INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2006/08–2–231–234
http://www.fspublishers.org

Leaf Protein Electrophoretic Profiles and Chromosome Numbers of Some Araceae

THORIA R. MOHAMED[1], SAYED F. KHALIFA† AND REEM M. SALAH EL-DINE
Departments of Botany Faculty of Girls, and †Science, Ain Shams University, Cairo, Egypt
[1]Corresponding author's e-mail: thoria.rashad@hotmail.com

ABSTRACT

Chromosome number of 19 species representing 14 genera of Araceae we recorded, of which 9 counts were novel. Different levels of polyploidy were evident in 5 species. The combination of chromosome numbers and the profiles of leaf proteins of the 19 species (as revealed by numerical analysis of SDS-PAGE results) highlighted some hitherto un-recognized relationships among the genera and species. While there was positive correlation between chromosome numbers and protein patterns in *Aglaonema* and *Anthurium* so that they seemed taxonomically homogeneous, such correlation was not evident among the species representing *Syngonium* and *Philodendron*.

Key Words: Aracea; Chromosome count; Protein electrophoresis

INTRODUCTION

The Araceae are a relatively large family with more than 105 genera and 3300 species (Mayo *et al*., 1997) of tropical and temperate distribution (Croat, 1981). Many species are of immense economic importance. The large starchy tubers of *Colocasia esculenta*, *Alocasia indica* and *Xanthosoma sagittifolium*, and the fruits of *Monstera deliciosa* are edible tropical crops. A considerable number of species are used widely for medicinal purposes (Croat, 1994). The family includes a number of ornamental plants on account of their variegated leaves (e.g. *Pothos*, *Aglaonema*, *Philodendron*, *Syngonium*, *Diffenbachia*), fenestrated leaves (e.g. *Monestra deliciosa*; Melville & Wrigley, 1969) or colourful spathe of the inflorescence (e.g. *Anthurium*).

Cytologically, the family is distinguished by wide variation in chromosome numbers (2n = 14 to 140) and in chromosome size (Marchant, 1973). Some genera have only one chromosome number e.g. *Anubias* with 2n = 48, others may have both diploid and tetraploids e.g. *Spathiphyllum* with 2n = 30, 60, or more. Rarely a combination of euploid and aneuploid numbers are found of which the most extreme example is *Cryptocoryne* with 2n = 20, 22, 30, 33, 34, 36, 42, 54, 66, 68, 72, 85, 88, 102, 112, and 32 (Marchant, 1973). The numbers 2n = 24, 26, 28, 30, 32, 34, and 36 are common in the family, which would suggest a primary basic number of x = 6, 7, or 8, (Mabberley, 1997). However, in several cases the numbers may represent an aneuploid series of secondary basic numbers e.g. n = 12, 13, 14, 15, 16, 17 and 18.

Chromosomal data have played an important role in the study of plant systematics and evolution at all levels (Mabberley, 1997). It has been used to solve many problems in the delimitation of various plant taxa. The taxonomic value of chromosomal features within and between taxonomic units was discussed by Moore (1978).

The differences between species should be based on gene differences, but direct comparison of genes is difficult and time-consuming. Therefore, the differences can probably be measured by comparing the products of gene activity, i.e. by using proteins as genotype markers (Miege, 1982).

Electrophoretic techniques are used on large scales in protein and enzyme analysis to identify and characterize the genotype differences among plant species and varieties. Matta *et al.* (1981) studied *Vicia* legumin structure by polyacrylamide gel electrophoresis. Many authors used the electrophoretic tool to characterize the differences and similarities between plants. Among these authors are: Abdel-Tawab *et al*. (1982), Giannasi and Crawford (1986), Gamal El Din *et al.* (1988), Eweda (1989), Vries (1996) and Kamel and Hassan (2001).

The present study was undertaken to evaluate the relationships among selected species of Araceae using both cytological and electrophoretic criteria.

MATERIALS AND METHODS

Nineteen species from 14 genera of the Araceae were studied and the localities from, which they were collected are given in Table I. Root tips were pretreated with 0.027% 8 -hydroxy-quinoline kept at 4°C for 3 h, the roots were fixed in acetic acid-ethyl alcohol (1:3 v/v) for 24 h and stored in 70% ethyl alcohol in the refrigerator. At the time of squashing, the roots were rinsed in a series of descending alcohol concentrations 70%, 30% and distilled water for 30 min. in each concentration. Hydrolysis followed 1N hydrochloric acid at 60°c for 3 - 12 min. The roots were placed in distilled water for 10 min. the root tips were

squashed in a drop of aceto-carmine on slides after removing the root cap. The squashes were made permanent according to Sharma and Sharma (1980).

