IDENTIFICATION OF SEEDS FROM VARIOUS SPECIES OF *STROPHANTHUS*

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In a search for differential specific characters, the outer epidermis of the testa from *Strophanthus* seeds of 15 species has been examined. A "trichome index" has been defined, and the method of its determination described. It enabled the species studied to be divided into six groups.

The genus *Strophanthus* includes over 60 species, the seeds of about 30 of which have been subjected to chemical examination. Since the first study of the pharmacognosy of *Strophanthus* by Elborne[1] in 1887, many workers have searched for characters by which the seeds of one species might be readily distinguished from another. The seeds are morphologically similar, hence much reliance has been placed on chemical tests as a means of distinguishing them. In 1927, Mathiesen[2] studied authentic seeds of several species, checking details of anatomical structure, and searching for diagnostic characters for samples of commercial seed. Results of the sulphuric acid colour test on fourteen species were recorded, showing the variety of colours produced during the course of the reaction on a single species, and the similarity of colours produced in several different species. Fourteen colour tests were investigated by Smelt[3], who recommended the use of four tests to distinguish between *S. kombé* Oliver and *S. emini* Aschers.

The numerous chemical studies of Jacobs, of Stoll and of Reichstein have shown the variation in constituents according to species. The possible use of sarmentogenin in cortisone syntheses caused a period of renewed interest in the genus, during which some confusion between seeds of the various species occurred. Several expeditions explored Africa from 1949 to 1951 to collect as many species as possible[4,5] and the material had been studied botanically by Monachino[6,7], who reviewed the genus, observing polymorphism in species having a wide geographical distribution. Youngken and Simonian[8] illustrated the morphological characters of four species.

Bush and Taylor[9] found the sulphuric acid test to be unreliable and largely dependent on substances other than cardiac glycosides. They developed a paper chromatographic method for the routine semi-micro investigation of the easily hydrolysable glycosides of the seeds, and classified 24 species into three groups. This method was suggested as a test for seed samples to replace the sulphuric acid colour test.

Experimental

Although such structural differences as do occur in the seeds of various species of *Strophanthus* are of degree rather than of kind, methods depending on numerical relationships between tissues, cells or cell inclusions have

not been reported. Fifteen species were examined, showing that the outer epidermis of the testa was the most suitable tissue for numerical differences.

The lengths of trichomes on surface preparations could not be measured because of the dense mass of tangled trichomes in certain species, and experiments were made to determine the most suitable method of preparing a suspension of separated intact epidermal cells. The testa was removed from seeds previously softened in water and portions were disintegrated by several reagents. The reagent selected was an aqueous solution containing 5 per cent chromic acid and 5 per cent sulphuric acid. It was found possible to control the degree of disintegration easily with this reagent, by varying the concentration, time and temperature. The testa was placed in a screw-capped bottle of 15 ml. capacity with 1 ml. of the reagent for 5 hours at 20°. The trichomes were separated by centrifugation, washed until acid free and stained by suspending in 50 per cent v/v aqueous glycerol, containing 0·001 per cent methylene blue. The lengths were measured by micro-projection[10].

Results

Five hundred trichomes from each of 24 samples of disintegrated material were measured, and the results summarised in Table I.

TABLE I

Length of trichomes on testa of strophanthus seeds

Strophanthus kombé Oliver		
Sample number	Seed number	Length (microns)
1	1	60–390–900
	2	50–300–700
	3	50–390–800
	4	40–440–900
	5	70–310–700
2		70–350–800
3		60–380–700
4		60–360–700
5		40–290–700
6		50–400–900
Other species		
S. amboensis Engl. et Pax.		80–185–435–600
S. courmontii Sacleux.		40–160–440–700
S. emini Aschers.		100–345–935–1300
S. gerrardii Stapf.		50–150–330–500
S. grandiflorus (Brown) Gilg.		40–180–400–500
S. gratus Baill.		—
S. hispidus A.P.DC.		50–155–325–500
S. hypoleucus Stapf.		40–140–340–500
S. intermedius Pax.		100–240–500–700
S. nicholsoni Holmes.		200–440–1100–1700
S. preussii Engl. et Pax.		40–140–310
S. sarmentosus A.P.DC.		60–145–275–400
S. speciosus Reber.		30–50–120
S. welwitschii K. Schum.		50–165–415–700

From these data it appeared possible to distinguish certain species by length of the trichome. The number, per cent, of trichomes exceeding a selected critical length was tested, using several levels of the critical length, and termed the 'trichome index'. This is defined as the number, per cent, of trichomes which exceed a certain critical length. The latter is specified by prefix, for example: "100 micron trichome index".

The results of trichome index determinations using several critical lengths are given in Table II.

