Mindanao, molecular characterization changed into executed the use of RAPD evaluation. A general of 10 random primers had been used to make bigger gene loci in the genome of the one-of-a-kind turmeric accessions. RAPD evaluation has in advance been used to check genetic variety in turmeric<sup>9,10</sup>.

From the electrophoregrams of the PCR amplicons (records now no longer shown), the ten random primers produced a complete of 209 amplification merchandise with sizes ranging from one hundred thirty bp to 2 a hundred bp. The variety of scored bands consistent with primer from 14 (OPN06) to 25 (OPB07 and OPC05), the median was 20.9 according to the primers (Table 1). Almost all bands generated with the help of primers were polymorphic, with the exception of OPN06 and OPA11, which were 14 and 24 general generated bands, respectively, changed into monomorphic (Table 2). The discovered excessive possibilities of polymorphic bands generated by the use of the one-of-a-kind primers imply the lifestyles of genetic variety with inside the turmeric accessions.

Table 1. Description of primers and PCR amplicons generated from RAPD analysis of the turmeric accessions and two outgroup species belonging to Family Zingiberaceae.

| RAPD Primer | Sequence (5'-3') | Amplified bands | Size range of amplicons (bp) |
|-------------|------------------|-----------------|------------------------------|
| OPN 06      | GAGACGCACA       | 14              | 250 to 1 200                 |
| OPD 08      | GTGTGCCCA        | 24              | 310 to 1 400                 |
| OPA 18      | AGGTGACCGT       | 20              | 150 to 1 400                 |
| OPB 07      | GGTGACGCAG       | 25              | 490 to 2 000                 |
| OPA 11      | CAATCGCCGT       | 24              | 300 to 1 600                 |
| OPA 12      | TCGGCGATAG       | 16              | 270 to 1 300                 |
| OPC 02      | GTGAGCGGTC       | 21              | 160 to 1 400                 |
| OPC 05      | GATGACCGCC       | 25              | 340 to 2 100                 |
| OPN 04      | GACCGACCCA       | 17              | 260 to 1 000                 |
| OPN 16      | AAGCGACCTG       | 23              | 130 to 1 000                 |
| Total       |                  | 209             |                              |
| Average     |                  | 20.9            |                              |

Table 2. Summary statistics for markers used in RAPD analysis of turmeric accessions together with two outgroup species belonging to Family Zingiberaceae.

| Marker | Nei's gene diversity | Polymorphic information content (PIC) | Number of loci detected (N) | Number of polymorphic loci | % Polymorphic loci (B) | Effective multiplex ratio (EMR) (=N × B) | Marker index (MI) (=PIC × EMR) |
|--------|----------------------|---------------------------------------|-----------------------------|----------------------------|------------------------|------------------------------------------|--------------------------------|
| OPN 06 | 0.43                 | 0.37                                  | 14                          | 13                         | 0.92857                | 13                                       | 4.81                           |
| OPD 08 | 0.57                 | 0.48                                  | 24                          | 24                         | 1                      | 24                                       | 11.52                          |
| OPA 18 | 0.48                 | 0.42                                  | 20                          | 20                         | 1                      | 20                                       | 8.4                            |
| OPB 07 | 0.55                 | 0.46                                  | 25                          | 25                         | 1                      | 25                                       | 11.5                           |
| OPA 11 | 0.56                 | 0.47                                  | 24                          | 23                         | 0.95833                | 23                                       | 10.81                          |
| OPA 12 | 0.55                 | 0.46                                  | 16                          | 16                         | 1                      | 16                                       | 7.36                           |
| OPC 02 | 0.21                 | 0.18                                  | 21                          | 21                         | 1                      | 21                                       | 3.78                           |
| OPC 05 | 0.42                 | 0.46                                  | 25                          | 25                         | 1                      | 25                                       | 11.5                           |
| OPN 04 | 0.57                 | 0.41                                  | 17                          | 17                         | 1                      | 17                                       | 6.97                           |
| OPN 16 | 0.53                 | 0.45                                  | 23                          | 23                         | 1                      | 23                                       | 10.35                          |
| Total  |                      |                                       | 209                         | 207                        |                        |                                          |                                |

The polymorphic statistics content material (PIC) of the ten random primers is likewise supplied in Table 2. Six of the ten primers, specifically OPD08, OPB07, OPA11, OPA12, OPC05 and OPN16, had PIC values near 05. The PIC is taken into consideration in maximum research as the man or woman of hobby in the choice of beneficial or informative markers. The PIC fee displays the variety of detected alleles and the relative distribution of their frequency. Low values for PIC imply that the marker is not able to discriminate among genotypes and a PIC of zero.five and above is indicative of a terrific marker<sup>11</sup>. Effective multiplex ratio (EMR) and marker index (MI), each of which measures of the capability of markers to discriminate genotypes, had been additionally distinctly excessive for the equal markers besides for OPA12. Surprisingly, primer OPC02, which changed into capable of making bigger DNA sequences in all accessions, had the bottom PIC of 0.18.

