ABSTRACT

The morphometric study was conducted during 2009 to 2010. About 28 morphological characters were measured under 13 natural locations of *D. hatagirea* (D. Don) Soo. Geographic variation in morphology reflects phenotypic responses to environmental gradients and evolutionary history of populations and species. At points, beside its broad geographic range (Nubra, Suru and Indus valley) characterization of *Dactylorhiza* phenotype was normally accomplished by use of morphological descriptors, hence as a first step, phenotype collection and its morphometric analysis was assessed for the first time. However, plant height, leaf length, lowermost leaf length, length of second leaf from base and mean length from lowest bract to the top of inflorescence were presented to account for the remarkable variation in morphological traits. Tirith location showed more values of this trait while Skurru showed less value. From this, it was concluded that Tirith showed great morphometric variation as compared to other location. Multivariate morphometric techniques, principal component analysis (PCA), multidimensional scaling (MDS) and cluster analysis were used to determine whether these locations can be reliably considered as morphologically similar or dissimilar. The first two principal components derived more than 75% variation among population. The results of PCA and MDS analysis were comparable to that of cluster analysis, which showed considerable phenotypic variation in morphological and horticultural traits that can be utilized for its genetic improvement. To support this study, further constructive information have been provided in the present study on the status of the populations of *D. hatagirea* which may increase the conservation value of this population and may resolve the taxonomic and nomenclatural controversies related to the suitable areas.

RAPD and ISSR marker analysis have been used for the first time to characterize the population genetic structure and differentiation within and among thirteen locations of *D. hatagirea*. The genetic diversity of *D. hatagirea* has been revealed by Nei’s diversity index (H), Shannon’s diversity index (I), polymorphic loci and percentage of polymorphic loci (PPL). Pair-wise location genetic distances ranged from 0.05 to 0.48. Although, both the molecular markers revealed high percentage of polymorphism, ISSR marker detected more diversity than RAPD marker. Analysis of molecular variance (AMOVA) revealed that 57% RAPD and 60% ISSR variability was partitioned among population with moderate level of genetic differentiation and gene flow. Both Principal coordinate’s analysis (PCoA) and neighbour joining (NJ) cluster analysis supported the grouping of all 136 sample sizes of
thirteen locations into two collection groups. Model based Bayesian clustering, principal coordinate analysis and neighbour-joining analysis highlighted the role of high mountain Ladakh range (6500m amsl) as an important geographic barrier for this species at the studied site. Two main gene pools have been observed one in Nubra- Indus valley and other in Suru valley. The present level and pattern of genetic diversity and structure of *D. hatagirea* are assumed to result largely from its habitat fragmentation, its unique biological traits and evolutionary history. The genetic structure could be attributed to an earlier period of more pronounced gene flow when the species had a more continuous distribution. A Mantel test revealed no significant positive correlation between genetic distances and geographic distances.

The population status of *D. hatagirea* was investigated for the first time which covers Ladakh region. At random 20 quadrates in four habitats of each location were drawn and vegetation was measured accordingly. This study is also supported with soil data for identifying appropriate measures for the vegetation of this endangered orchid. Result showed that 21 species belonging to 16 families were encountered and it was found that 1812 individuals of *D. hatagirea* were present in our surveyed area with low density i.e. 6.9% (Unprotected area). For improving the conservation status of the species Vegetation analysis, potential area and habitat for reintroduction were predicted using Maximum Entropy (MaxEnt) distribution modelling algorithm. The model was developed using data from 13 locations in the native range of Ladakh region along with 13 environmental parameters including enhanced vegetation index (EVI) and digital elevation data. The model predicted that suitable habitats of *D. hatagirea* were restricted to an area of 485 km in the Indus and Suru valley of Ladakh region. Population status was positively correlated with higher model thresholds in the undisturbed habitats confirming the usefulness of the habitat model in population monitoring, particularly in predicting the successful establishment of the species. The study describes the potential habitats in the elevation of Indus and Suru Valley within the native range where the species can be reintroduced.

The study was carried out where immature seeds cultured on ten different media for germination. Maximum germination was achieved in Lindeman orchid medium (37.12%) within 17 days of culturing. Protocorms with leaf primordia were cultured on BM-2 and seven different modifications of MS media with various hormone combinations (0-3 mg/L IBA and 0-3 mg/L Kin) for plantlets regeneration and mass multiplication. Maximum number of shoots (18.12 ± 2.3), highest shoot length (17.80 cm ± 2.16), maximum root number (8.25 ± 0.69) and highest root length (8.02 cm ± 1.45) were found in MS medium with 3 mg/L IBA.
and 1 mg/L Kin. Plantlets with 2-3 shoots were transferred to different potting mixtures for acclimatization to field conditions and further multiplication. 100% survival was obtained in C-8 potting mixture consisting of Cocopeat + Vermiculite + Perlite (1:1:1) which produced 75 number of shoots (25 plantlets) after one month of transplantation in the glass house. The current study reports for the first time a rapid in vitro protocorm development and mass multiplication protocol for D. hatagirea which holds robust potential for large-scale propagation and metabolite production from the plant.