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Morphotaxonomy and Genetic diversity of different *Oxalis* species in Jharkhand, India

Sangita Kumari[1,3], Sumit Kumar Pathak[1,3], Charupriya Chauhan[2] & VK Yadav[3]*

[1]Department of Botany,Yogoda Satsanga Mahavidyalaya, Ranchi-834 008, Jharkhand, India

[2]Department of Genetics & Plant Breeding, School of Agriculture, Lovely Professional University, Phagwara-144 402, Punjab, India

[3]Department of Botany, Ranchi University, Ranchi-834 008, Jharkhand, India

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The present study deals with the genetic diversity and morphological distribution within a population of *Oxalis* which are commonly grown in Jharkhand. Five species, namely *Oxalis corniculata*, *Oxalis latifolia*, *Oxalis debilis*, *Oxalis triangularis* (herbaceous plants) , and *Averrhoa carambola* (a tree species) , have been considered in the present study. The external morphology of *Oxalis corniculata* includes a trailing stem, rooting at the nodes, and a tap root, while the three herbaceous species are colonial, stoloniferous, and bulbous, lacking upright stems. Genetic diversity was analyzed using AFLP marker (Amplified Fragment Length Polymorphism) , revealing high genetic variation among the species. Results showed that *Oxalis latifolia* and *Oxalis triangularis* are closely related, whereas *Averrhoa carambola* is genetically distinct. This study highlights how genetic diversity and morphological traits are interconnected, providing insights into plant adaptation and evolution. The findings have implications for local biodiversity conservation and management.

Keywords: AFLP, Genetic diversity, Jharkhand, Morphology, *Oxalis*,Plant adaptation

Oxalis with around 900 species is the largest genus in the wood-sorrel family. Except in Polar Regions, it thrives globally and among several species which have been reported in India almost 8 in peninsular region and 4 in Keralaare well known. The genus has been thoroughly studied with new taxa including the separate genus *Xanthoxalis* for the Corniculates section. *Oxalis* are often regarded as troublesome weeds flourishing in lawns, arable lands, waste areas, and gardens and can pose challenges in greenhouse environments[1,2]. The unique characteristic of short monoadelphous stamens distinguishes the Oxalidaceae family from others. Understanding the morphology of *Oxalis* is crucial for accurate plant identification and classification. Key traits such as leaf, stem, flower, and fruit characteristics help differentiate species and reflect their adaptation strategies[3]. These morphological features are closely tied to genetic diversity and environmental interactions which are essential for survival in varied habitats. The study was conducted in Jharkhand, where the ecological context and local biodiversity provide a unique setting for researching *Oxalis* species. These diverse environments of this region offer insights into how *Oxalis* species adapt to different conditions[4,5]. The study of *Oxalis* morphology and genetics in Jharkhand contributes valuable knowledge about local plant diversity and adaptation mechanisms, which can have implications for conservation and management strategies in the region[6].Genetic analysis using AFLP (Amplified Fragment Length Polymorphism) was employed to obtain fingerprint profiles for five plant samples, including four *Oxalis* species and one outlier species, *Averrhoa carambola*. Sixteen EcoRI/MseI primer combinations were used, resulting in 445 bands with 437 (98.2%) polymorphic bands[7,8]. The highest genetic similarity was observed between *Oxalis latifolia* and *Oxalis triangularis*, while the lowest was between *Oxalis debilis* and *Averrhoa carambola*. The average genetic similarity of 0.468 indicates significant genetic diversity among the samples, reflecting their geographical distribution and classification. The morphological and genetic analysis of *Oxalis* species in Jharkhand enhances our understanding of their adaptation strategies and genetic relationships[9,10]. This research is particularly relevant for local biodiversity conservation, ecological studies, and potential applications in agriculture and horticulture in the region[11-16].