Leaf protein electrophoresis. Electrophoretic profiles were revealed using continuous polyacrylamide gel electrophoresis in the presence of sodium-dodecyle sulphate (i.e. SDS-PAGE). One gram of clean leaves for each sample was ground in a mortar with liquid nitrogen and 200 µL of extraction buffer (100 mm Tris-HCl (pH-7.5), 4 mm 2 - mercaptoethanol, and 0.1 mm EDTA-NA. The extract was centrifuged at 18000 rpm at 10°C for 30 min. The supernatant was mixed with activated charcoal to eliminate pigments. Preparation of gel was as described by Laemmli (1970). Electrophoresis was performed in a vertical slap mold (Hoefer Scientific Instrument; San Fransisco, CA, USA, Model LKB 2001, measuring 16 × 18 × 0.15 cm). Electrophoresis was carried out at 30 mA at 10°C for 3 h. The silver staining method for protein described by Sammons *et al.* (1981) was used. The resulting database was analyzed using SPSS (statistical Package for Social Scientists) software packages in order to elucidate the genetic relationship of the species under study in the light of protein banding pattern homology.

The grouping of Operational Taxonomic Units (OUT's) produced from the analysis were examined and compared with the current taxonomic classification of the family. For the numerical analysis the NTSYS-PC, version 1.50 program (Rohlf, 1989) was used.

RESULTS AND DISCUSSION

Somatic and possible basic chromosome numbers as well as previous counts of the19 species of Araceae are presented in Table I. The chromosome numbers recorded in the present study for *Alocasia macrorrhiza, Anthurium andraeanum, Anthurium hookeri, Colocasia antiquorum, Monstera deliciosa, Scindapsus aureus, Xanthosoma violaceum, Philodendron selloum* and *Zantedeschia aethiopica* agree with those repoted by Darlington and Wylie (1955), Fedorov (1969), and Goldblatt and Johnson (1981 - 1997). Novel chromosome counts are recorded in *Aglaonema pseudobracteatum, Dieffenbachia ameona, Nephthytis podophyllum, Philodendron hastatum, Syngonium podophyllum*, and *Syngonium standleyanum.* It is worthly to mention that the chromosome counts recorded for *Aglaonema commutatum* did not agree with the previous counts. Polyploidy was recorded in *Aglaonema commutatum, A. pseudobracteatum and Dieffenbahcia amoena.* All these species have a basic number of x = 8 and a tetraploid was recorded in *Monstera deliciosa* and *Spathiphyllum wallisii.* The remaining, were diploid.

The Araceae showed a wide range of basic chromosomes numbers ranging from 8 to 22. The most common basic number in the family is x = 14. Even though the haploid number 7 has not so far been found, x = 7 was assumed to be the original basic number of the family and that x = 14 was derived by polyploidy (Mookerjea, 1955; Jones, 1957). The presence of chromosome number as low as n = 8 in *Theriophonum* and *Typhonium* supports this view. Ramachandran (1978) stated that the evolution of an aneuploid series (x = 6, 7, 8, 9 & 10) from the original number x = 7, followed by polyploidy might well have given rise to the wide range of basic numbers now observed in the family in the present study.

Subramanian and Munian (1988) concluded that both aneuploidy and euploidy play an important role in the origin and evolution of the species of the south Indian Araceae. Raven (1975) found that the predominant chromosome numbers were n = 14 and n = 21, other common numbers were n = 13 and n = 12. He suggested that the original basic number is possibly x = 7, but this number is not known in recent taxa.

Electrophoretic analysis of proteins revealed a total of 57 protien bands in the leaves of the 19 species under investigation. The number of bands differed from a species to another, with the largest number (18) in *Aglaonema commutatum* and the lowest (5) in *Diffenbachia amoena* (Fig. 1). For ease of comparison, the 57 protien bands were lumped together into 7 categories of molecular masses and the number of bands from each category was scored for every species (Table II). However, relationships between the species were determined numerically on the basis of the distribution of individual protein bands (Fig. 1) and the results were hierarchically depicted in Fig. 2.

