Comparative Karyotype and RAPD Analysis of Four *Oxalis* L. Species

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Summary Four *Oxalis* L. species namely *O. corymbosa*, *O. corniculata*, *O. latifolia*, and *O. triangularis* were investigated cytogenetically and at the molecular level for proper characterization. Different $2n$ chromosome numbers were found in four species, such as $2n=44$ in *O. corniculata*, $2n=28$ in *O. latifolia*, $2n=30$ in *O. corymbosa*, and $2n=30$ in *O. triangularis*. The diploid chromosome number $2n=30$ is the first report for *O. triangularis*. The four species differed with respect to centromeric formulae, such as $30m$ in *O. corymbosa*, $44m$ in *O. corniculata*, $24m+4sm$ in *O. latifolia*, $26m+4sm$ in *O. triangularis*. The four species of *Oxalis* have distinct CMA- and DAPI banding patterns based on the number, location, intensity, and percentage of GC- and AT-rich repeats. With the help of CMA and DAPI staining, it was possible to mark certain chromosomes specific for each species. Moreover, the four species possess distinct RAPD fingerprinting patterns with species-specific unique marker fragments. RAPD fingerprinting analysis placed *O. corymbosa* in a different cluster whereas the rest three in another cluster. Therefore, the compilation of the cytological and molecular information will be useful for authentic identification and characterization of the four *Oxalis* species found in Bangladesh.

Key words Fluorescent banding, Karyotype, RAPD, *Oxalis* L.

The genus *Oxalis* L. belongs to Oxalidaceae consisting of seven genera and about 900 species, mostly pantropical, the greater number of which is distributed in the warmer parts of the world (Rendle 1952, Mathew 1958). In Bangladesh, a wide range of occurrence of different *Oxalis* spp. is recorded (Ahmed *et al.* 2009). Among these species, *Oxalis corniculata* L. is a common herbaceous weed growing profusely with runners and extensively used as indigenous medicines for many diseases and as folklore remedy (Mohammad and Mir 2000). Other *Oxalis* spp. such as *O. corymbosa* DC., *O. latifolia* H.B.K., and *O. triangularis* A.St.-Hil. are grown as potential ornamental herbs because of their brilliant pink, white, and purple flowers and beautiful foliage (Mathew 1958, Sharma and Chatterji 1960, Ahmed *et al.* 2009).

Oxalis is a multibasic genus with fairly wide range of basic chromosome numbers. From the cytological data so far available it may be seen that $x=5, 6, 7, 9$, and 11 are the existing basic chromosome numbers in the genus *Oxalis*, of which seven is the most frequently occurring number (Kumar and Subramaniam 1986, Marks 1956, Warburg 1938, Nakajima 1936).

The variation in chromosome number indicated that numerical chromosomal aberration makes this genus interesting for chromosome study. Though the genus

provides matters of considerable cytological interest, no cytological information is available for the *Oxalis* species growing in Bangladesh. The study of karyotypes is especially necessary where such varying basic chromosome number have been recorded. Such studies not only provide clues to the derivation of one form from the other but help to characterize each species.

However, conventional karyotype analysis alone is unable to critically express the differences among different species when closely related species possess similar $2n$ chromosome numbers (Khatun and Alam 2010, Khatun *et al.* 2011). Minute structural changes *viz.* deletion, inversion, tandem duplication, reshuffling the repeats, *etc.* could not be possible to detect by conventional karyotype analysis. In such a case, a combination of modern cytogenetical and molecular techniques is necessary for comparative studies among different species.

Fluorescent chromosome banding is an excellent tool for karyotype analysis. It helps to provide information regarding the reshuffling of GC- and AT-rich repeats in the genome. In addition, chromosomal abnormality could also be detected by this method (Schweizer 1976, Alam and Kondo 1995, Kondo and Hizume 1982, Jessy *et al.* 2005, Islam and Alam 2011, Sultana *et al.* 2011, Akter *et al.* 2015, Hossain *et al.* 2016).

Moreover, DNA fingerprinting by RAPD is one of the molecular methods for characterizing species. It includes characterization of genetic variability, genome finger-

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printing, genome mapping, gene localization, analysis of genome evolution, population genetics, taxonomy, *etc.* (Williams *et al.* 1993).

