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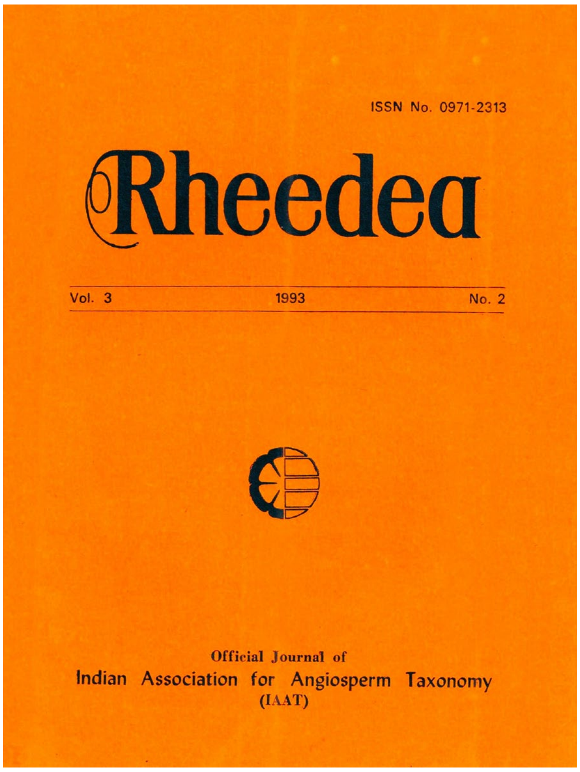

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Defence compounds and their taxonomic significance in Euphorbiaceae

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Abstract

The products of shikimate (proanthocyanidins, tannins, caffeic acid, chlorogenic acid, alkaloids) and acetate mevalonate (steroids, terpenoids, iridoids) pathways in 86 members of Euphorbiaceae are analysed and interpreted in terms of their advancement. The incidence of caffeic acid or its isomer in a number of taxa of the present study adduces its efficacy. The evolutionary strategies among some of the ecotypes/cultivars are discussed in the light of biosynthetic pathways.

INTRODUCTION

It has been observed that the evolution of secondary chemical constituents, including repellents produced in different biosynthetic pathways is closely connected with the morphological evolution (Cronquist 1977; Gottleib 1982). Thus, the shikimate pathway, which is in operation in primitive angiospermous taxa, produces lignin for mechanical strength and proanthocyanidins (PAC) and tannins for defence in the same route. With the blockage of final steps of the pathway and subsequent oxidation of the accumulated cinnamate, the benzyl isoquinoline alkaloids-(Magnoliales), betalains-(Caryophyllales) and caffeic acid (CA, in several taxa) are produced. Further complexity is achieved with the production of chlorogenic acid (Chl A) and depside rosmarinic acid which are the derivatives of caffeic acid. There is a shift from shikimate pathway to a more sophisticated and elaborate acetate metabolism either via malonate leading to the synthesis of polyacetylenes in Araliales and Asterales or mevalonate leading to iridoids, terpenoids and steroids in several other advanced taxa (Dahlgren 1980). It is presumed that the sharp taste and instantaneous effect are the reasons for the choice of these compounds (Kaplan and Gottleib 1982; Molgaard 1985b).

In the present communication, 86 Euphorbiaceous taxa are analysed and assessed against this backdrop.

MATERIALS AND METHODS

As delineated in Table 1, the 86 taxa of Euphorbiaceae, spread over 27 genera and 10 tribes were collected from different localities of India (except *Euphorbia degenereii* which was obtained from Hawaiian Islands) and deposited in the Herbarium, P. G. Department of Botany, Nizam College, Hyderabad, India. The various secondary metabolites were screened following standard phytohcemical procedures of Amarasingham *et. al.* (1964), Gibbs (1974) and Santaram (1983). Using fresh vegetative aerial parts, Leucoanthocyanin Test 'A' for leucoanthocyanins, HCl/Methanol Test or Isenberg Buchanan test for condensed tannins, Trimhill test for iridoids and Ehrlich test for aucubin compounds (monoterpenoids) were conducted. Using 80% methanolic extract of the shade dried material, ellagic acid test for ellagic acid, tannin test 'A' for hydrolysable tannins and Liebermann Burchard test for triterpenoids/steroids were carried out. For the detection of phenolic acids like caffeic and chlorogenic acid, bidirectional ascending paper chromatographic technique was followed using the solvent systems 1% HCl and Propanol : Ammonia : Water (20: 1: 4 v/v) in first and second directions respectively. The dried chromatograms were observed under UV light (with ammonia) and sprayed with 0.2% diazotised sulphanilic acid and compared them with the R_f values and spot colours of the authentic standard samples chromatographed under identical conditions.

