A karyotypic and anatomical study of an unidentified liliaceous plant

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ABSTRACT

An unidentified sterile liliaceous plant and three possible relatives were studied karyotypically and anatomically. All these plants have a somatic chromosome number of 2n = 14. The possibility of the sterile plant having arisen as a result of a mutation appears unlikely, when compared with the possibility of hybrid origin. Chromosome morphology rules out Bulbine latifolia (L.f.) R. & S. and Aloe arborescens Mill. as possible parents. The sterile plant and Aloe marlothii Berger have similar karyotypes and, therefore, A. marlothii may be one of the parents. A close relationship between the sterile plant and the genus Aloe is further confirmed by their similar epidermal structure.

INTRODUCTION

A curious, liliaceous plant, apparently unknown to science, was found in a neglected garden in Pretoria during 1970. The plant has distichous leaves and a short almost prostrate stem and obviously belongs to the Liliaceae, but flowering material was required for a positive identification. The plant was transplanted to the Pretoria National Botanical Garden but, for the past 12 years, all attempts to induce flowering have proved unsuccessful and it therefore appears to be sterile.

The sterility of the plant may be due to genetic factors, or may indicate a hybrid origin. A search for possible parents revealed only three likely plants in close proximity to the sterile plant, namely Aloe arborescens Mill., A. marlothii Berger and Bulbine latifolia (L.f.) R. & S. This investigation attempted, by karyotypic and anatomical methods, to establish whether hybridization between any of these species could have resulted in this unidentified plant.

MATERIALS AND METHODS

Root tip material of Aloe arborescens (Hardy 6045), A. marlothii (Henderson 352), Bulbine latifolia (Mauve 4995) and the supposed hybrid (Hardy 6055) were collected in the Pretoria National Botanical Garden and pretreated in monobromo-naphthalene at room temperature. After four hours, the root tips were washed in running water and fixed in Carnoy fixative (Darlington & La Cour, 1976) for 24–36 hours. After hydrolysis for 10 minutes at 60°C in 1N HCl, the material was stained in leucobasic fuchsin (modified formula after Coleman, 1938; Darlington & La Cour, 1976) for 2 hours. The darkly stained parts were then squashed in aceto-orcein (La Cour, 1941). Slides were made permanent by placing them in a fridge for ±10 minutes, removing the coverslip with a needle, dehydrating through an alcohol series and mounting in Euparal. Ten cells per plant were studied.

Leaf segments were boiled for 10 minutes in concentrated HNO₃ to prepare epidermal peels. The epidermis was separated from the rest of the leaf during this process and was removed from the debris, stained in Methylene Blue, dehydrated in alcohol and mounted in Euparal.

Installation specimens of the material studied are lodged in the National Herbarium, Pretoria.

RESULTS

(a) Bulbine latifolia

A somatic chromosome number of 2n = 14 for this species, as reported by Snoad (Darlington & Wylie, 1955) and Jones & Smith (1967), was confirmed. The haploid karyotype (Fig. 1) consists of two large metacentric to submetacentric, two large subtelocentric and three small subtelocentric chromosomes. An idiogram (Fig. 1a) has been compiled from the data given by Jones & Smith (1967). The results of the present study are shown in Fig. 1b.

The two idiograms illustrate small differences in the karyotypes. The present study revealed the second chromosome pair to be submetacentric, whereas the published data show a metacentric chromosome pair. The fourth chromosome pair was found to be of similar length to the third pair, but with the centromeric index slightly lower. Although Jones & Smith also found the fourth pair to be more subtelocentric than the third, the two pairs could also be distinguished by a marked variation in length. The third difference observed in this study lies in the presence of satellites on the long arms of the fifth chromosome pair. No satellites were reported by Jones & Smith (1967).

These karyotype differences between the published and observed data indicate the existence of chromosomal variability in B. latifolia. A cytotoxic study of this species should prove valuable.

(b) Aloe arborescens

A somatic chromosome number of 2n = 14 was observed and confirms reports in the literature (Taylor, 1925; Ferguson, 1927; Resende, 1937; Muller, 1941; Snoad, 1951; Sharma & Mallick, 1966). The haploid idiogram (Fig. 2) illustrates four long subtelocentric chromosomes and three short subtelocentric ones. Satellites are present on the distal part of the long arms of the fourth chromosome pair. Apart from the SAT-region on the fourth chromosome pair, no secondary constrictions, as described by Sharma & Mallick (1966), were observed.