Fig. 1. SDS-PAGE analysis of leaf proteins of 19 species of Araceae represented by lanes 1-19. M = Marker. Molecular masses of marker proteins are given in KD. 1- Aglaonema commutatum, 2- A. pseudobracteatum, 3- Alocasia macrorrhiza, 4- Anthurium andraeanum, 5- Anthurium hookeri, 6- Colocasia antiquorum, 7- Dieffenbachia amoena, 8- Monstera deliciosa, 9- Nephthytis podophyllum, 10- Philodendron hastatum, 11- Philodendron devansayeanum, 12- Philodendron selloum, 13- Pothos sp., 14- Scindapsus aureus, 15- Spathiphyllum wallisii, 16- Syngonium podophyllum, 17- Syngonium standleyanum, 18- Xanthosoma violaceum, 19- Zantedeschia aethiopica

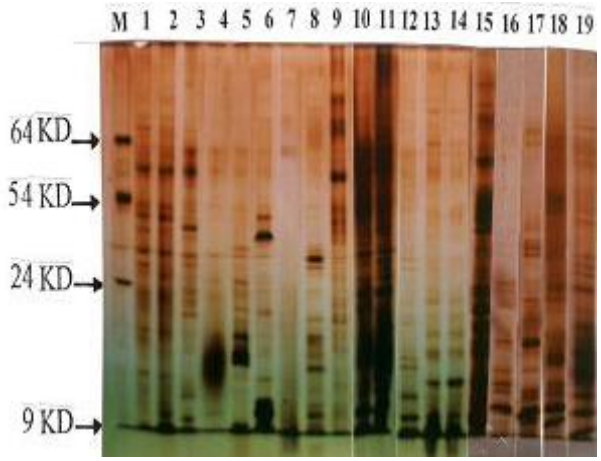


Table I. Chromosome counts of 19 species of Araceae. Sources of materials taken from botanic gardens: 1= Botany Department, Faculty of Science, Ain Shams University; 2= Botany Department, Faculty of Science, Alexandria University; 3= El-Ageena Farm, Kalyobeya Governorate

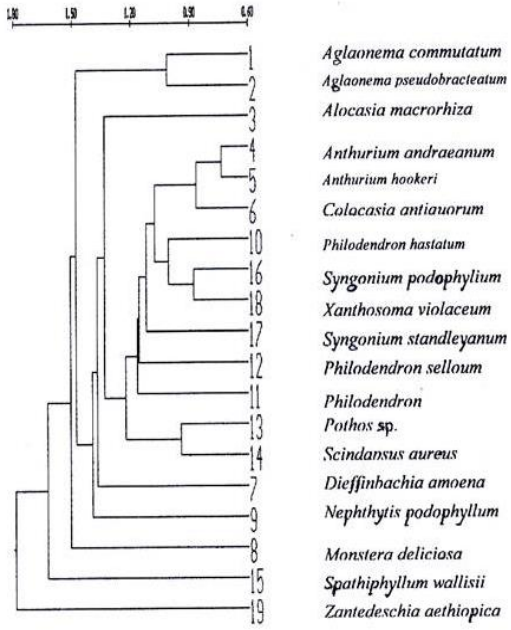
Species	Source of Material	Chromosome number	X	Previous counts	Reference
Aglaonema commutatum Schott	1	80	8	14	(Fedorov, 1969)
A. pseudobracteatum Schott	1	64	8	None	
Alocasia macrorrhiza Schott	1	26	13	26	(Darlington & Wylie, 1955)
Anthurium andraeanum Linden	1	30	15	30	(Sheffer & Kamemoto, 1976)
Anthurium hookeri Kunth	1	30	15	30	(Sheffer & Kamemoto, 1976)
Colocasia antiquorum Hubbard & Rehder	3	28	14	28	(Darlington & Wylie, 1955)
Dieffenbachia amoena Genitl	1	72	8	None	
Monstera deliciosa Liebm.	1	56	14	56.58	(Goldblatt & Johnson, 1991)
Nephthytis podophyllum Schott	1	24	12	None	
Philodendron hastatum C. Koch	1	30	15	None	
Philodendron devansayeanum Linden	1	30	15	None	
Philodendron selloum C. Koch	1	32	16	23.34	(Fedorov, 1969)
Pothos sp.	1	48	24	None	
Scindapsus aureus Engler	1	48	24	48	(Subramanian & Munian 1988)
Spathiphyllum wallisii Hooker	1	36	9	None	
Syngonium podophyllum Schott	1	22	11	None	
Syngonium standleyanum Schott	1	22	11	None	
Xanthosoma violaceum Schott	1	26	13	24.26	Goldblatt & Johnson(1991)
Zantedeschia aethiopica Sprengel	2	26	13	26	(Darlington & Wylie,1955)

