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HYBRIDIZATION BETWEEN *ACACIA NILOTICA* SUBSP. *INDICA* AND *A. NILOTICA* SUBSP. *CUPRESSIFORMIS*

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Abstract

The hybridization between *Acacia nilotica* subsp. *indica* and *A. nilotica* subsp. *cupressiformis* is substaintiated by the study of phenolic constituents. The hybrids have additive or some new compounds, not present in either parent. Small number of phenolic constituent and their uniform pattern indicate that the Indian population of *A. nilotica* subsp. *indica* and the populations of *A. nilotica* sub sp. *cupressiformis* from N. Punjab genetically were purer than the populations from Sindh which have higher number of phenolic constituents and a variable pattern.

Introduction

Acacia nilotica (L.) Willd. ex Delile, widely distributed in Africa, Arabia and the Indo-Pakistan subcontinent, consists of 9 subspecies (Ali & Qaiser, 1980; Brenan, 1983). In some earlier papers (Ali & Faruqi, 1969; Ali & Qaiser, 1980) the variability met within Pakistan in this complex is discussed in the light of hybridization between *A. nilotica* subsp. *indica* and *A. nilotica* subsp. *hemispherica*. Though probable hybridization between *A. nilotica* subsp. *indica* and *A. nilotica* subsp. *cupressiformis* was discussed, yet the evidence available at that time was not conclusive enough because most of the samples analysed were derived from Sindh only. In view of this it seemed necessary to collect additional evidence from those regions where *A. nilotica* subsp. *cupressiformis* is available in pure form.

Material and Methods

The plant material used in the present study was either collected by the authors or was obtained mostly from the Karachi University Herbarium (KUH), National Herbarium, Pakistan Agriculture Research Council (RAW), Punjab University Herbarium, Patiala (PUN), National Botanic Gardens, Lucknow (LUG) and Botanical Survey of India, Arid Zone Circle, Jodhpur (BSJO).

Phenolic constituents present in the leaves of the dried herbarium specimens were studied in all cases. The standard methods of Bate-Smith (1962) and Harborne (1973) were used for the separation and the identification was made by comparing with the authentic markers along with the Rf values and colour in U.V. light with and without ammonia.

Observations and Results

The data dealing with phenolic constituents are presented in Tables 1, 2 and 3. In each case the values given in the Table indicate the percentage of plants in which the substance concerned was observed.

Table 1. Phenolic constituents of *Acacia nilotica* (data expressed in terms of the number of plants having a particular phenolic substance per 100 plants (%) belonging to each taxon)

		A. nilotica subsp. indica (India)	A. nilotica subsp. indica (Pakistan: Sindh)	A. nilotica subsp. cupressiformis (Pakistan:Sindh)	A. nilotica subsp. cupressiformis (Pakistan:N.Punjab)
Sample size		7	11	16	13
	Caffeic acid	0	63.6	81.25	0
	Ferulic acid	100	100	100	100
	P-Coumeric acid	100	81.8	62.5	53.8
Phenolic	PA 1	0	18.18	0	0
acids	PA 2	0	0	31.25	0
	PA 3	57.14	63.63	81.25	0
	PA 4	0	9.09	0	0
Leuco	Malvidin	100	100	100	0
anthocya-	Peonidin	100	72.27	100	100
nidins	Delphinidin	0	90.9	62.5	0
	Quercetin	100	100	100	100
	Kaempferol	0	9.09	12.5	0
	Isorhammetin	0	36.36	18.75	0
	Myricetin	0	18.18	0	0
Flavonoids	Luteolin 5-glucoside	0	90.9	81.25	100
	Quercetin 3-rutinoside	100	9.09	18.75	0
	Fl 3	0	0	25	0
	Fl 5	42.85	9.09	0	0
	Spot 1	0	0	6.25	0
	Spot 3	0	9.09	0	0
	Spot 4	0	0	18.75	0
Unknown	Spot 5	0	27.27	25	0
	Spot 6	0	0	0	15.3
	Spot 7	0	9.09	0	0
	Spot 8	0	18.18	0	0
Total number of compounds detected:		8	20	17	6

