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ELUCIDATION OF INTERSPECIFIC RELATIONSHIPS THROUGH CHROMATOGRAPHIC STUDIES

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Abstract

Paper and thin layer chromatographic studies of the three species of *Corchorus*, namely, (a) *C. hirtus* L. of Argentina, (b) *C. olitorius* L. var. C.G. and (c) *C. trilocularis* L. show that (a) and (c) share more common spots than (b) shares with (a) or (c). Although *hirtus* belongs to a continent different from Pakistani species of *olitorius* and *trilocularis*, morphologically it is similar to *trilocularis*. Chromatographic studies lead one to the same conclusion that *trilocularis* is more closely related to *hirtus* compared to *olitorius*.

Chromatographic partition techniques for analysis of species and hybrids of plants and animals are providing to be significant tools in taxonomy, phylogeny, and genetics (Hadorn 1956; Turner and Alston 1959; Buzzati-Traverso 1960; Hubby and Throckmorton 1960; Riley and Bryant 1961).

The preliminary work of Buzzati-Traverso suggested that the simple method of squashing tissues on filter paper, followed by one dimensional chromatographic separation of nin-hydrin positive and ultraviolet flourescent substances could yield results of value in determining the phylogenetic relationships of taxa whose affinities cannot be established through the study of morphological characters alone.

The work of Alston and Turner (1962) showed that by partition paper chromatography the introgressent progeny of natural species hybrids can be recognised through comparison of spots which one or the other parental species contains. Stebbins *et al.* (1963) have compared chromatograms obtained from the extracts of dried leaves of various entities of the *Viola purpurea* complex as well as of *V. pedunculata*. Such studies enabled them to elucidate the relationships between various complexes of *V. purpurea* which conventional methods of study failed to reveal.

In the study of the crossing relationships between *Corchorus olitorius, C. trilocularis* and *C. hirtus* it was observed that the first two fail to produce any hybrid and the latter two resemble closely in their morphology and produce sterile hybrids. We wanted to find out whether the similarity as found in the morphology of *trilocularis* and *hirtus* also extends to the biochemical level. Comparison of organic compounds e.g. phenols of the above three species by means of chromatography was therefore carried out.

Materials and Methods

The leaves of the three species of *Corchorus*, namely, colchicine induced tetraploid *C. olitorius* L. var. C.G., *C. hirtus* L. obtained from Argentina and *C. trilocularis* L. were tested for their contents by paper chromatography and their flowers by thin layer chromatography.

(a) *Thin layer chromatography:* The tissue was killed by plunging it into hot ethanol for five min. and then crushed in a mortar. The resultant slurry was filtered through cotton wool and the filtrate was preserved in a small tube.

By means of capillary tube the plant extract was applied upon a slide containing a thin film of silica gel. After applying sufficient amount of plant extract, the slide was transferred into a beaker containing chloroform-benzene (1:1) mixture without letting the drop of the plant extract come in direct contact with the solvent.

After the liquid reached the end of the slide, the latter was removed from the beaker and was placed in another beaker containing iodine crystals (Stahl 1965). It was in the vapours of iodine that the spots became distinct and clear. With the help of this method the Rf values of differernt compounds were measured. The experiment was repeated six times.

(b) *Paper chromatography:* Twenty g of leaf tissue of each species were immersed in 50 ml of 95% ethanol. The extraction was made in 2 ways i.e. by means of (i) Soxhlet apparatus and by (ii) direct heating on a water bath.

The spotting of one species was made on the same paper at 5 different places, each spot containing 25 lambda of the extract. Chromatograms were run in only one (ascending) direction using butanol-acetic acid-water (4:2.2:1) as solvent system. The spots on the dried chromatograms were detected with the help of an ultraviolet lamp at 2537Å and marked off. The experiment was repeated eight times.

Observations

Thin layer chromatography

(i) *For hydrophilic compounds:* The Rf values of hydrophilic compounds of *olitorius*, *hirtus* and *trilocularis* have been compared and are given below:

TABLE 1

Distribution of hydrophilic spots from the flower extracts

Sr. no. & solvent	Name of plant	Solvent system used	No. of spots	Rf values
I.	c-4x *olitorius*	Chloroform + benzene (50 + 50)	Three light yellow	0.31, 0.5, 0.6
	hirtus	,,	Two yellow	0.16, 0.19
	trilocularis	,,	Two yellow	0.16, 0.20
II.	c-4x *olitorius*	Chloroform + ethyl acetate + formic acid (50 + 40 + 1)	Nil	— Nil
	hirtus	,,	Two	0.70, 0.81
	trilocularis	,,	Two	0.72, 0.95

The three light yellow spots obtained in *olitorius* were similar to those reported by Frost and Bose (1966) in the same material i.e. *olitorius*.