TABLE II

TRICHOME INDEX AT DIFFERENT CRITICAL LENGTHS

Trichome index	100 μ	200 μ	300 μ	400 μ	500 μ	1000 μ
S. amboensis	98	80	47	23	7	0
S. courmontii	95	72	48	25	7	0
S. emini	99	93	84	77	67	11
S. gerrardii	95	65	24	3	0	0
S. grandiflorus	96	67	20	2	0	0
S. gratus	0	0	0	0	0	0
S. hispidus	96	67	20	2	0	0
S. hypoleucus	93	63	27	6	1	0
S. intermedius	100	89	69	38	15	0
S. kombé	94	80	60	40	20	0
S. nicholsoni	100	100	97	89	80	24
S. preussii	76	17	1	0	0	0
S. sarmentosus	98	52	7	0	0	0
S. speciosus	2	0	0	0	0	0
S. welwitschii	95	76	46	20	5	0
Strophanthus kombé					300 μ	400 μ
Limits of variation between seeds					64–70	45–55
Limits of variation between samples					55–70	35–50

TABLE III

SEPARATION TABLE, USING TRICHOME INDEX (T.I.) TO GROUP *Strophanthus* SPECIES; ALSO COMPARING BUSH AND TAYLOR'S CLASSIFICATION[9], AND COLOURS REPORTED WITH 80 PER CENT SULPHURIC ACID ON THE ENDOSPERM OF SEED SECTIONS

Group	Species	Bush and Taylor's classification	80 per cent sulphuric acid test
I. 1000 μ T.I. exceeds 18	*S. nicholsoni*	2	red
II. 1000 μ T.I. less than 18 400 μ T.I. exceeds 70	*S. emini*	2	red or orange
III. 400 μ T.I. less than 70 300 μ T.I. exceeds 35	*S. amboensis* *S. courmontii* *S. grandiflorus* *S. intermedius* *S. kombé* *S. welwitschii*	3 3 — 3 2 3	red or orange orange red or orange — green red
IV. 300 μ T.I. less than 35 200 μ T.I. exceeds 37	*S. gerrardii* *S. hispidus* *S. hypoleucus* *S. sarmentosus*	3 2 2 3	red or orange green red or orange red or orange or green
V. 200 μ T.I. less than 37 100 μ T.I. exceeds 35	*S. preussii*	2	—
VI. 100 μ T.I. less than 35	*S. gratus* *S. speciosus*	1 —	red red or orange

DISCUSSION

The fifteen species of strophanthus seeds studied were divided into six groups, using the proposed trichome index (Table III). Variations between seeds, and between samples, are shown by the measurements upon *S. kombé* to be less than the variation between the six groups proposed in Table III. The use of this separation table is proposed as an aid to the identification of the seeds from various species of *Strophanthus*.

REFERENCES

1. Elborne, *Pharm. J.*, 1887, **17**, 743.
2. Mathiesen, *Pharm. Act. Helvet.*, 1927, **2**, 228; *ibid.*, 1928, **3**, 21; 34.
3. Smelt, *Quart. J. Pharm. Pharmacol.*, 1933, **6**, 467.
4. Callow, *Brit. med. J.*, 1950, **1**, 1484.
5. Meikle, *Pharm. J.*, 1950, **165**, 142.
6. Monachino, *Phytologia*, 1950, **3**, 366.
7. Monachino, *ibid.*, 1951, **3**, 476.
8. Youngken and Simonian, *J. Amer. pharm. Ass. Sci. Ed.*, 1950, **39**, 615.
9. Bush and Taylor, *Biochem. J.*, 1952, **52**, 643.
10. Hall and Melville, *J. Pharm. Pharmacol.*, 1951,**3**, 934.

DISCUSSION

The paper was presented by Mr. W. G. Thomas.

THE CHAIRMAN. Could the method be applied to powdered seed? He had not found the sulphuric acid test to be unreliable. Was it possible that seeds of *Strophanthus sarmentosus* had been confused with *S. kombé* as he believed the green colour with the latter to be characteristic?

MR. S. G. E. STEVENS (London). It would be difficult to differentiate clearly between *S. kombé* and *S. emini* solely on length of trichomes. Would the treatment have the same effect on the short stubby trichomes as on the tenuous ones?

DR. T. E. WALLIS (London). Insufficient information about the procedure had been given. *S. nicholsoni* would give difficulties because of its matted, twisted trichomes. Those of *S. hespidus* were brittle and were broken off during commercial handling. It would be difficult to measure the length when the trichomes were twisted or overlapped. Did the length include the base embedded in the epidermis? Was the apex always complete? Was the number measured for each sample a chance number or did it represent the total hairs present on the seed? By examination of the endosperm and the crystals, with one exception, he had, not had any difficulty in detecting seeds of other species when present in samples of kombé seeds.

DR. B. P. JACKSON (Sunderland). For kombé, 10 seeds were mentioned, were the other determinations carried out on only one seed? Were they young seeds with hairs probably not fully developed or were they mature seeds?

MR. THOMAS replied. The colour test had been carried out on botanically authenticated samples. The variation with *S. sarmentosus* appeared to be due to geographical variation in the species and there had been work which suggested that there should be one or two new varieties of this species. The suggested method could be applied to the powdered seed. The trichomes had been measured by micro-projection and damaged trichomes would be revealed. He agreed that the groupings were fine and that the method must be used in conjunction with other methods. The short stubby trichomes had survived the treatment. The matted trichomes of *S. nicholsoni* could be seen clearly after applying the method for separation. He agreed that the fragility of the trichomes of *S.*

hespidus would give rise to difficulties in commercial samples. The length measured included the base itself. The actual number of hairs on the seed had not been determined. The figures quoted were the results from many hundreds of seeds. It was considered that 500 was the minimum number of trichomes which must be measured from one seed.