*Correspondence:
E-mail: vinodbhu89@gmail.com

Materials and Methods

Sample preparation and morphological observation

Plant samples were collected from their natural habitats to study morphological and genetic variations. Samples were collected during different growth stages including vegetative, flowering, and fruiting periods. Observations such as plant abundance, flower colour, fruit and seed size, and plant height were recorded. The specimens were verified with references and analysed under a microscope.

DNA isolation and quantification

DNA was extracted from lyophilized leaves using a CTAB method. The leaves were ground into powder, mixed with CTAB buffer, and heated at 65°C for 1 h. After incubating the samples mixed with buffer was for 5 min at room temperature, equal volume of chloroform isoamyl alcohol (24:1) was added. The samples were centrifuged for 10 min at 10,000 rpm. The upper aqueous phase was isolated smoothly in separate Eppendorf tube without disturbing the other phase of the tube[17, 18]. The isolated aqueous phase was mixed with isopropanol and mixed gently to avoid the breakage of nucleic acids. The tubes were kept at -20°C for 1 h and centrifuged at 10,000 rpm for 10 min thereafter[19]. The isopropanol was removed and 500 microlitre 70% ethanol was added into each tube. Again, tubes were centrifuged at 10,000 rpm for 10 min. The ethanol was removed and pellet at the bottom of the tubes were dried at room temperature. After drying the pellet, 30 microlitre TE buffer was added and pellet was dissolved[20]. The quality of the isolated DNA was assessed by agarose gel electrophoresis and quantified by nanodrop 2000 (Thermo scientific).

PCR amplification using AFLP markers and data analysis

Genomic DNA (300 ng) was digested with EcoRI and MseI enzymes. The resulting fragments were ligated to adapters and amplified with primers matching the adapter sequences. The amplification included20 cycles at 90°C for 30 seconds, 56°C for 60 seconds, and 72°C for 60 sec. The pre-amplified product was checked on a gel to confirm AFLP library quality. EcoRI primers were labelled with IR700 and IR800 dyes[21,22]. A diluted pre-amplified AFLP sample was used with EcoRI + 3 and MseI + 2 primers for selective amplification. The PCR conditions were 94°C for 30 sec, 65°C for 30 sec (12 cycles) , and 72°C for 60 sec, followed by 12 cycles with a decreasing annealing temperature and 23 more cycles at 94°C for 30 sec, 56°C for 30 sec, and 72°C for 60 sec. After PCR, formamide dye was added, and samples were analysed on a polyacrylamide gel. Amplified fragments were manually scored as '1' for presence or '0' for absence for each primer[23]. Jaccard's dissimilarity matrix was analysed using the unweighted pair group method of arithmetic averages (UPGMA) to create a dendrogram with NYSys software (version 2.2) .

Results

The five species of the genus *Oxalis* collected include *Oxalis corniculata, Oxalis latifolia, Oxalis debilis, and Oxalis triangularis*, which are herbaceous species, while *Averrhoa carambola* is a tree. *Oxalis corniculata* features slender stems with nodes and a tuberous root system. In contrast, the other three herbaceous species (*Oxalis latifolia, Oxalis debilis, and Oxalis triangularis*) have bulbous and stoloniferous growth forms, with horizontal stems close to the soil surface. These species reproduce in different ways: *Oxalis corniculata* and *Averrhoa carambola* spread mainly by seeds, while the other three species reproduce through bulbils[24,25].

The species show the morphological traits, inflorescences are cymose with involucre bracts and flowers on pedicels. The flowers are complete, actinomorphic, and have bisexual reproductive organs. They feature pentamerous floral parts, gamosepalous sepals, and a persistent calyx. There are five petals with twisted aestivation and a caryophyllaceous corolla with nectary appendages. The flowers have ten stamens with didynamous filaments. Stamens are inserted, locules are monothecous, and filaments are dorsifixed to the anthers with discrete connectives. The ovary is pentacarpellary[26,27].