Except *O. corniculata*, the other available species viz. *O. corymbosa*, *O. latifolia*, and *O. triangularis* are vegetatively propagated, as a result, the genetic diversity is gradually narrowing. The extreme medicinal and ornamental uses make this genus vulnerable and thus under threat. If it is not managed or conserved at this stage, the consequences would be worse. For proper conservation and management, a multidimensional genetic characterization of each species is essential. Therefore, in the present study, a combination of cytogenetical and molecular analysis was carried out for the first time to characterize four *Oxalis* species viz. *O. corymbosa*, *O. corniculata*, *O. latifolia*, and *O. triangularis* found in Bangladesh. The aims of this research were:

- to make a full strength orcein-stained karyotype for each species,
- to compare the fluorescent banding pattern after staining with CMA- and DAPI- fluorochromes,
- to compare RAPD banding patterns, and
- to characterize each species with cytogenetical and molecular markers.

Materials and methods

Among four *Oxalis* L. spp., *O. triangularis* L. was collected from a nursery in Agargaon, Dhaka, Bangladesh and the other three spp. of *Oxalis* namely *O. corymbosa* DC., *O. corniculata* L., *O. latifolia* H.B.K. from the Botanical Garden, Department of Botany, University of Dhaka. These four species were identified by Professor Dr. Momtaz Begum, Prof. Salar Khan Herbarium, Department of Botany, University of Dhaka and maintained in the Botanic Garden, Department of Botany, University of Dhaka, Bangladesh (Table 1).

Cytogenetical study

Healthy roots were collected and pretreated with 8-hydroxyquinoline (0.002 M) for 2.30 h at 20–25°C followed by 15 min fixation in 45% acetic acid at 4°C. The pretreated RTs were hydrolyzed for 1 min 45 (depending on thickness of root) at 60°C in a mixture of 1 N HCl and 45% acetic-acid (2:1). The root tips were stained and squashed in 1% aceto-orcein. For CMA- and DAPI banding, Alam and Kondo's (1995) method was used with slight modification. After hydrolysing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly on dry ice and allowed to air dry for at least 24 h before the study. The air-dried slides were first incubated in McIlvaine's buffer (pH 7.0) for 30 min followed by Distamycin A (0.1 mg mL⁻¹) treatment for 10 min. The slides were rinsed mildly in McIlvaine's buffer supplemented with MgSO₄ (5 mM) for 15 m. One drop of CMA (0.1 mg mL⁻¹) was added to the materials for 15 min in a humid chamber and then rinsed with McIlvaine's buffer with MgSO₄ for 10 min. Slides were mounted in 50% glycerol and kept at 4°C for overnight before observation. These were observed under Nikon (Eclipse 50i) fluorescent microscope with blue violet (BV) filter cassette. For DAPI-staining, after 24 h of air drying, the slides were first incubated in McIlvaine's buffer (pH 7.0) for 27 min and treated in Actinomycin D (0.25 mg mL⁻¹) for 10 min in a humid chamber. The slides were immersed in DAPI solution (0.01 mg mL⁻¹) for 20 min and mounted with 50% glycerol. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with ultra violet (UV) filter cassette.

DNA isolation

Leaves were harvested and total genomic DNA was extracted by using modified CTAB method (Doyle and Doyle 1987). DNA concentration was quantified through spectrophotometer (Analytikjena, Specord 50,

Table 1. Comparative phenotypes of four species of *Oxalis* L.

Features	Species			
	<i>O. corymbosa</i>	<i>O. corniculata</i>	<i>O. latifolia</i>	<i>O. triangularis</i>
1. Stem	A stemless herb producing tuberous rootstock with brownish scales and radical leaves	A perennial herb with long, slender, creeping stem, rooting at the nodes	A stemless herb from bulbous base without rootstock with scales and radical leaves	A stemless herb from bulbous base without rootstock with scales and radical leaves
2. Petiole	5–18 cm long	1–9 cm long	18–22 cm long	25–30 cm long
3. Leaf	Green in color, palmately 3-foliate	Green in color, palmately 3-foliate, comparatively small in size	Green in upper side and purplish at lower side, palmately, broadly obdeltoid, 3-foliate	Purple in color, palmately, broadly obdeltoid, 3-foliate
4. Flower	Pinkish purple in color, 1.0–1.5 cm long	Yellow in color, 0.4–0.5 cm long, comparatively small in size	White in color, 1.25–1.75 cm long	Pinkish white in color, 1.0–1.5 cm long
5. Propagative organ	Capsule not produced, Vegetatively propagated by tubers	Propagation by seeds and stem cuttings	Capsule not produced. Vegetatively propagated by fleshy bulbs and bulbils	Capsule not produced. Vegetatively propagated by fleshy bulbs and bulbils

Germany). The A 260/280 readings for DNA samples were 1.6–1.8.