Table II. SDS-PAGE protein patterns of leaves of 19 species of Araceae. The 57 protein bands revealed by the analysis are lumped into 7 categories of molecular masses and the number of bands in each category is given for every species

Molecular masses of bands (KDa)							
Under 20	**20 to 40**	**40 to 60**	**60 to 80**	**80 to 100**	**100 to 120**	**Above 120**	**Species**
5	4	3	4	1	—	1	*Aglaonema commutatum*
4	4	3	3	1	—	1	*A. pseudobracteatum*
4	6	2	1	—	1	1	*Alocasia macrorrhiza*
2	3	2	1	—	1	—	*Anthurium andraeanum*
5	5	2	1	—	1	—	*Anthurium hookeri*
4	4	1	1	—	1	—	*Colocasia antiquorum*
1	—	—	1	1	1	1	*Dieffenbachia amoena*
6	5	1	1	1	1	—	*Monstera deliciosa*
3	4	3	1	1	—	1	*Nephthytis podophyllum*
5	2	1	—	—	—	1	*Philodendron hastatum*
2	4	—	1	—	—	1	*Philodendron devansayeanum*
6	4	—	1	—	—	1	*Philodendron selloum*
5	2	2	1	1	—	1	*.Pothos sp*
5	3	1	2	1	—	1	*Scindapsus aureus*
1	3	1	1	1	1	—	*Spathiphyllum wallisii*
6	1	—	—	1	—	—	*Syngonium podophyllum*
5	4	—	1	—	—	—	*Syngonium standleyanum*
4	1	1	—	—	—	—	*Xanthosoma violaceum*
6	5	2	1	—	1	—	*Zantedeschia aethiopica*

A number of taxonomic outcasts with relatively high levels of dissimilarity were the first to bo cut off from the rest of the species. Thus, *Monstera deliciosa*, *Spathiphyllum wallisii* and *Zantedeschia aethiopica* seem to bear little resemblance to each other and to other species. They were also dissimilar in terms of choromosome counts (Table I). The remaining species from a relatively homogeneous assemblage, with the two representatives of *Aglaonema* showing the highest level of dissimilarity with other species and seem closest to each other than either of them to any other member of this assemblage; *Aglaonema* is apparently a homogeneous genus. Among the remaining genera and species, the only other seemingly homogeneous genus was *Anthurium*, since its two representative species join each other in a distinct group before joining representatives of other genera. Both *Aglaonema* and *Anthurium*, were cytologically homogeneous (Table I). Although the two representatives of *Syngonium* had the same chromosome number (22; Table I), their leaf protein profiles did not coincide sufficiently to combine them in the same group in Fig. 2, indicating a degree of heterogeneity in the genus. The same applies to the 3 representative species of *Philodendron*. The pattern of relationships between the 6 genera represented by one species each (*Colocasia, Xanthosoma, Pothos, Scindapsus, Diffenbachia & Nephthytis*) was not supported by their chromosome

Fig. 2. UPGMA- dendrogram, based on the 57 SDS-PAGE of leaf protein characters, illustrating average taxonomic distance between the studied taxa



numbers: for instance, *Colocasia antiquorum* (2n = 28) was closest to *Anthurium* spp. (2n = 30) and *Xanthosoma violaceum* (2n = 26) was nearest to *Syngonium standleyanum* (2n = 22). Clearly, there was much disparity between chromosome counts and leaf protein patterns of some genera as there was agreement between them in others.

Although the sample of genera and species in the present study was admittedly far from representative of the entire family, the results served the purpose of indicating that the Araceae are long overdue for an extensive taxonomic re-appraisal. To the best of our knowledge, the tool of SDS-PAGE analysis of proteins has not been previously applied to members of this family. The number of genera and species for which chromosome counts were on record is relatively small. A comprehensive study, where chromosome counts and protein patterns (preferably of seed proteins), which would be coupled with other types of taxonomic evidence in the Araceae would be well-rewarded.

Acknowledgments. We wish to thank Prof. Dr. Amal Shehab and Prof. Dr. Zakia Adam (Botany Department, Faculty of Girls, Ain Shams University) for continuous help and encouragement.

