INVESTIGATION ON THE POPULATION VARIATION OF *Drosera indica* L. COMPLEX USING COMBINED MORPHOLOGICAL AND MOLECULAR TECHNIQUES

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ABSTRACT

Drosera indica L. is an annual, tropical species of carnivorous plant exhibiting a considerable amount of morphological variability, including plant size, flower colour, stamen form, seed size, and seed coat ornamentation pattern. Thus far there has been no study dealing with these morphological variability. The present study, therefore, is aiming at investigating the pattern of morphological and genetic variability in this species to determine whether there are morphologically distinguishable groups, and whether these groups are genetically distinct.

Materials used in this study consisted of air-dried herbarium specimens, water- and silica sand-preserved plant, and glasshouse- and tissue culture-grown plants germinated from seeds. The assessment of morphological variation was carried out on sixty two accessions of *D. indica* based on 62 accessions based on 14 vegetative and floral characters, as well as 12 micromorphological seed characters examined using scanning electron microscope. Multivariate numerical analysis on morphological data was performed using cluster analysis and two ordination techniques: the multidimensional scaling and principal component analysis. The pattern of genetic variation was evaluated on 15 accessions of *D. indica* using random amplified polymorphic DNA (RAPD). The DNA for RAPD analysis was obtained from fresh materials only, either from glasshouse- or tissue culture-grown plants germinated from seeds. The other types of materials failed to produce DNA of sufficient amounts and quality.

Results of morphological data analysis indicated that there are six morphotypes, each representing a distinctive combination of seed type and other morphological characters. Examination on the geographic distribution of accessions, coupled with the geology and the average annual rainfall data suggested that these morphotypes occurred sympatrically, and that they did not exhibit distinct geographical and ecological patterns. Based on this

evidence, therefore, these morphotypes might represent varieties within *D. indica*, or possibly even distinct species.

Cluster analysis and multidimensional scaling ordination on RAPD data revealed a high degree of genetic dissimilarity between accessions and between different morphotypes. The grouping of accessions based on RAPD data did not correspond to that resulted from morphological analysis. A comparison on the same set of samples (15 accessions) indicated that accessions from different morphotypes grouped together in the same cluster generated from RAPD data, and that there was no consistent pattern in the grouping of these morphotypes. This result indicated that there were differences in the pattern of within-species morphological and genetic variation. The discrepancy between results from morphological and molecular data was discussed. The two data sets, however, are in general agreement in detecting the degree of similarity between accessions.

The high degree of genetic dissimilarity revealed from RAPD analysis confirms the inbreeding nature of *D. indica*, and provides evidence on the reproductive isolation between sympatric morphotypes. This result, therefore, supports the recognition of the six defined morphotypes as distinct species. Considering the wide range of distribution of *D. indica* across different habitats and continents, however, further examination of specimens covering as much as possible its range of geographic distribution and morphological variation is required to justify the suggested taxonomic treatment.

DECLARATION

This work contains no material which has been accepted for the award of any degree or diploma in any university or other tertiary institutions and, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Ratna Susandarini 21/6/2001

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3/2/4-5 '*D. ramentacea* Burch, ex DC., *D. madagascariensis* DC., *D. burmanni* Vahl and *D. peltata* Thunb. is recognised as the'
7/2/1 'areas, and' = 'areas, that'
7/2/10 'Asia to' = 'Asia, Japan'
9/3/1 remove 'L.'
9/3/2 remove 'a set of characters such as'
10/2 remove all 'L.'
11/2/1 'resulted in a new'
12/1/1 replace 'amount' with 'number'
13/2/5 'of a specific'
13/2/6 'by a factor'
15/2/8 'researchers: Welsh'
18/1/5 'from the heterozygote
18/3/2 replace 'overcome' with 'reduced'
19/1/9 'in conjunction with'
23/2/5 'kinds'
31/2/11 'data may have'
35/1/8 remove 'each'
35/2/1 remove 'research'
35/2/2 'variability between specimens'
36/1/6 replace 'Provided' with 'Combined'; 'evident' with 'evidence'
38/2/4 'either help to define'
38/2/14 'At the infraspecific level'
39/1/10 'species on seed morphology'
40/1/5 delete 'genus'
40/1/9 delete 'was'
40/2/7 delete 'the family'
40/2/9 'as in the study'
49/1/3 'SEO meant that'
51/1/4 'excavations'
52/2/2 'Type II seeds'
52/3/4 'belonging to'
53/1/2-3 'which no additional character was found to support the'
55/1/2 replace 'quality' with 'concentration'
55/2/5 delete 'cell'
66/3/6 'sources'
69/2/11 delete 'because'
69/2/12 'hydration, so the'
69/3/1 delete 'using'
69/3/4 delete 'from'
76/3/title 'plants'
77/3/4 '1986;'
79/2/5 'thus does not necessarily'
80/2/8 'character overlap with'
81/2/4 'replace 'to use' with 'that'
81/2/5 'analysis to be used in'
84/1/3 delete 'in the application of numerical phenetic methods'
84/2/12 delete 'two'
85/2/3 'fifty-nine complete specimens'
88/2/1 'dendrogram'
91/1/2 'of the clusters (Fig. 5.4)'
91/4/1 replace 'Despite' with 'In addition to'
102/1/3 'with a distinctive'
102/1/4 delete 'and thus'
102/1/8 'population systems'
102/1/17 'in the case of *D. indica*'
103/2/1 'that of the six defined morphotypes, 3 (A, B and C)
104/1/2 delete 'six defined'
104/2/1 'comprised three morphotypes and a further three subtypes'
105/1/10 'inbreeding'
105/1/11 ' the three morphotypes'
106/2/7 'similarity and difference'
108/1/1 replace 'in which the' with 'so that a'
108/2/1 'replace' from with 'of'
108/2/6 'which is a commonly used'
108/2/9 'reproductive'
109/1/1 'fingerprinting technique using RAPD'
110/1/2 delete 'random amplified polymorphic DNA'
112/2/2 'B2 and C, corresponding to the main clusters identified in the earlier morphological analysis'
112/2/3 'Cluster A (seed Type II)
112/2/5 'Cluster B1 (seed Type I)
112/2/7 'Cluster B2 (seed Type I)
112/2/9 'Cluster C (seed Type III)
112/2/10 'and red-striped petiolate leaves'
117/1/3 delete 'result'
118/1/5 '(Abbott *et al.* 1985)'
119/2/2 replace 'inbreeding' with 'inbred'
119/2/3 'that is responsible'
119/2/5 replace 'explaining' with 'explains'
119/2/10 'populations from exchanging genes'
120/2/10 'Whitkus, 1997), whereas'
121/2/2 'populations'
122/1/2 'difference, and'
122/1/8 'as much as'
122/1/9 'conclusion could'
124/2/8 replace 'defining' with 'studying'
126/1/1 delete 'are'
127/3/2 'cases where a pair'
128/1/14 'is the nature of morphology'
128/1/21 'and that with a large'
128/1/22 'there is a possibility that'
129/3/2 replace ', thus confirms its inbreeding nature' with 'providing evidence of reproductive isolation between accessions, possibly through inbreeding'
130/2/3 'distinct species. The anecdotal pollination observations where there is clear pollinator preference between the different morphs also supports the idea of genetic isolation. However, considering'
130/2/5 'as possible of its range'

***Drosera indica* L.**