CHARACTERIZATION OF FOUR SRI LANKAN *EXACUM* (BINARA) SPECIES BY RAPD

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Introduction

Exacums are endemic plant species found in Sri Lanka that grow mostly in wild. Classification systems of Sri Lankan *Exacums* during last 25 years are widely differing from each other, and are complex. Even plants of the same species growing in different localities show variation in morphological characters, and therefore, the analysis of genetic variation is important.

Detection of genetic polymorphism using molecular markers provides more reliable information than phenotypic analysis. The PCR based techniques like RAPD and microsatellites are more popular and convenient to investigate genetic polymorphism in our conditions. In this study, the RAPD technique was employed to assess the genetic variation of several *Exacum* populations in Sri Lanka.

Materials and Methods

Plants of *Exacum trinervium* subsp. *trinervium* were collected from nearby areas in the Kandy district (Pilimathalawa, Danture, and Mahakanda) of the central province of Sri Lanka, and planted in a green house of the Department of Agricultrual Biology of the University of Peradeniya. After flowering, the plants were subjected to artificial pollination, and pods were harvested within two months. Harvested seeds were planted in seed-trays and after two months, they were transplanted into pots. The fungicide 'Captan' was applied at two-week intervals to control botrytis, which is the major and devastating disease on *Exacum* at seedling stage.

Plants of the *Exacum trinervium* subsp. *macranthum* were collected at Illukkumbura, *E. petiolare* from Lenadora, *E. walkeri* from the Hortain plains in the central province of Sri Lanka, and the leaves of these species were collected and kept under refrigerated conditions at -21°C.

DNA of the four taxa of the genus *Exacum* was extracted using CTAB method from young, tender leaves (Weising *et al.*, 1995) and RAPD profiles were generated using 22 Random Primers. Polymerase chain reactions (PCR) for amplification of DNA preparations were carried out in 15µl volumes in a programmable Perkin-Elmer thermo cycler. The reaction tube contained 3.0µl of 25ng DNA, 0.2 µl of 5 units/µl Taq polymerase (AB gene) 1.5µl of 10X PCR reaction buffer (Birthon), 1.5µl 2.5mM $MgCl_2$ (Birthon), 1.2µl of 2.5mM dNTPs mix (Takara), 2.0 of primer (10pm/ µl) and 5.6µl of sterilized distilled water.

At the preliminary screening, total DNA from these 4 species was amplified first with 22 operon primers (OPN, OPQ and OPS- series). Based on the resolution and polymorphism produced, only five primers could be selected: OPQ 18, OPQ 11, OPS 17, OPN 1 and OPN 20 (Table 1) were used to analyze individual DNA samples from the collected species. The PCR programme consisted of an initial denaturation at 94 °C for 5 min, followed by 40 proper cycles comprising 94°C for 1 min, 35°C for 1 min, 72°C for 2 min, and a 10 min final extension at 72°C. A 1.4% agarose was used in gel electrophoresis.

Table 1. Primer sequences used in the PCR

OPQ 11	5' TCGCCGCAAA 3'
OPQ 18	5' AGGCTGGGTG 3'
OPS 17	5' TGGGGACCAC 3'
OPN 1	5' CTCACGTTGG 3'
OPN 20	5' GGTGCTCCGT 3'

Results and Discussion

In this study, only four species of the genus *Exacum* available in Sri Lanka could be deployed to evaluate the genotypic variation. Altogether 57 bands were scored of which 45 were polymorphic, while 12 were monomorphic (Figure 1).

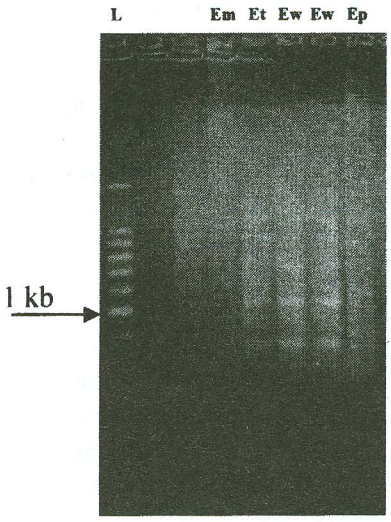

Figure 1. RAPD profiles of 4 taxa with OPQ 18 (Em – *E. macranthum*, Et – *E. trinervium*, Ew – *E. walkerii*, Ep – *E. petiolare*)

The cluster gram constructed, based on distant co-efficient of Jacaard, using Drawtree editor of PHYLIP software program (Version 3.69) showed *E. petiolare (Ep)* diverging early at 0.74 from the other three species. *E. walkerii (Ew)* separated at 0.51 and the two other species of this study, *E. macranthum (Em)* and *E. trinervium (Et)* branched only at 0.22 (Figure 2).

Conclusions

It was evident that among the four species tested, *E. trinervium and E. macranthum* are the closest. Despite the chromosome number differences (2n=60 for *E. trinervium* and 2n=54 for *E. macranthum*), these were the only 2 species that exhibited compatibility at fertilization amongst all Sri Lankan *Exacum* taxa in an earlier study (Riseman *et al,* 2006) implying that they are the closest

relatives. *E. petiolare* showed the highest divergence amongst the 4 species studied.

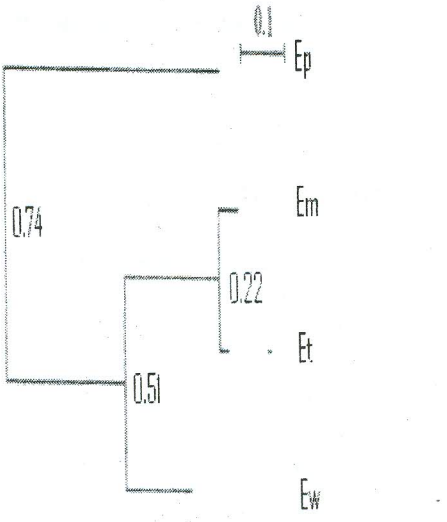

Figure 2. The Clustergram

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References

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