AFLP analysis was conducted to generate fingerprint profiles for five plant samples, including four *Oxalis* species and one outlier species (Table 1). We used 16 EcoRI/MseI primer combinations, producing 445 clear bands, of which 437 (98.2%) were polymorphic across all samples (Table 2). The highest genetic similarity (Jc = 0.266) was observed between *Oxalis latifolia* and *Oxalis triangularis*, while the lowest (Jc = 0.144) was between *Oxalis debilis* and *Averrhoa carambola*. The average genetic similarity was 0.468, indicating significant genetic diversity among the samples (Table 3)[28]. This genetic diversity aligns with the sample collection locations and species classifications (Fig. 1). The dendrogram

Table 1 — Sequence of the primers employed for the AFLP analysis

Preamplification Primers	Code	Sequence
Eco RI+1	E-A	5'-GAC TGC GTA CCA ATT C-A 3'
Mse I+0	M-0	5'-GAT GAG TCC TGA GTA A-3'
Selective Primers		
Eco RI+3 –ACA (IR-700 Labeled)	E-AAC	5'-GAC TGC GTA CCA ATT C ACA-3'
Eco RI+3 –AAG (IR-800 Labeled)	E-ACT	5'-GAC TGC GTA CCA ATT C AAG-3'
Mse I+2 –CG	M-CAC	5'-GAT GAG TCC TGA GTA A CG-3'
Mse I+2 –GT	M-CTA	5'-GAT GAG TCC TGA GTA A GT-3'
Mse I+2 –CA	M-CTA	5'-GAT GAG TCC TGA GTA A CA-3'

Table 2 — Primer combinations, total bands and polymorphic bands obtained

Sl. No.	Primer Combinations	Total Bands Scored	Polymorphic Bands	% Polymorphism
1	E-ACA X M-CG	75	75	100.00
2	E-AAG X M-CG	94	93	98.94
3	E-ACA X M-GT	60	60	100.00
4	E-AAG X M-GT	60	59	98.33
5	E-ACA X M-CA	75	73	97.33
6	E-AAG X M-CA	81	77	95.06
		445	437	98.28

Table 3 — Similarity matrix of the samples used in the study

	Oxalis corniculata	*Oxalis latifolia*	*Oxalis debilis*	*Oxalis triangularis*	*Averrhoa carambola*
Oxalis corniculata	1.000				
Oxalis latifolia	0.216	1.000			
Oxalis debilis	0.152	0.224	1.000		
Oxalis triangularis	0.229	0.266	0.223	1.000	
Averrhoa carambola	0.192	0.199	0.144	0.175	1.000

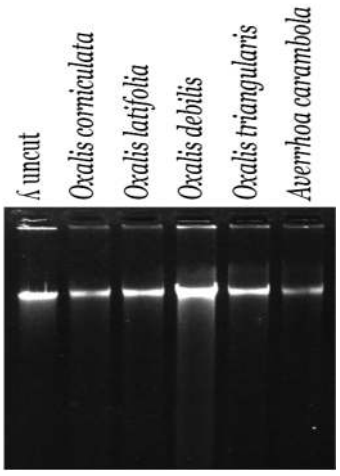


Fig. 1 — *Oxalis spp.* Genomic DNA quantification on agarose gel

constructed using binary data from neighbor-joining clustering, grouped the samples into two clusters (Fig. 2). All four *Oxalis* species were clustered together, while *Averrhoa carambola* was identified as an outlier (Fig. 4). A representative AFLP profile with six primer combinations is shown in (Fig. 3). The genetic analysis of the *Oxalis* species shows that all four herbaceous species *Oxalis corniculata, Oxalis latifolia, Oxalis debilis*, and *Oxalis triangularis*are closely related, which matches their similar morphological traits like bulbous and stoloniferous growth forms[29]. *Averrhoa carambola*, being a tree with a different growth form and reproductive strategy was identified as an outlier in the genetic analysis, reflecting its distinct morphology. The highest genetic similarity was between *Oxalis latifolia* and *Oxalis triangularis*, which is consistent with their similar physical characteristics. On the other hand, *Oxalis debilis* and *Averrhoa carambola* showed the lowest genetic similarity, which aligns with their notable differences in morphology and reproduction[14].

