

ABSTRACT

In the present research work, 665 plant specimens belonging to 247 species, 62 families of 27 different orders in 5 major classes of plant kingdom i.e. Mangolopsida (Dicot), Lilipopsida (Mononcot), Pinidae (Gymnosperms), Pteropsida and Bryopsida were collected from different parts of district Dera Ghazi Khan, Punjab, Pakistan. Herbarium sheets with mounted and identified specimens of all the species were deposited in Dr Sultan Ahmad herbarium, GC University, Lahore after posting voucher numbers. The ethnobotanical knowledge on the use of local plants was collected from four hundred pertinent people belonging to all walks of life through their interviews using a semi-structured questionnaire. The plants were found in use by locals as food, feed, medicine, source of fuel and furniture wood, etc. The leaves of the plants were found most frequent in use for treating various ailments, as compared to whole plant or other parts of the plant. Tissue sampling and DNA barcode sequencing was carried out at NIBGE, Faisalabad and Canadian Centre of DNA Barcoding (CCDB), University of Guelph, Canada, respectively. At Family level, 61/62 (98.39%) families were found having at least one sequence of *rbcl* while no *matK* sequence in 6/27 (22.22%) orders and 13/62 (19.67%) families. But 49/61(80.33%) families were found to have both *rbcl* and *matK* sequences. A total of 1015 barcodes were sequenced, with 630/665 (94.73%) barcodes having *rbcl* sequences of 235/247 (95.14%) species representing 176 genera and 62 families while 385/665 (57.90%) *matK* sequences of 185/247 (74.90%) species from 146 genera and 49 families. The data analysis was accomplished on the basis of three criteria; PCR Amplification and Sequencing Success of *rbcl* and *matK*; Barcode Gap Analysis (Nearest Neighbor (NN) Analysis) and Neighbor Joining (N/J) Cluster Analysis. PCR and sequencing success were found very high for the *rbcl* region, i.e. 94.73% (630 / 665 specimens) while *matK* had the lowest overall rate of recovery, i.e. 57.9% (385 / 665 specimens). The *matK* showed the highest sequence quality for almost all the recovered samples. Barcode Gap Analysis revealed that 95.22% sequences of *matK* and 96.63 % of *rbcl* exhibited no intra-specific variation. The inter-specific divergence varied from 0.0% to 19.06% for *rbcl* while 0.0% to 4.12% for *matK*. Out of 105 congeneric species with *rbcl* sequences, 69/105 (65.71%) were identified while in congeneric species with *matK* sequences 54/71 species (73.24%) were successfully differentiated. In pair wise divergence across all the species (non-congeneric), both

matK and rbcL sequences showed clear boundaries between the 84.21% (96/114) and 70.45% (93/133) of the species, respectively. In Barcode Gap Analysis, matK showed more discriminatory power than rbcL. Among taxa, patterns of sequence divergence was visualized by means of Neighbor-Joining (NJ) cluster on MEGA6. In all three trees of rbcL, matK and rbcL+matK, assignment of families within their respective orders was 83.61% (51/61), 79.59% (39/49) and 83.67% (41/49), respectively. In all three trees of rbcL, matK and rbcL+matK, species were identified on the basis of "Bootstrap Threshold Value" and "Monophyly". In neighbor trees (N/J) of rbcL, matK and rbcL + matK, the number of monophyletic families were 49/61(80.33%), 40/49 (81.63%) and 40/47 (85.11%), respectively. In neighbor joining (N/J) trees of rbcL, matK and rbcL + matK, the number of monophyletic families were 49/61(80.33%), 40/49 (81.63%) and 40/47 (85.11%), respectively. Therefore, on the basis of monophyletic recovery and node support, all three neighbor joining trees were found best resolved monophyletic trees having more than 80% monophyletic families.