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Optimization of Protocol for Isolation of Genomic DNA from Leaves of *Selaginella* Species Suitable for RAPD Analysis and Study of their Genetic Variation

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ABSTRACT.—A simple and efficient protocol for isolating genomic DNA from leaves of *Selaginella* spp. (*S. delicatula*, *S. repanda*, *S. bryopteris*, *S. plana*, *S. monospora*) was developed, involving a modified CTAB protocol of Rogers and Benedich (1994). Increasing the incubation time with the precipitation buffer (1X CTAB) from 1–3 hours to 12–14 hours helped achieve higher quantity genomic DNA from the specimens, when compared with DNA extracted by protocols reported by Dellaporta *et al.* (1983), Murray and Thompson (1980) and Doyle and Doyle (1987). The DNA yield ranged from 846–1836 µg/ml from fresh and herbaria-preserved leaf samples. The DNA samples were found suitable for genetic diversity analysis with Random Amplified Polymorphic DNA (RAPD) markers. Nine random primers (OPA A17, OPB 4, OPB13, OPC 2, OPC 11, OPD 5, OPG 2, OPG 19 and OPK 10) were studied, of which two primers (OPD 5 and OPG 2) yielded reproducible amplification profile of polymorphic fragments.

KEY WORDS.—DNA extraction, RAPD, *Selaginella*, modified protocol

Selaginella (spike moss) is an enigma in the plant kingdom. At present only one genus is recognized in the Selaginellaceae, i.e., *Selaginella* (Family Selaginellaceae, Order Selaginellales, Class Lycopsidea). The genus *Selaginella* is cosmopolitan in distribution and contains approximately 700 species that include temperate, tropical, frost-tolerant arctic, and drought-tolerant desert species. Such extremes are very rarely found in the same genus, and hence the family Selaginellaceae has been treated differently and sub-divided into myriad taxa by researchers (Spring, 1850; Braun, 1857; Baker, 1883; Hieronymus, 1901; Walton and Alston, 1938; Jermy, 1986).

Selaginella shows morphological variation within species and as such it is difficult to distinguish species depending on traditional morphology only. Thus, researchers have concentrated on molecular phylogenetic analysis to gain information about its evolutionary relationships. A recent molecular phylogenetic analysis of the genus has revealed that rates of molecular evolution among species are remarkably high, including when compared to the angiosperm families (Korall and Kenrick, 2004). Although many subtle morphological and developmental differences exist between species, few of these differences are phylogenetically useful markers for classifying the species in a way that is consistent with molecular data (Korall and Kenrick

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