The genetic divergence between *Oxalis* species and *Averrhoa carambola* supports their distinct reproductive methods *Oxalis* species reproduce via bulbils, while *Averrhoa carambola* spreads through seeds. This genetic and morphological divergence highlights how closely related species share similar physical traits and how differing traits correspond to genetic differences[30, 31].

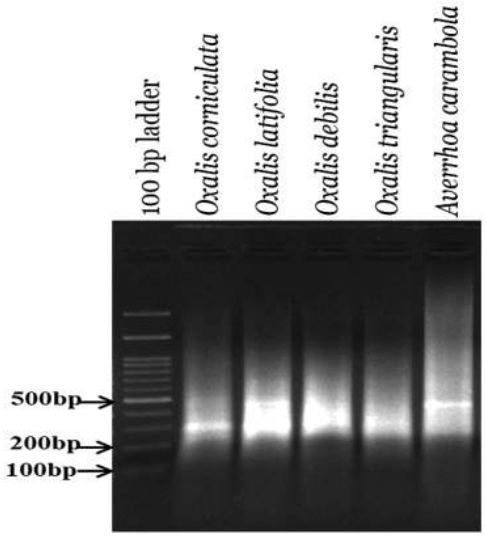


Fig. 2 — Preamplification of adaptor ligated libraries

Discussion

The correlation between morphological traits and genetic variations observed in the species of *Oxalis* provides valuable insights into their evolutionary relationships and diversity[32]. This study analyzed the morphological characteristics and genetic profiles of five species, including *Oxalis corniculata, Oxalis latifolia, Oxalis debilis, Oxalis triangularis, and Averrhoa carambola.*

Firstly, the morphological traits, such as stem structure and growth form, showed clear patterns that corresponded with genetic data. *Oxalis corniculata*, with its slender stems and tuberous root system, differed significantly from the bulbous and stoloniferous growth forms of *Oxalis latifolia*, *Oxalis debilis*, and *Oxalis triangularis*. Recent research by Zietsman *et al*., (2008) supports this observation suggesting that these growth