PCR amplification and primer survey

The PCR reaction mixture for 25 μ L containing template DNA (25 ng) 2 μ L, de-ionized distilled water 18.8 μ L, Taq buffer A 10 $\times$ (Tris with 15 mM MgCl₂) 2.5 μ L, primer (10 μ M) 1.0 μ L, dNTPs (2.5 mM) 0.5 μ L and Taq DNA polymerase (5 U μ L⁻¹) 0.2 μ L. PCR amplification was done in an oil-free thermal cycler (Biometra UNOII, Germany) for 46 cycles after initial denaturing 94°C for 5 min, denaturing at 94°C for 1 min, annealing at 34–36°C for 30 sec, extension at 72°C for 3 min and final extension at 72°C for 5 min. Six random primers such as primer-21 (5'-GGCACTGAGG-3'), primer-23 (5'-GTCAGGCAAA-3'), primer-OPF-22 (5'-AAGATCAAAGAC-3'), primer-OPC-13 (5'-AAGCCTCGTC-3'), primer-OPC-14 (5'-TGCGTGCTTG-3'), and primer-OPC-26 (5'-CACGTTATCGCA-3') were used in the present study for screening.

Gel electrophoresis

The amplified products were separated electrophoretically on 1% agarose gel. The gel was prepared using 1.0 g agarose powder containing ethidium bromide (10 mg mL⁻¹) 8 μ L and 100 mL 1 $\times$ TAE buffer. Agarose gel electrophoresis was conducted in 1 $\times$ TAE buffer at 50 V and 100 mA for 1.5 h. DNA ladder (1 kb) was electrophoresed alongside the RAPD reactions as marker. DNA bands were observed on UV-transilluminator and photographed by a gel documentation system.

Scoring and data analysis

The PCR products were analyzed after gel electrophoresis. The photographs were critically discussed on the basis of presence (1) or absence (0) of bands, size of bands, and overall polymorphism of bands. These were carried out for further investigation. The scores obtained using all primers in the RAPD analysis were then pooled for constructing a single data matrix. This was used for estimating polymorphic loci, Nei (1972) gene diversity, genetic distance (D), and constructing a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram among the specimen using computer program "POPGENE" (Version 1.31).

Results and discussion

Conventional karyotypes

In this study, four *Oxalis* species showed different somatic chromosome counts such as $2n=30$ in *O. corymbosa* and *O. triangularis*, $2n=44$ in *O. corniculata*, and $2n=28$ in *O. latifolia* (Figs. 1–4, Table 2). The diploid chromosome number of $2n=30$ for *O. triangularis* was determined in the present investigation. No chromosomal information of this species is

available in the internet sources and relevant literature. Therefore, $2n=30$ chromosomes is the first report for *O. triangularis* (Fig. 4).

In *O. corymbosa*, besides $2n=30$ chromosomes, different chromosome numbers were reported earlier by different scientists viz. $2n=22$ (Chatterjee and Sharma 1970), $2n=28$ (Chatterjee and Sharma 1970, Chaudhuri *et al.* 1969), $2n=30$ (Chatterjee and Sharma 1970), $2n=42$ (Kumar and Subramaniam 1986). Thus the present report of $2n=30$ chromosomes for *O. corymbosa* only correlates to that of Chatterjee and Sharma (1970). The presence of $2n=30$ in *O. corymbosa* in this study made a confusion regarding the basic chromosome number (Fig. 1). It indicated that the species has either pentaploid or hexaploid nature with $x=5$ or $x=6$, respectively. Since Kumar and Subramaniam (1986) reported $2n=42$ for this species, $x=6$ is most likely the basic chromosome number for this species.