Discussion

In four subspecies of *Acacia nilotica* fruits are narrowly and regularly constricted between seeds. Of these only *A. nilotica* subsp. *indica* and *A. nilotica* subsp. *cupressiformis* are present in Pakistan. *A. nilotica* subsp. *indica* occurs in the P.D. R. Yemen, the Yemen Arab Republic, Oman, Pakistan (Punjab, Sindh), India (Punjab, Uttar Pradesh, Bengal, Madhya Pradesh, Madras and Bombay) and Burma. In Africa it is known from Ethiopia, Somalia, Angola and Tanzania, but probably it is not truly indigenous in Africa. *A. nilotica* subsp. *cupressiformis* is present in Pakistan (Punjab, Sindh) and India (Deccan, Punjab, Uttar Pradesh, Bombay, Gujrat). It is most abundant and most typical in the upper part of Jech Doab, between Gujrat and Jhelum and near Jhang (Stewart, 1869). From there it may have spread to other parts in Pakistan and India.

In Sindh, judging by the plants which exhibit an intermediate appearance, low pollen fertility and the production of very few fruits, *A. nilotica* subsp. *cupressiformis* hybridizes with *A. nilotica* subsp. *indica*. This is further confirmed by the study of phenolic constituents.

A study of phenolic constituents has been found very useful for the elucidation of hybridization in a number of plants (Alston & Turner, 1963; Hunter, 1967; Quinn & Rattenbury, 1972; Harborne, 1975; Leach & Whiffinn, 1978; Ali & Qaiser, 1980). Generally the hybrid populations show an additive pattern and some hybrid-specific substances not present in either parent are also expected to occur (Harborne, 1975). Thus the total numbers of phenolic compounds present in hybrids is generally much larger than the number of compounds present in either parent. Further the same substances are not present in all the hybrids because genetically the hybrids are much more heterogenous than either parent. Hence, as discussed earlier (Ali & Qaiser, 1980) the results are presented in terms of percentage of plants containing a particular phenolic substance in each population with a view to compare different populations and determine the extent of introgression.

In the populations analysed, ferulic acid and quercetin are present in all the samples. These substances are reported to be widely distributed in Angiosperms (Swain & Bate-Smith, 1962; Harborne & Simmonds, 1964). A comparison of the pattern of phenolic constituents found in the samples of the subspecies *indica* from India and Pakistan (Table 1) shows that apart from one unidentified phenolic acid (PA3) and one unidentified flavonol glucoside (Fl5), all the constituents are present in all the samples from India, whereas in Pakistani samples, the only compounds consistently present are ferulic acid, quercetin and malvidin. Further only 8 compounds are recorded in the samples from India whereas 20 compounds could be detected in Pakistani samples. Both these criteria indicate that Indian populations are genetically much purer than Pakistani samples, where the genes from the hybrid swarms have infiltrated. This is substantiated by the fact that Indian samples were obtained from regions where only one subspecies occurs and the chances of infiltration of genes from other subspecies are rather remote.

A comparison of the pattern of phenolic constituents found in *A. nilotica* subsp. *cupressifomis* from Sindh and N. Punjab (Pakistan) shows that apart from ferulic acid, peonidin and quercetin, only one substance is present in all the samples from N. Punjab whereas in samples from Sindh, the compound consistently present is malvidin. Further only 6 compounds are present in the samples from N. Punjab whereas 17 compounds

Table 2. Phenolic constituents of *Acacia nilotica* present in one parent but absent from the other (data expressed in terms of the number of plants having a particular phenolic substance per 100 plants).

Subspecies		*indica* (India)	*indica* (Pakistan:Sindh)	*cupressiformis* (Pakistan:Sindh)	*cupressiformis* (Pakistan:N. Punjab)
Phenolic Acids - PA_3		57.14	63.63	81.25	0
Flavonols	Luteolin 5-glucoside	0	90.9	81.25	100
	Quercetin 3-rutinoside	100	9.09	18.75	0
	F1 5	42.85	9.09	0	0
Unknown	Spot 6	0	0	0	15.3

Table 3. Hybrid specific phenolic constituents of *Acacia nilotica* (data expressed in terms of the number of plants having a particular phenolic substance per 100 plants

Subspecies		*indica* (India)	*indica* (Pakistan:Sindh)	*cupressiformis* (Pakistan:Sindh)	*cupressiformis* (Pakistan:N. Punjab)
	Caffeic Acid	0	63.63	81.25	0
Phenolic Acids	PA 1	0	18.18	0	0
	PA 2	0	0	31.25	0
	PA 4	0	9.09	0	0
Leucoanthocya-nidins	Delphinidin	0	90.9	62.5	0
	Kaempferol	0	9.09	12.5	0
	Isorhamnetin	0	36.36	18.75	0
Flavonols	Myricetin	0	18.18	0	0
	Fl 3	0	0	25	0
	Spot 1	0	0	6.25	0
	Spot 3	0	9.09	0	0
	Spot 4	0	0	18.75	0
Unknown	Spot 5	0	27.27	25	0
	Spot 7	0	9.09	0	0
	Spot 8	0	18.18	0	0

could be detected in samples from Sindh. These criteria obviously indicate that the samples from N. Punjab are genetically much purer as compared to the samples from Sindh.