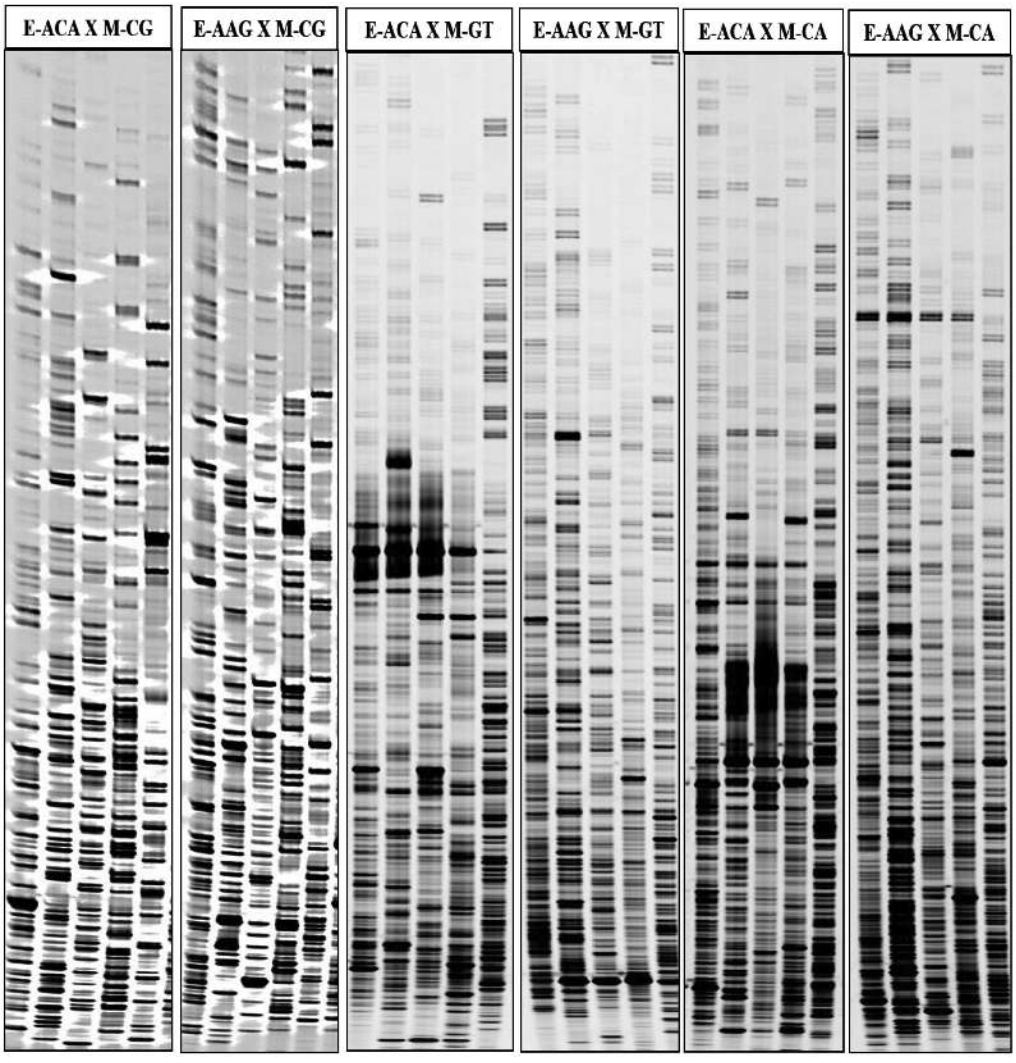


Fig. 3 — AFLP markers profile of *Oxalis* with six primer combinations

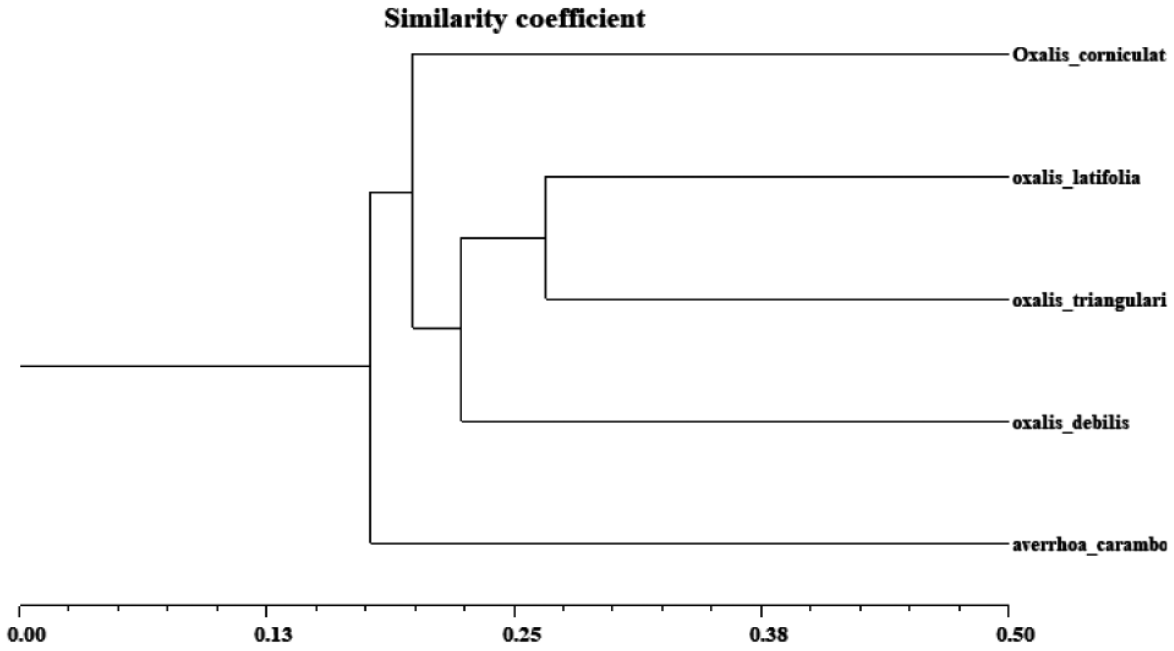


Fig. 4 — Dendrogram revealed genetic relationships among five *Oxalis spp*.

forms are often linked to specific genetic adaptations that enhance survival and reproduction in varying environmental conditions[33-35]. The reproductive strategies also revealed interesting correlations. *Oxalis corniculata* and *Averrhoa carambola* primarily reproduce by seeds, while the other three species reproduce through bulbils. This variation in reproductive methods aligns with findings fromVeldkamp (1972) and Gebregziabher (2004) which indicate that different reproductive strategies can lead to distinct genetic diversity patterns, reflecting adaptations to different ecological niches and varying reproductive success[36].

Flower morphology, characterized by actinomorphic flowers with pentamerous parts and a persistent calyx, is another trait that shows a connection with genetic diversity. Research by Zietsman (2007) highlighted that such floral traits are often conserved across related species due to their role in attracting pollinators and ensuring successful reproduction. This conservation of floral traits across Oxalis species suggests an evolutionary advantage and stability in their reproductive strategies[37].

The AFLP analysis revealed that the highest genetic similarity was between *Oxalis latifolia* and *Oxalis triangularis*, while the lowest was between *Oxalis debilis* and *Averrhoa carambola*. These findings align with the study of Oberlander *et al (*2009) which demonstrated that morphological and genetic data often show clustering patterns that reflect evolutionary relationships and genetic exchange among species. Finally, the cluster analysis, which grouped all *Oxalis* species together while identifying *Averrhoa carambola* as an outlier, supports the observations of Pissard, A., *et al*. (2008). This study noted that distinct morphological and genetic traits often lead to clear clustering patterns, separating species