(c) Aloe marlothii

A somatic chromosome number of 2n = 14 was found and agrees with reported observations (Resende, 1937; Riley, 1959). The haploid idiogram (Fig. 3) shows four long and three short subtelocen-
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A comparison of the haploid idiomgram of the possible hybrid with that of *Bulbine latifolia* reveals large differences. The presumed hybrid has no chromosomes that are nearly metacentric as in *B. latifolia* and the short-satellited chromosomes of *B. latifolia* are absent in the hybrid. If *B. latifolia* was one of the parental species, the resulting hybrid must have received at least two large metacentric chromosomes and a short chromosome with satellites from this source. This observation indicates that *B. latifolia* could not have been one of the parents. Further evidence for this is illustrated by the epidermal structure, where *B. latifolia* has a totally different epidermal structure from the hybrid which has a typical *Aloe* pattern (Figs 9–12).

The epidermal structure of the hybrid corresponds closely with the patterns found in *Aloe arborescens* and *A. marlothii* (Figs 10–12). A closer relationship between the hybrid and the Aloes studied is further confirmed by the similarity in their karyotypes. However, as the hybrid has two satellited chromosome pairs and *A. arborescens* only one, *A. arborescens* is an unlikely parent. The karyotypes of *A. marlothii* and the hybrid agree in almost all respects. The only observed differences were in the arm ratios of the first two chromosome pairs. These small differences might result from a statistically inadequate sample, or from the fact that the first two pairs are of almost similar length and the first and second pair might be interchanged in the presumed hybrid.

If the sterile plant is the product of mutation, the parents must have a karyotype similar to *A. marlothii*. The chances of a mutation transforming a fertile plant, a few metres tall, to a 6 cm sterile plant and hard thorny leaves to smooth fleshy leaves at the same time seems very unlikely. The suggestion of hybridization seems more probable.

The restricted growth habit and absence of flowers in the sterile plant suggest hybridization rather than a new species. The similarity between the sterile plant and *A. marlothii* with regard to

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The restricted growth habit and absence of flowers in the sterile plant suggest hybridization rather than a new species. The similarity between the sterile plant and *A. marlothii* with regard to
karyotype and epidermal structure make it reasonable to suggest that *A. marlothii* might be one of the parents. Although no other possible parent was found in the vicinity of the supposed hybrid plant, a hybrid origin for the sterile plant is still possible. The second parent might have died by the time the hybrid was discovered. It is also possible that the hybrid was introduced into the area where it was found.

The results of this study indicate, therefore, that the sterile plant is a hybrid between *A. marlothii* and another species of *Aloe*.

REFERENCES

Coleman, L. C., 1938. Preparation of leucobasic fuchsin for use in the Feulgen reaction. *Stain Technol.* 13: 123–124.

Darlington, C. D. & Wylie, A. P., 1955. *Chromosome atlas of flowering plants*. London: Allen & Unwin.

Ferguson, N., 1927. The Aloinae: a cytological study, with especial reference to the form and size of the chromosomes. *Phil. Trans. R. Soc. Ser. B* 215: 225–253.

Jones, K. & Smith, J. B., 1967. The chromosomes of the Liliaceae: I. The karyotypes of twenty-five tropical species. *Kew Bull.* 21: 31–38.

La Cour, L. F., 1941. Acetic-orcein. *Stain Technol.* 16: 169–174.

Miller, F. S., 1941. *'n Sitologiese studie van 'n aantal Aloe-soorte*. I. cromosoomgetalle. *Tydskr. Wet. Kuns* 2: 99–104.

Reende, F., 1937. Über die ubiquität der SAT-Chromosomen bei den Blütenpflanzen. *Planta* 26: 757–807.

Riley, H. P., 1959. Chromosome numbers in *Aloe*. *J. S. Afr. Bot.* 25: 237–246.

Sharma, A. K. & Mallick, R., 1966. Interrelationships and evolution of the tribe Aloineae as reflected in its cytology. *J. Gener.* 59: 20–47.

Snoad, B., 1951. *'n Sitologiese studie van 'n aantal Aloe-soorte*. I. cromosoomgetalle. *Tydskr. Wet. Kuns* 2: 99–104.

Taylor, W. R., 1925. Cytological studies on *Gasteria*. 2. A comparison of the chromosomes of *Gasteria*, *Aloe* and *Haworthia*. *Am. J. Bot.* 12: 219–223.