The following substances are present only in one parent (Table 2), subspecies *indica* and the hybrids including Pakistani forms of subsp. *indica* and subsp. *cupressiformis* from Sindh: phenolic acid (PA3), quercetin 3-rutinoside and an unidentified flavonol (Fl5). Similarly luteolin 5-glucoside is present in subsp. *cupressiformis* from N. Punjab, Sindh and subsp. *indica* from Sindh but absent from subsp. *indica* from India, whereas an unidentified substance (Spot 6) is present in 15.3% of the samples of subsp. *cupressiformis* from N. Punjab, but absent from all the other samples. Subspecies *indica* and subspecies *cupressiformis* from Sindh have 20 and 17 phenolic compounds respectively including the following hybrid specific compounds which are not present in both these subspecies: Caffeic acid, delphinidin, kaempferol, isorhamnetin and an unknown spot (spot 5). Whereas three unidentified phenolic acids (PA1 and PA4), myricetin and three unknown spots (Spot 3, spot 7 and spot 8) are present in subsp. *indica* from Sindh and an unidentified phenolic acid (PA2), an unidentified flavonol (Fl3) and two unidentified spots (spot 1 and spot 4) are present in subspecies *cupressiformis* from Sindh (Table 3).

The hybridization between *A. nilotica* subsp. *indica* and *A. nilotica* subsp. *cupressiformis* is thus substantiated by the study of phenolic constituents. The suspected hybrids not only show additive pattern but some hybrid specific compounds as well.

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References

Ali, S. I. and S. A. Faruqi. 1969a. A taxonomic study of *Acacia nilotica* Complex in West Pakistan. *Pak. J. Bot.*, 1: 1-8.

Ali, S. I. and S. A. Faruqi. 1969b. Hybridization in *Acacia nilotica* Complex. *Pak. J. Bot.*, 1: 119-128.

Ali, S. I. and M. Qaiser. 1980. Hybridization in *Acacia nilotica* (Mimosoideae) Complex. *Bot. J. Linn. Soc.*, 80: 69-77.

Alston, R. E. and B. L. Turner. 1963. *Biochemical Systematics*. New Jersey: Prentice- Hall.

Bate- Smith, E. C. 1962. The Phenolic Constituents of Plants and their taxonomic significance. Dicotyledons. *Bot. J. Linn. Soc.*, 58: 95-173.

Brenan, J. P. M. 1983. Manual of Taxonomy of *Acacia* species. FAO, Rome.

Harborne, J. B. 1973. *Phytochemical Methods*. London: Chapman & Hall.

Harborne, J. B. 1975. Biochemical Systematics of Flavonoids. In J. B. Harborne, T. J. Mabry & H. Mabry, (Eds.). *The Flavo noid*: 1056-1095. London: Chapman & Hall.

Harborne, J.B. and N.W. Simmonds. 1964. The natural distribution of Phenolic aglycones. In: J.B. Harborne (Ed.), *Biochemistry of Phenolic Compounds:* 77-127. Academic Press, London and New York.

Hunter, G. E. 1967. Chromatographic documentation of interspecific hybridization in *Vernonia*: Compositae. *Amer. Jour. of Bot.*, 54: 473-477.

Leach, G. H. and T. Wahiffin. 1976. Analysis of hybrid between *Acacia brachybotrya* and *A. calamifolia* (Leguminosae) *Bot. J. Linn. Soc.*, 76: 53-69.

Quinn, C. J. and J.A. Rattenbury. 1972. Structural hybridity in New Zealand Dacrydium. *N. Z. J. Bot.*, 10: 427-436.

Stewart, J. L. 1869. *Punjab Plants*. Lahore: Government Press.

Swain, T. and E.C. Bate-Smith. 1962. In: M. Florkin & H.S. Mason (Eds.), Comparative Biochemistry, 3: 755-809. Academic Press, London and New York.

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