REFERENCES

Abdel-Tawab, F.M., A.K. Selim, K.R.F. Hussein and M.A. Rashad, 1982. Phylogenetic relationship in the genus *Zea* and related genera. II Electrophoretic patterns and molecular weight of protein. *Egypt J. Genet. Cytol.*, 11: 265–73

Croat, T.B., 1981. The distribution of Araceae. *In*: Larsen, K. and H. Nielsen (eds.), *Tropical Botany*, Pp: 291–308, Academic Press, London

Croat, T.B., 1994. The use of new world Araceae as drug plants. *J. Japan Bot*., 69: 185–203

Darlington, C.D. and A.P. Wylie, 1955. *Chromosome Atlas of Flowering Plants*. Pp. 272–6. London

Eweda, M.A., 1989. Genotypic variation in the electrophoretic banding pattern of seed storage proteins of Soybeen. *The 1st Arab. Conf. of Genetics*. Pp. 289–300. Fayoum, 25 – 27 Oct

Fedorov, M.A., 1969. *Chromosome Number of Flowering Plants*. V.L. Komarov Botanical Inst., Leningrad

Gamal El–Din, A.Y., E.H.A. Hussein and M.A. Eweda, 1988. Variation in chromosome numbers and its bearing on electrophoretic protein banding pattern in *Vicia*. *Bull. Fac. Agric*., (Cairo Univ.) 39: 143–53

Giannasi, D.E. and D.J. Crawford, 1986. Biochemical systematic II. Areprise. *In*: Hecht, M.K., B. Wallace and G.T. Prance (eds.), *Evolutionary Biology*, Vol. 20, Pp: 25–148. Plenum Press, New York

Goldblatt, P. and D.E. Johnson, 1981-1997. *Index to Plant Chromosome Numbers. Monographs in Systematic Botany*, Vol. 40. Missouri Botanical Garden, Missouri, U.S.A

Jones, E.J., 1957. Chromosome number and phylogenetic relationships in the Araceae. *Ph. D. Thesis*, University of Virginia, Virginia, U.S.A

Kamel, E.A. and L.M.A. Hassan, 2001. The significance of cuticular features, petiole anatomy and SDS - PAGE in the Taxonomy of the Lauraceal. *Pakistan J. Biol. Sci*., 4: 1094–1100

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature*, 277: 680–5

Mabberley, D.J., 1997. *The plant Book, A portable Dictionary of the Vascular Plants*. Cambridge University Press, Cambridge

Marchant, C.J., 1973. Chromosome variation in Araceae. I. Pothoideae to Slylochitoneae. *Kew Bull.*, 24: 315–22

Matta, D.N., J.A. Gatehouse and D. Boulter, 1981. The structure of legumin of Vicia faba L. *J. Exp. Bot.*, 32: 183–97

Mayo, S.J., J. Bogner and P.C. Boyce, 1997. *The genera of Araceae*. Kew Royal Botanical Gardens

Melville, R. and F.A. Wrigley, 1969. Fenestration in the leaves of *Monsters* and its bearing on the morphogenesis and colour patterns of leaves. *Bot. J. Linn. Soc*., 62: 1–16

Mookerjea, A., 1955. Cytology of different species of Aroids with a view to trace the basis of their evolution. *Cytologia*, 7: 271–91

Moore, D.M.P., 1978. The Chromosomes and Plant Taxonomy. *In*: H.E. Street (ed.), *Essays in Plant Taxonomy*, Pp: 39–56, Acad. Press, London

Ramachandran, K., 1978. Cytological studies on south Indian Araceae. *Cytologia*, 43: 289–303

Raven, P.H., 1975. The Bases of Angiosperm Phylogeny. *Ann. Missou. Bot. Grad*., 62: 724–64

Rohlf, J.F., 1989. *NTSYS-PC*. Pp: 1–29. Numerical taxonomy and multivariat analysis. Setauket, New York

Sammons, D.W., L.D. Adamas and E.F. Nishizawe, 1981. Ultrasensitive silver based color staining of polypeptides in polyacrylamide gels. *Electrophoresis*, 2: 135–40

Sharma, A.K. and A.L. Sharma, 1980. *Chromosome Technique. Theory and Practice*. 3rd ed. Pp: 145–50. Butterworth and Co. Publishers Ltd., London

Sheffer, R.D. and H. Kamemoto, 1976. Chromosome number in the genus Anthurium. *American J. Bot.*, 63: 74–81

Subramanian, D. and M. Munian, 1988. Cytotaxonomical studies in south Indian Araceae. *Cytologia*, 53: 59–66

Vries, I.M., 1996. Characterization and identification of Lactuca sativa cultivars and wild relatives with SDS–electrophoresis (Lactuca sect. Lactuca, Compositae). *Genet. Resour. Crop Eval.*, 43: 193–202

(Received 21 November 2005; Accepted 14 February 2006)