The results presented in Table 1 clearly show that two morphologically similar species i.e. *hirtus* and *trilocularis* have three common hydrophilic compounds of the Rf values of 0.7, 0.16 and 0.20. No spot of the same Rf value could be detected in c-4x *C. olitorius* indicating that this species does not only show important morphological differences with *hirtus* and *trilocularis* but also differences in the presence of hydrophilic compounds.

(ii) *For organic acids:* The same method as outlined in the extraction of phenol was followed here.

Mixtures of carboxylic acids may be separated on silica gel layer with basic polar solvents. This experiment was set up in order to compare organic acids present in the above mentioned species of *Corchorus*. The results are given in Table 2.

TABLE 2

Distribution of organic acids from the flower extracts

Sr. no. & solvent	Name of plants	Solvent system used	No. spots	Rf value
I.	c-4x *olitorius*	Methanol-5N+ammonia solution (80+20)	One	0.8
	hirtus	,,	One	0.71
	trilocularis	,,	One	0.73
II.	c-4x *olitorius*	Ethanol (96%) + water + ammonia solution 25% (100+12+16)	Two	0.82, 0.88
	hirtus	,,	Three	0.60, 0.66, 0.71
	trilocularis	,,	Three	0.62, 0.66, 0.84
III.	*olitorius*	Benzene+methanol+ glacial acetic acid (90+16+8)	One	0.88
	hirtus	,,	Two	0.59, 0.62
	trilocularis	,,	Two	0.61, 0.66

The above results indicate that *olitorius* does not share any of its organic acid spots with *hirtus* and *trilocularis* regardless of the solvent system used. *C. hirtus* and *trilocularis*, on the other hand, share the same spot in solvent I, two out of three in solvent II and two out of two in solvent III. The slight variation in the Rf value of spot may be due to experimental error.

Paper chromatography

The results of paper chromatography indicate that the three species of *Corchorus* used in the present work have not more than seven phenolic compounds. *C. trilocularis* was seen to have the largest number of spots i.e. 7 and both *hirtus* and *olitorius* were seen to have six each. Two phenolic compounds having Rf values 0.33 and 0.78 were common between *hirtus* and *trilocularis*. One spot of 0.8 Rf value was common between all the 3 species (Table 3).

TABLE 3

Distribution of phenolic compounds from leaf extracts

Name of plants	Solvent system used	No. of spots	Rf value
c-4x *olitorius*	BAH (4:2.2:1)	6	0.12, 0.18, 0.19, 0.20, 0.21 and 0.25
hirtus	,,	6	0.18, 0.14, 0.16, 0.33, 0.41 and 0.76
trilocularis	,,	7	0.17, 0.33, 0.37, 0.48, 0.52, 0.65 and 0.78

Discussion

Of the three species used in the present investigation two, *olitorius* and *trilocularis* are sympatric in Pakistan and the third, *hirtus* being of Argentinian origin is allopatric in relation to the first two. *C. olitorius* and *trilocularis* overlap in their distribution in Pakistan but natural hybrids have not been recorded so far. Artificial hybridization between the two have failed, the pollinated flower having been observed to drop 2-3 days after the crossing (Islam 1965). When the crossing in this combination was tried using indole-3-acetic acid (300 PPM), there was fruit set with *olitorius* as female only but the fruits were small and empty (Islam & Ali 1967). Between *hirtus* and *trilocularis* reciprocal hybrids were obtained without the use of any hormone (Mughal 1967). The hybrids were sterile but sterility might have been more due to their triploid nature (it was a cross between a diploid and tetraploid species) rather than the disharmony between the two genomes. Another important aspect of the relationship is that reciprocal hybrids such as has been obtained in the above combination has been reported only once before i.e. between *olitorius* and *pseudo-olitorius* (Islam and Kadir 1960).

The conclusion based on crossing relationship found support in the results obtained through paper and thin layer chromatography. The data on paper chromatography indicate that there are more common spots of phenolic compounds between *hirtus* and *trilocularis* than between *olitorius* and *trilocularis*. Those of the thin layer chromatography also showed that more spots are common between *hirtus* and *trilocularis*. Morphologically also *trilocularis* was found to resemble *hirtus* more than it resembles *olitorius*. The combined study of morphology,

hybridization and chromatography therefore points to the same conclusion i.e. closer relationship of *hirtus* to *trilocularis*.

It is not always that the morphological distinctions parallel with genetical diversities but in the present case it seems that the evolution of different isolating mechanisms in morphological, genetical and biochemical characteristics went hand in hand.

As regards the question whether *trilocularis*—a wild diploid and *hirtus*—a new world tetraploid share a common genome, it cannot be decided till the crossing is made at tetraploid level i.e. between artificially induced tetraploid *trilocularis* and the natural tetraploid *hirtus* and their meiosis studied.

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