with significant differences and highlighting the evolutionary divergence of *Averrhoa carambola*. In summary, integrating morphological traits with genetic analysis provides a comprehensive view of plant diversity and evolution[38]. The correlations observed in this study reinforce the idea that while morphological traits offer insights into adaptation and classification, genetic data provides a deeper understanding of evolutionary relationships and genetic variation[39,40].

Conclusion

The study on the morphotaxonomy and genetic diversity of *Oxalis* species in Jharkhand has revealed significant insights into the relationships and adaptations of these plants. By examining both morphological traits and genetic profiles through AFLP analysis, we found that the herbaceous species *Oxalis corniculata, Oxalis latifolia, Oxalis debilis*, and *Oxalis triangularis* share close genetic ties, which align with their similar bulbous and stoloniferous growth forms. In contrast, *Averrhoa carambola*, a tree species with distinct reproductive

methods and morphology, was identified as a genetic outlier, highlighting its unique evolutionary path. The AFLP analysis showed that *Oxalis latifolia* and *Oxalis triangularis* are the most closely related, reflecting their similar physical characteristics, while *Oxalis debilis* and *Averrhoa carambola* displayed the lowest genetic similarity, consistent with their notable differences in morphology and reproduction. This genetic divergence underscores the influence of reproductive strategies bulbils versus seeds on genetic diversity. Overall, the study emphasizes the value of integrating morphological and genetic data to understand plant diversity and evolutionary relationships. The findings contribute to the broader knowledge of local plant species in Jharkhand, offering insights into their adaptation mechanisms and potential applications for conservation and management.

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Conflict of interest

All authors declare no conflict of interest.

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