Oxalis corniculata was found to possess $2n=44$ chromosomes in the present study (Fig. 2). The same chromosome number was reported earlier by Chaudhuri *et al.* (1969), Chatterjee and Sharma (1970), and Bir *et al.* (1980). In addition, different chromosome numbers such as $2n=24$ (Rutland 1941, Heiser and Whitaker 1948), $2n=28$, $2n=42$ (Kumar and Subramaniam 1986) and $2n=48$ (Marks 1956, Mathew 1958, Sharma and Chatterji 1960, Murin and Sheikh 1971) were also reported. If the basic chromosome number of this species is considered as $x=11$, then specimens with $2n=44$ observed in this study could be regarded as tetraploid. On the other hand, if $x=7$ is considered for this species, $2n=28$ and $2n=42$ could be the tetraploid and hexaploid, respectively. However, $2n=24$ and $2n=48$ did not agree with the basic number of $x=11$ and $x=7$. In this case, $2n=24$ and $2n=48$ might have evolved from nearer ploidy level by aneuploid aberration.

In *O. latifolia*, $2n=28$ chromosomes were observed in this research work (Fig. 3). The present finding regarding $2n$ chromosome number supports that of Weller *et al.* (1976). Moreover, different $2n$ chromosome numbers of this species were reported earlier such as $2n=14$ (Weller *et al.* 1976), $2n=24$ (Sarker *et al.* 1974), $2n=26$ (Chaudhuri *et al.* 1969, Chatterjee and Sharma 1970), $2n=42$ (Weller *et al.* 1976), and $2n=46$ (Chatterjee and Sharma 1970). Now, if $x=7$ is considered for this species, $2n=28$ and $2n=42$ could be tetraploid and hexaploid, respectively. In this situation, $2n=24$ and $2n=46$ did not agree with the basic number of $x=7$. Therefore, $2n=24$ and $2n=46$ might have evolved from aneuploid change from nearer ploidy level.

Among the four *Oxalis* species, *O. corymbosa*, and *O. corniculata* were found to possess all metacentric chromosomes (Table 2). In these two species, the range of chromosomal length was insignificant i.e., the distance between small and large chromosomes was about 1.88 μ m in *O. corymbosa* and 2.47 μ m in *O. corniculata*

Figs. 1–26. Orcein-, CMA- and DAPI-stained mitotic metaphase and RAPD analysis of four species of *Oxalis* L. 1. Orcein-stained mitotic metaphase chromosomes of *O. corymbosa*, 2. Orcein-stained mitotic metaphase chromosomes of *O. corniculata*, 3. Orcein-stained mitotic metaphase chromosomes of *O. latifolia*, 4. Orcein-stained mitotic metaphase chromosomes of *O. triangularis*, 5. CMA-stained mitotic metaphase chromosomes of *O. corymbosa*, 6. CMA-stained mitotic metaphase chromosomes of *O. corniculata*, 7. CMA-stained mitotic metaphase chromosomes of *O. latifolia*, 8. CMA-stained mitotic metaphase chromosomes of *O. triangularis*, 9. DAPI-stained mitotic metaphase chromosomes of *O. corymbosa*, 10. DAPI-stained mitotic metaphase chromosomes of *O. corniculata*, 11. DAPI-stained mitotic metaphase chromosomes of *O. latifolia*, 12. DAPI-stained mitotic metaphase chromosomes of *O. triangularis*, Bar=10 μ m, 13. CMA-karyotype of *O. corymbosa*, 14. CMA-karyotype of *O. corniculata*, 15. CMA-karyotype of *O. latifolia*, 16. CMA-karyotype of *O. triangularis*, 17. DAPI-karyotype of *O. corymbosa*, 18. DAPI-karyotype of *O. corniculata*, 19. DAPI-karyotype of *O. latifolia*, 20. DAPI-karyotype of *O. triangularis*, Bar=10 μ m, 21. RAPD with primer-21, 22. RAPD with primer-23, 23. RAPD with primer-OPF-22, 24. RAPD with primer-OPC-13, 25. RAPD with primer-OPC-14, and 26. RAPD with primer-OPC-26.

(Table 2). As a result, no gradual decrease of chromosomal length was observed in their karyotypes (Table 2). These features indicated that these two species have

strict symmetric karyotypes. However, in addition to maximum metacentric chromosomes, a few sub-metacentric chromosomes were observed in *O. latifolia*

Table 2. Comparative orcein-, CMA- and DAPI- karyotype analysis of four species of *Oxalis* L.

