

Karyomorphology of *Incarvillea emodi* and its phylogenetic position in the genus

Karyotypic variations are important criteria to distinguish related species in a genus. The study of karyomorphology in the genus *Incarvillea* shows that karyotypic variations are the main feature of chromosomes and involved in the evolution of the genus. In general, the evolutionary tendency of this genus seems to be an increase in the asymmetry of the karyotype¹. The four sub-genera in the genus *Incarvillea* Juss. include *Amphicome*, *Incarvillea*, *Pteroscleris* and *Niedzwedzkia*². Among these, *Niedzwedzkia* is described as an independent genus in *Flora Unionis Rerumpublicarum Socialisticarum Sovieticarum*³. Sub-genera *Amphicome* has two species, *Incarvillea* has three, while *Pteroscleris* has 10 species. The present study was undertaken to determine the phylogenetic position of *Incarvillea emodi* (which belongs to *Amphicome*) in the genus based on its karyomorphology.

The seeds used for the study were collected from plants domesticated at the Institute of Himalayan Bioresource Technology (IHBT, CSIR), Palampur. The voucher specimens have been deposited in the Herbarium (PLP 5963) of IHBT. Root tips from germinated seeds were used for cytogenetic studies. The roots were pretreated with colchicine (0.01% aqueous solution) for 4 h and fixed in Carnoy's fluid (1:3 acetic acid: absolute alcohol) at about 4°C for 24 h. The fixed roots were hydrolysed in 1 N HCl at 60°C for 8–10 min, followed by staining with 2% aceto-carmine and then squashed for cytological observations. The slides were examined using a Nikon biological research photomicroscope (model 80i), equipped with a digital camera and good preparations photographed and prints made, from which the metaphase chro-

mosomes of five cells were measured. Standard procedures were followed to present symbols for the description of metaphase chromosomes⁴ and classification of asymmetry of the karyotype⁵.

A total of 22 chromosomes were observed in *I. emodi* and all the chromosomes were less than 4 µm long. The karyotype formula of the species is $2n = 22 = 14m + 8sm$, and the ideogram of the species is shown in Figure 1. The ratio of the longest to the shortest chromosome is 1.82 and 9% of the chromosomes have an arm ratio of more than 2.0. The asymmetry of the karyotype is type 2A. The number of diploid chromosomes in *I. emodi* is the same as in all other species belonging to sub-genera *Amphicome*, *Incarvillea* and *Pteroscleris*. Based on their size, chromosomes of eukaryotic organisms can be divided into four grades⁶: (I) length less than 1 µm; (II) length between 1 and 4 µm; (III) length between 4 and 12 µm, and (IV) length more than 12 µm. The chromosomes of *I. emodi* are categorized under grade II of chromosomes. Earlier studies involving different species of *Incarvillea* confirm that chromosomes of the sub-genera *Amphicome* and *Incarvillea* are less than 4 µm long and smaller than those of sub-genus *Pteroscleris*¹. Further, two populations of *I. arguta* in the sub-genus *Amphicome*, viz. Yunan population and Sichuan population have been reported¹ with karyotype formula $18m + 4sm$. In the present study, the karyotype formula of *I. emodi* was observed as $14m + 8sm$, implying greater degree of karyotype asymmetry in comparison with *I. arguta* populations and suggesting that *I. emodi* is evolutionarily advanced compared to *I. arguta*. However, all the species of sub-genera *Incarvillea* and

Pteroscleris are asymmetric to a greater extent, with higher frequencies of sub-median and sub-telomeric chromosomes¹.

Based on the degree of asymmetry of the karyotype, sub-genera *Amphicome* (asymmetry type 2A) and *Incarvillea* (asymmetry type 3A) are considered primitive to *Pteroscleris* (asymmetry type 3A), which has higher number of sub-telomeric chromosomes. The present study highlights the distinct evolutionary status of *I. emodi*, which is intermediate between *I. arguta* and other species in the sub-genera *Incarvillea* and *Pteroscleris*. Molecular phylogenetic studies based on ITS and TRNL-F sequences⁷ also support the karyomorphological observation that *I. emodi* is intermediate between *I. arguta* (Yunan and Sichuan) populations and sub-genera *Incarvillea* and *Pteroscleris*.



Figure 1. Metaphase chromosomes of *Incarvillea emodi* ($2n = 22$) and ideogram.

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