RESULTS AND DISCUSSION

The distribution pattern of various repellents prodcued by the shikimate and acetate-mevalonate pathways is shown in Table 1, from which it is evident that *Baliospermum solanifolium* produces exclusively PAC. It can be regarded as the most primitive taxon among the members studied. Since PAC cannot be hydrolysed and reinvested in the primary metabolism, the plants have switched over to the production of such substances like hydrolysable tannins, CA or its complex derivatives in the shikimate pathway which can be hydrolysed avoiding the toxicity (Molgaard 1985b). It may be pointed out that the taxa, producing condensed tannins (Hemalatha and Radhakrishnaiah 1993) also occupy the similar lowest rung of the evolutionary ladder. The tanniniferous taxa as evidenced by the possession of ellagic acid (a hydrolysed product of tannins), such as *Euphorbia elegans, E. rosea, E. thymifolia, Phyllanthus pinnatus, P. reticulatus, Gelonium multiflorum* and *Chrozophora rottleri,* represent the second step in evolution, in this direction, as held earlier (Hemalatha and Radhakrishnaiah 1993).

A few taxa of the present study, like *Givotia rottleriformis, Bridelia crenulata, Manihot esculenta* (with variegated leaves) and *Euphorbia caracasana,* which represent the synthesis of toxicants like caffeic acid/or its isomer chlorogenic acid together with PAC, represent slightly advanced situation. The benzyl isoquinoline alkaloids and CA or its derivatives, found in *Acalypha wilkesiana* (green variety). *Acalypha ciliata, Phyllanthus emblica* and *Macaranga denticulata* constitute the next logical step, as the synthesis of PAC is bypassed.

There is a widespread incidence of caffeic acid (or chlorogenic acid)

Table 1 Members of Euphorbiaceae showing different biosynthetic pathways

I Shikimate Pathway					II Acetate-Mevalonate Pathway		III Dual pathways					
*PAC	EA	PAC+CA/ Chl. A	CA/Chl.A +Alk.	CA/Chl.A	Ster./ Terp.	Irid.	PAC+CA/ Chl. A. + Ster. /Terp.	PAC+CA Chl. A. +Irid.	CA/Chl.A +Ster. /Terp.	Alk.+ Ster. /Terp.	Alk. + Irid.	CA/ Chl.A+ Terp
@12	22,29	15,24	4,10,60	1, 3, 6, 7	66	23	18,46,50	49,55	2,5,9	17	8	19,34
	42,44	48,64	73	16,21,27	74	40	75,81	70,76	11,13	83		37,43
	47,78			28,30,31	77				14,20			51,52
	79			33,36,38					25,26			
				39,41,53					32,35			
				54,56,57					45,58			
				63,65,68					59,61			
				69,80,82					62,67			
				84,85,86					71,72			

*For details see the text.

@ 1. *Antidesma acuminatum* wall. 2. *Aporusa acuminata* Thw. 3. *Acalypha alnifolia* Klein 4. *A. ciliata* Forsk. 5. *A. godseffiana* J. J. Smith 6. *A. hispida* Burm. 7. *A. Indica* L. 8. *A. lanceolata* Willd. 9. *A. paniculata* Miq. 10. *A. wilkesiana* Muell. Arg. (green cultivar) 11. *A. wikesiana* Muell. Arg. (red cultivar) 12. *Baliospermum solanifolium* (J. Burm.) Suresh. 13. *Breynia patens* Benth. 14. *B. rhamnoides* Muell. Arg. 15. *Bridelia crenulata* Roxb. 16. *B. montana* Willd. 17. *Cleistanthus collinus* Benth. 18. *C. patulus* Muell. Arg. 19. *Codiaeum variegatum* Blume. 20. *Croton bonplandianum* Baill. 21. *Chrozophora parvifolia* Klotzsch. 22. *C. rottleri* Juss. 23. *Euphorbia antiquorum* L. 24. *E. caracasana* Boiss. 25. *E. cotinifolia* L. 26. *E. cyathophora* Murray 27. *E. degeneri* Shreff. 28. *E. dracunculoldes* Lam. 29. *E. elegans* Spreng. 30. *E. hirta* L. (green ecotype) 31. *E. hirta* L. (purple ecotype) 32. *E. heterophylla* L. (green ecotype) 33. *E. heterophylla* L. (purple ecotype) 34. *E. hypericifolia* L. 35. *E. leucocephala* Lotsy. 36. *E. milii* Des. Moul. 37. *E. nerifolia* L. 38. *E. prostrata* L. (green ecotype) 39. *E. prostrata* L. (purple ecotype) 40. *E. pulcherrima* Willd. (red cultivar) 41. *E. pulcherrima* Willd. (white cultivar) 42. *E. rosea* Retz. 43. *E. rothiana* Spreng. 44. *E. thymifolia* L. 45. *E tirucalli* L. 46. *Excoecaria bicolor* Hassk. 47. *Gelonium multiflorum* A. Juss. 48. *Givotia rottleriformis* Griff. 49. *Glochidion lanceolarium* Dalz. 50. *C. zeylanicum* Juss. 51. *Hura crepitana* L. 52. *Jatropha curcas* L. 53. *J. glandulifera* Roxb. 54. *J. gossypifolia* L. 55. *J. heyneii* Balakr. 56. *J. integerrima* Jacq. (red cultivar) 57. *J. integerrima* Jacq. (pink cultivar) 58. *J. multifida* L. 59. *J. podagarica* Hook. 60. *Macaranga denticulata* Muell. Arg. 61. *M. indica* Wt. 62. *M. peltata* Muell. Arg. 63. *Manihot esculenta* Crantz. (green cultivar) 64. *M. esculenta* Crantz. (variegated) 65. *Mallotus philippensis* (Lamk.) Muell. Arg. 66. *Monadenium heteropodum* N. E. Br. 67. *Pedilanthus tithymaloides* Poit. (green cultivar) 68. *P. tithymaloides* Poit. (variegated) 69. *P. tithymaloides* ssp. *angustifolius* Poit. 70. *Phyllanthus acidus* Skeels 71. *P. angustifolius* Swartz. 72. *P. debilis* Herb. Ham. 73. *P. emblica* L. 74. *P. gardnerianus* Baill. 75. *P. macraei* Muell. Arg. 76. *P. maderaspatensis* L. 77. *P. fraternus* Webster 78. *P. pinnatus* Webster. 79. *P. reticulatus* Poir. 80. *P. simplex* Retz. 81. *P. urinaria* L. 82. *Ricinus communis* L. 83. *Sebastiana chamaelea* Juss. 84. *Securinega leucopyrus* Muell. Arg. 85. *Synadenium grantii* Hook. 86. *Tragia cannabina* L.