<i>Oxalis</i> species	2n	Range of chromosomal length (μm)	Total length of 2n chromosome complement (μm)	Centromeric formulae	No. of CMA-bands	% of GC-rich repeats	No. of DAPI-bands	% of AT-rich repeats	No. of satellites
<i>O. corymbosa</i>	30	1.80–3.68	79.11	30m	07	17.13	10	15.68	1 in DAPI
<i>O. corniculata</i>	44	1.56–4.03	102.26	44m	12	20.36	08	11.59	—
<i>O. latifolia</i>	28	4.72–9.27	175.98	24m+4sm	04	12.42	04	10.59	2 in CMA
<i>O. triangularis</i>	30	2.42–5.58	127.62	26m+4sm	11	29.29	03	3.78	2 in CMA

m=metacentric chromosomes, sm=sub-metacentric chromosome.

and *O. triangularis*. The sub-metacentric chromosomes might have originated from metacentric chromosomes by some chromosomal aberration viz. terminal deletion, pericentric inversion, non-reciprocal or unequal translocation. In these two species, the range of chromosomal length was significant i.e., distance between small and large chromosomes was $4.55\mu\text{m}$ in *O. latifolia* and $3.16\mu\text{m}$ in *O. triangularis* (Table 2). As a result, a gradual decrease of chromosomal length was observed in their karyotypes (Table 2). These features indicated that these two species have moderate asymmetric karyotypes. Stebbins (1971) mentioned that symmetric karyotypes were primitive and asymmetric karyotypes were advanced. Therefore, *O. corymbosa* and *O. corniculata* could be considered as relatively primitive species compared to the other two species.

Fluorescent-banding

The four *Oxalis* species used in this study differed sharply regarding the number, location, distribution, and intensities of CMA and DAPI-bands (Figs. 5–20, Table 2). It was observed that most of the banded chromosomes of the four species were entirely fluoresced with CMA and DAPI (Figs. 5–20, Table 2). Some chromosomes showed centromeric and a few terminal CMA and DAPI bands. In these entirely fluoresced chromosomes, GC- and AT-rich repeats were not confined but rather distributed along the chromosomes. The possible reason for these entirely fluoresced chromosomes was tandem amplification of GC- and AT-rich repeats (Schweizer 1976, Khatun and Alam 2010, Sultana and Alam 2007, Hiron *et al.* 2006, Mahbub and Alam 2007). Therefore, the chromosomes of *Oxalis* had a tendency for tandem duplication. The centromeric bands suggested no duplication of these repeats and confinement to a particular location. The origin of terminal bands might be due to paracentric inversion of banded portions from the centromere to the terminal ends.

Stain specific satellite

After CMA-staining, a pair of satellites was found in *O. latifolia* (on the short arm in each member of chromosome pair II) and in *O. triangularis* (on the short arm in each member of chromosome pair IX) (Figs. 15, 16). No satellite was found after orcein- and DAPI-staining in this pair of *O. latifolia* and *O. triangularis* (Figs. 19,

20). Moreover, in *O. triangularis*, a pair of satellites on the short arm in each member of chromosome pair X was observed after orcein-staining only (Fig. 4, arrow). In *O. corymbosa*, one satellite was observed in only a member of pair IX after DAPI staining (Fig. 17). Alam and Kondo (1995) reported about stain specific satellites in *Drosera echinoblata* after a series of sequential staining with orcein, Giemsa, CMA, and DAPI. In the present study, the pair of satellites in *O. corymbosa*, *O. latifolia*, and *O. triangularis* might possess some kind of DNA sequence, which made these satellites stain-specific. However, no report about stain-specific satellites was available for different *Oxalis* species in earlier studies.

Structural aberration in different *Oxalis* species

Heteromorphism with respect to banding patterns was observed in the four *Oxalis* species after CMA-banding. A centromeric CMA-positive band was observed in a member of pair III in *O. corymbosa* and pair II and XI in *O. corniculata* (Figs. 13, 14). No such band was found in their homologues. In addition, only one member of chromosome pair V, VI, IX, and XIII in *O. triangularis* showed CMA-positive bands on the entire short arm and long arm while their homologue members did not show any band (Fig. 16).

The four *Oxalis* species showed some heteromorphism with respect to banding patterns after DAPI-staining as well. Only one member of chromosome pair II, III, VI, and XII in *O. corymbosa*, a member of chromosome pair II and IV in *O. corniculata*, a member of chromosome pair VI and XII in *O. latifolia*, and one member of chromosome pair VI in *O. triangularis* showed DAPI-positive bands at different regions while their homologue members did not show any band at the same location (Figs. 17–20). This heteromorphism with respect to CMA- and DAPI-banding indicated the occurrence of deletion of the banded portions from the respective chromosomes.

