PART-II

NUMERICAL CHEMOTAXONOMIC STUDIES
INTRODUCTION

In the earlier part of the present century, too much dependence on chromosome data resulted in considerable activity on the study of chromosomes. This led to the realization that chromosome information in specific situation may be quite inadequate and additional data from other disciplines may be necessary to resolve the possible mechanism of evolution. It is because of this realization, the new multidisciplinary branch of biology has emerged. Nowadays, a hybrid discipline between phytochemistry and taxonomy has won increasing recognition as a useful tool, together with cytology, to be used in the analysis of genetic variation with plant populations (Moore et al., 1970; Moore, 1972). However, it has been repeatedly emphasized that phytochemical results cannot be employed for chemotaxonomy unless the chromatogram spots are definitely identified. Since it is not always possible, the problem is whether or not biochemical data can usefully be employed without identification of the compounds. Grant (1968) and Gupta (1978) remarked that chromatographic spots are excellent markers and are much more important than mere chromosome numbers. If chromosome numbers and/or size could be of taxonomic value without knowing any details about the base sequences in the contained DNA or the aminoacid
sequences of chromosomal proteins, these spots which in all probabilities should have same structure in different species, should be of good taxonomic value as well. The identification of the compound would no doubt inform us whether or not a particular compound in one species could be derived from a related compound found in another species and will thus be of significance in understanding evolution. But in the absence of this information, presence or absence and relative intensity along with area of spots provide very useful information. Perhaps the most useful class of compounds for such study is the phenolics because of their relative ease of extraction and handling (Earborne, 1967, 1970, 1972 and also because they are regarded "chemically privileged" (Bate-Smith, 1952). A large amount of information is already available about plant phenolics as taxonomic guides (Bate-Smith and Lerner, 1954; Bate-Smith, 1954, 1956, 1958, 1959, 1961, 1962; Bate-Smith and Metcalfe, 1957; Bate-Smith and Ribereau-Gayen, 1959). These chemical constituents when used as diagnostic characters have a particular high taxonomic value (delimiting and diagnostic) for they have been shown "to be stable, unambiguous and not easily, if at all changeable" (Davis and Heywood, 1967). Further, these chemical characters will show chemical relationship in the same way as
morphological characters show morphological relationships. They may also provide a 'new source' of information to supplement the observations based on morphology. Alston and Irwin (1961) have shown that the patterns of variation of secondary substances offer a greater potential in taxonomic work than do aminoacids. Harborne (1971), in his review article "Distribution of flavonoids in the Leguminosae", has pointed out the inestimable value of flavonoid patterns in systematic studies.

Recently, phenolic substances are being employed to study phylogenetic affinities, species relationships and ancestry of natural hybrids in higher plants. (Riley and Bryant, 1961; Alston and Turner, 1963; Smith and Levin, 1963; Stebbins et al., 1965; Harney and Grant, 1964, 1965; Brehm and Ownbey, 1965; Grant and Whetter, 1966; Grant and Zandstra, 1968; Belzer and Ownbey, 1971; Rajhathy et al., 1971; Iiyama and Grant, 1972 and Chennaveeraiah and Razdan 1980). A comparative study of flavonoid patterns in case of barley has yielded very impressive results and provided a clue for the origin of barley itself (Frost and Holm, 1971; Asker, 1972; Asker and Frost, 1973 and Frolst and Asker, 1973).
Two dimensional thin-layer chromatography has been used in Brassica (Dass and Nybom, 1967), Saxifraga (Jaworska and Nybom, 1967), Prunus (Muzysnak and Nybom, 1968), Secale (Dedio et al., 1969a), Triticale (Dedio et al., 1969b), Aegilops (Kaltsikes and Dass, 1970a,b), Hordeum (Dass, 1972a), Triticinae (Dass, 1972b), Cucumerinae (Dass et al., 1974), Petunia (Natarliza and Sink, 1974), Annona (Dass et al., 1975), Hordeum (Frost, 1975, 1979), Setaria (Gupta and Singh, 1977), Blumea (Singh and Dakshini, 1977), Citrus (Dass et al., 1978), for the analysis of phenolic compounds in conjunction with numerical taxonomy (Sokal and Sneath, 1963). The biochemical study in Orchidaceae is surprisingly not attempted except by Lining (1974) and Hegle and Jorapur (1975).

Thus, the present study is designed to assess the usefulness of 2-dimensional TLC technique (Balsgard System-Nybom, 1964) to complement the morphological and cytological studies in evaluating the taxonomic status of the three genera viz., Habenaria Willd., Peristylus Bl. and Platanthera L.O.Rich. The results obtained on TLC profiles have also been subjected to numerical assessment as an aid in establishing phenetic relationships amongst the genera and species.
It may, however, be pointed out here that the present investigation is undertaken with special emphasis on phenolics for chemotaxonomic purposes, and study is not meant to be a detailed analysis and chemical characterization of compounds.

Numerical assessment

As mentioned in earlier lines, thin-layer chromatographic studies on phenolic compounds of higher plants have been useful in solving problems of taxonomical and genetical nature. Satisfactory results can be obtained where differences between the two taxa are large and mostly qualitative in nature (viz., presence or absence of spots). In cases where differences are small or are quantitative in nature (viz., differences in the intensity of spots), more critical methods of evaluation are needed. Thin layer chromatography, combined with methods of numerical taxonomy (Bikel and Sneath, 1963) have been found to be generally helpful in such cases. Use of numerical taxonomy in combination with biochemical systematics started with the publications of Allison et al. (1962), Lorenz and Schulz Schaeffer (1964) and Matthews (1966). Wismark (1972) has pointed out the merits and demerits of different numerical methods utilized for analysing unidentified phenolic
patterns in chemotaxonomy. Dass et al. (1976) again discussed the relative merits of different available numerical methods using spot intensity and spot area and suggested that biochemical distance without squaring the difference calculated from the spot areas are most reliable.

It is felt in the present investigation that a similar approach would be suitable to supplement the karyomorphological information if the available data on chromatographic profiles are subjected to numerical assessment.