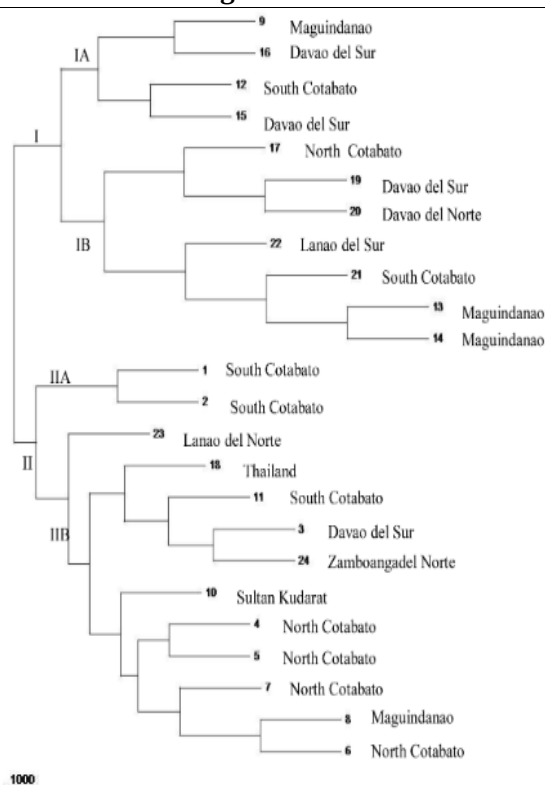


Fig. 1. Phylogenetic tree of the 22 turmeric accessions together with two outgroup species belonging to Family Zingiberaceae obtained from different provinces in Mindanao according to the UPGMA with similarity index of.

Figure 1 indicates the phylogram built primarily based totally at the polymorphisms detected the use of the ten random primers. This ends up used to determine the genetic relatedness of the turmeric accessions collectively with the outgroup species (Acc 23 and 24). To Cluster 1 belonged eleven accessions even as to Cluster II become grouped the relaxation of the thirteen accessions. At a similarity index of round, a hundred percent each cluster had been in addition divided into sub-clusters. The clustering of the accessions primarily based totally at the phylogram did now no longer monitor any full-size sample in step with the places from wherein the accessions had been amassed besides for Acc 4, 5, 6, and seven which constitute the turmeric series of the University of Southern Mindanao, Kabacan, North Cotabato; they had been grouped collectively in sub-cluster IIB. Accessions amassed from the equal province belonged to special clusters. However, there had been additionally accessions that had been amassed from the equal province (Acc 1 and 2, Acc thirteen and 14), which had been genetically comparable. A comparable end result become suggested when<sup>9</sup> evaluated the genetic range of 5 cultivars and fifty-five turmeric accessions from 10 special agro-

climatic areas the use of RAPD and ISSR markers. The authors attributed the absence of region specificity amongst the amassed genotypes to danger migration of rhizome seed cloth with the aid of using growers from one location to another. This can also be the purpose for the determined clustering of the special turmeric accessions. The creation of special genotypes in the shape of planting substances for cultivation might also additionally have been the purpose for the various genotypes found in a specific province.<sup>(12)</sup>

#### 4. CONCLUSION

Six of the ten random primers used for RAPD evaluation exhibited highly excessive PIC values, EMR and MI (besides for OPA12) indicating that the primers had been correct and had been capable of discriminate among turmeric genotypes. The clustering of the accessions primarily based totally at the phylogram commonly did now no longer monitor any full-size sample in step with the places from wherein the accessions had been amassed. Absence of region specificity many of the amassed genotypes and version in the genotypes gift in a precise province might also additionally be due to the change and creation of special genotypes withinside the shape of planting substances from one province to another.<sup>(12)</sup>

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