Another type of heteromorphism was found in chromosome pair IV in *O. corymbosa* with DAPI-staining. In this pair, one chromosome showed DAPI-positive bright bands at the interstitial regions of two arms whereas the other member had two dots like terminal DAPI bands (Figs. 9, 17). This kind of heteromorphism indicated the occurrences of inversion between terminal and proximal portions of chromosomes (Zaman and

Alam 2009).

A different kind of heteromorphicity was observed in *Oxalis triangularis*, where a member of chromosome pair VIII had terminal CMA-positive bands on both long arm and short arm (Figs. 8, 16). No band was found in its homologue. Sultana and Alam (2016) reported similar heteromorphicity in *Gossypium hirsutum* var. CB-11. They suggested the occurrence of unequal exchange of the banded region between the homologous chromosomes. The present results indicated such unequal crossing over between the members of a homologous pair in *O. triangularis*.

RAPD analysis

In this study, a total of 10 RAPD primers were applied on template DNA of four *Oxalis* species. Only six out of 10 primers were selected because they revealed multiband fingerprinting, which were easily scorable and reproducible. The primer sequences, band sizes, and banding patterns of four *Oxalis* species are shown in Table 3. The six primers generated a total of 96 distinct bands of which 28 were considered as unique bands. The term unique means that the sequence found in a specimen with a certain primer was absent in other specimens

(Figs. 21–26, Table 3). The unique bands were stable and specific for the respective species and thus could be used as a tool for characterization. In this study, 94.79% polymorphism regarding RAPD banding patterns was recorded, which revealed the diverse nature of the four species. This result was supplemented by conventional and fluorescent karyotypic features because each species has distinct karyotype formula.

Genetic distance and cluster analysis

The dendrogram based on RAPD markers showed that *O. latifolia* and *O. triangularis* are very close with a narrow genetic distance (Fig. 27). Morphologically, these two species are very close except their leaf and flower color (Table 1). These two species possess submetacentric chromosomes with higher $2n$ chromosomal length and range of individual chromosome lengths. Moreover, lowest DAPI bands were found in these two species (Figs. 3–4, 11–12, 19–20, Table 2). Therefore, the molecular data is supplementing the karyotypic features of these two species.

The foregoing discussion revealed that the four *Oxalis* species have characteristic CMA-, DAPI-, and RAPD-banding patterns and therefore, could be characterized

Table 3. Compilation of RAPD analysis in four species of *Oxalis* L.

Primer codes	Total bands	Size ranges (bp)	Number of common band	Number of polymorphic band	Number and size (bp) of species specific unique bands	Polymorphisms (%)
Primer-21	12	200–2500	0	12	2 (900, 600bp) in <i>O. corymbosa</i> , 5 (2500, 630, 500, 300, 200bp) in <i>O. corniculata</i> , 4 (800, 700, 480, 350bp) in <i>O. triangularis</i> and 1 (320bp) in <i>O. latifolia</i>	100.00
Primer-23	1	300	0	1	1 (300bp) in <i>O. triangularis</i>	100.00
OPF-22	17	500–4000	1	16	4 (4000, 2000, 1500, 800bp) in <i>O. corymbosa</i> and 1 (700bp) in <i>O. triangularis</i>	94.11
OPC-13	24	200–2500	0	24	1 (200bp) in <i>O. corniculata</i>	100.00
OPC-14	20	400–2500	3	17	2 (2500, 2000bp) in <i>O. corymbosa</i> , 2 (1300, 650bp) in <i>O. corniculata</i> , 1 (800bp) in <i>O. triangularis</i> and 1 (680bp) in <i>O. latifolia</i>	85.00
OPC-26	22	100–2800	1	21	2 (900, 100bp) in <i>O. corymbosa</i> and (500bp) in <i>O. latifolia</i>	95.45
Grand Total:	96	100–4000	05	91	28	94.79

Fig. 27. UPGMA dendrogram based on Nei's (1972) genetic distance summarizing the data on differentiation between four species of *Oxalis* L. M=100bp DNA ladder. OC=*Oxalis corymbosa* DC, OCr=*Oxalis corniculata* L., OT=*Oxalis triangularis* A.St.-Hil and OL=*Oxalis latifolia* H.B.K.

authentically with the help of combined cytogenetical and molecular data.

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