unaccompanied by PAC or other products, in a majority of the members studied, concurring with observations of Dahlgren (1980). It reinforces the view of Mohanty *et al.* (1982), Didry *et al.* (1982) and Molgaard (1985b) that caffeic acid is an efficient repellent and toxicant. The taxa in possession of the same represent a climax situation among those with shikimate pathway.

The presence of triterpenoids/steroids in *Phyllanthus gardnerianus*, *P. fraternus*, and *Monadenium heteropodum* and iridoids in *Euphorbia antiquorum* and *E. pulcherrima* (red variety) indicate the operation of the acetate-mevalonate pathway. There is suppression of synthesis of the products of shikimate pathway, which are considered as primitive compounds on the basis of correlation with such primitive characters as stipulate leaves and crassinucellate ovules (Sporne 1969, 1980). Bate-Smith (1962) and Harborne (1977), therefore, find it reasonable to consider their presence as a primitive trait and disappearance as an evolutionary advancement. Thus, the operation of the advanced pathway in the aforesaid taxa to the total exclusion of the archaic one, can be reckoned as advanced.

However, in some of the Euphorbiaceous taxa studied, there appears to be simultaneous or parallel operation of both shikimate and acetate-mevalonate pathways as shown in Table 1. Such taxa as *Cleistanthus collinus* and *Sebastiana chamaelea* produce alkaloids and steroids/triterpenoids and *Acalypha lanceolata*, the iridoids in lieu of steroids/triterpenoids. The simultaneous operation may be in response to the requirement of their flavonoids, mechanical strength and occurrence of specific predators (Molgaard 1985a; Swain 1978). Though the situation appears to be intermediate, it can be regarded as advanced according to Molgaard (1985b, p. 209) who observed "... an advanced compound is never found in a primitive taxon, but a primitive compound is likely to occur, sporadically in advanced taxonomic group". Thus, the occurrence of shikimate pathway, though archaic, if found in taxa with acetate-mevalonate pathway, the products of the former can be regarded as biochemical relicts In advanced taxa.

From the results, it is also evident that there are different evolutionary strategies among the ecotypes/garden cultivars of *Acalypha*, *Euphorbia*, *Jatropha*, *Manihot* and *Pedilanthus*. Thus, *Acalypha wilkesiana* (red variety), *Euphorbia heterophylla* (green ecotype) and *Pedilanthus tithymaloides* (green cultivar) are highly evolved on account of production of CA/or Chl. A accompanied by steroids/triterpenoids, which are efficient defence compounds against predators. *Euphorbia pulcherrima* (red) in which alkaloids and iridoids are produced could also be included in a similar category.

Euphorbia prostrata, *E. hirta* and *Jatropha integerrima*, each with green and pink forms, uniformly produce the most efficient predator deterrant, the caffeic acid or its isomer.

It can be concluded that *Acalypha wilkesiana*, *Euphorbia Pulcherrima* (red variety), *E. heterophylla* and *Pedilanthus tithymaloides* (both green and variegated cultivars) can be reconed as relatively more evolved on the basis of operation of advanced biosynthetic pathway while their counterparts are equally successful survivors due to to the production of caffeic acid or its isomer.

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