INDUCED TETRAPLOIDY IN *SOLANUM KHASIANUM* CLARKE

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Abstract

Tetraploidy has been successfully induced for the first time by colchicine treatment in *Solanum khasianum* an important source of solasodine which is an intermediate in the synthesis of cortisone. A comparative study of the cytology and morphology of the diploid and tetraploids is reported in this paper.

Introduction

The genus *Solanum* is of importance since it is a source of intermediates in the synthesis of cortisone. It yields solasodine, a nitrogen analogue of diosgenine. Chopra and Handa (1963) reported 4·8% and 3·5% glycoalkaloids in *Solanum indicum* and *Solanum xanthocarpum* respectively. An even more promising species of *Solanum* for the exploitation of this important raw material was found by Maiti *et al.* (1964) in *Solanum khasianum* Clarke a prickly way side weed found along the Terai region from Dehra Dun through Nepal to Assam and the Khasia Hills. In South India it occurs on the Nilgiris at 7,000 ft, in Coonoor. The total yield of fruits in this species is from 100–140 per plant and Maiti *et al.* (1964) reported 5·4% alkaloid content on dry weight basis. There has been conflicting reports on the alkaloid content of the fruits of *S. khasianum.*

Saini *et al.* (1965) analysed fruits of the same physiological age at weekly intervals and found there was a progressive accumulation of glyco-alkaloids and that its concentration was at its maximum when the colour of the fruit changed from green to yellow. Extraction from fresh fruits yielded 6% solasonine and 2% solasodine (Saini, 1966).

The production of still higher solasodine yielding races of *Solanum khasianum* suitable for large-scale cultivation in India has attained great importance in recent years. One great disadvantage in the cultivation of

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this plant is the abundance of spines on leaves, stem and even the calyx, which makes harvesting of fruits both difficult and uneconomical. In order to get rid of these spines and at the same time improve on the alkaloid content, a project was undertaken using colchicine, gamma-rays, and diethyl sulphate. The present report is concerned with the results obtained by the application of colchicine.

Material and Methods

Seeds of *S. khasianum* collected by the senior author from Dehra Dun were sown in pots on 1–12–1969, and young seedlings in which the plumule had not developed were treated with 0·1% and 0·2% aqueous solution of colchicine for 2 days, twice daily. They were transplanted into paper cups when one month old and planted out in the field after 2 months. For detection of tetraploids leaf tip squashes were made after fixation in modified Carnoys fixative (6 chloroform: 3 absolute alcohol : 1 acetic acid) to which iron acetate was added to improve staining with acetocarmine. Maceratin with con. HCl + 95% alcohol in the ratio of 1 : 1 for 1 minute helped in separating cells.

Results and Discussion

Out of 70 plants treated with 0·1% colchicine 4 tetraploids ($2n = 48$) and 5 mixaploids were produced while 0·2% colchicine produced 6 tetraploids and 21 mixaploids in a population of 84 plants. Figure 1 is that of a typical diploid control plant and Fig. 2 that of a tetraploid, which could be easily distinguished by its dark and thick foliage and lesser lobing of leaves. Among the 10 tetraploids 3 had few or no spines on the leaves, petiole and stem (Fig. 3). The range of spinlessness in leaves at different stages of development in diploid and tetraploid is shown in Fig. 4. Figure 5 shows relative size of flowers in tetraploids and diploids.

A chimera, in which half the plant was diploid with marked lobing of leaves and the usual heavy spine formation and the other half tetraploid, with almost rounded leaf margins and lesser spines, gives a good illustration of the difference between diploid and tetraploid in *S. khasianum* (Fig. 6).

Control plants in the field started to flower early in March 1970, while flowering in tetraploid plants was delayed by almost a month.

Pollen mother cell studies in diploids showed 24 chromosomes associated as 12 bivalents (Fig. 7). In the tetraploid the 48 chromosomes associated

themselves as quadrivalents, trivalents, bivalents, and univalents in varying frequencies, as shown in Table I. 100 pollen mother cells were scored from each plant except No. III-4 where only 50 cells were available. Pollen stainability observed using acetocarmine, also included in the same table, ranged from 2% to 60% in tetraploids, while it was almost 100% in diploids.

TABLE I

Frequencies of various chromosome associations per cell and pollen stainability

Plant No.	IV	III and I	II	Pollen stainability
II-6	2·22	0·07	19·42	28%
III-4	6·18	0·14	11·34	10%
V-3	3·44	0·00	17·22	2%
V-6	3·70	0·02	16·56	60%
XI-3	2·78	0·02	18·36	20%
XII-6	4·28	0·07	15·38	30%

Figures 9 and 10 show the relative sizes of pollen grains in diploid and tetraploid.

As expected from its late flowering, fruit formation was delayed in tetraploids. Tetraploid fruits were smaller than diploids and mixaploids, however, their seeds were larger than those in the diploids. The average weight of fruits in the diploid was 4·8 gm. while in the tetraploid it was only 3·1gm. Mixaploids had larger fruits than diploids—the average weight being 5·2 gm.

An interesting feature noticed was the presence of 3–5 fruits at some nodes, instead of the usual single fruit indicating development of more than one hermaphrodite flower. This feature may turn out to be an added advantage in commercial cultivation of *Solanum khasianum*.

Further breeding and selection from C_2 generation, already underway, is expected to reveal the fullest use of induced tetraploids for commercial exploitation of this important species of *Solanum*. The potentialities of the tetraploids in terms of solasonine is under investigation.

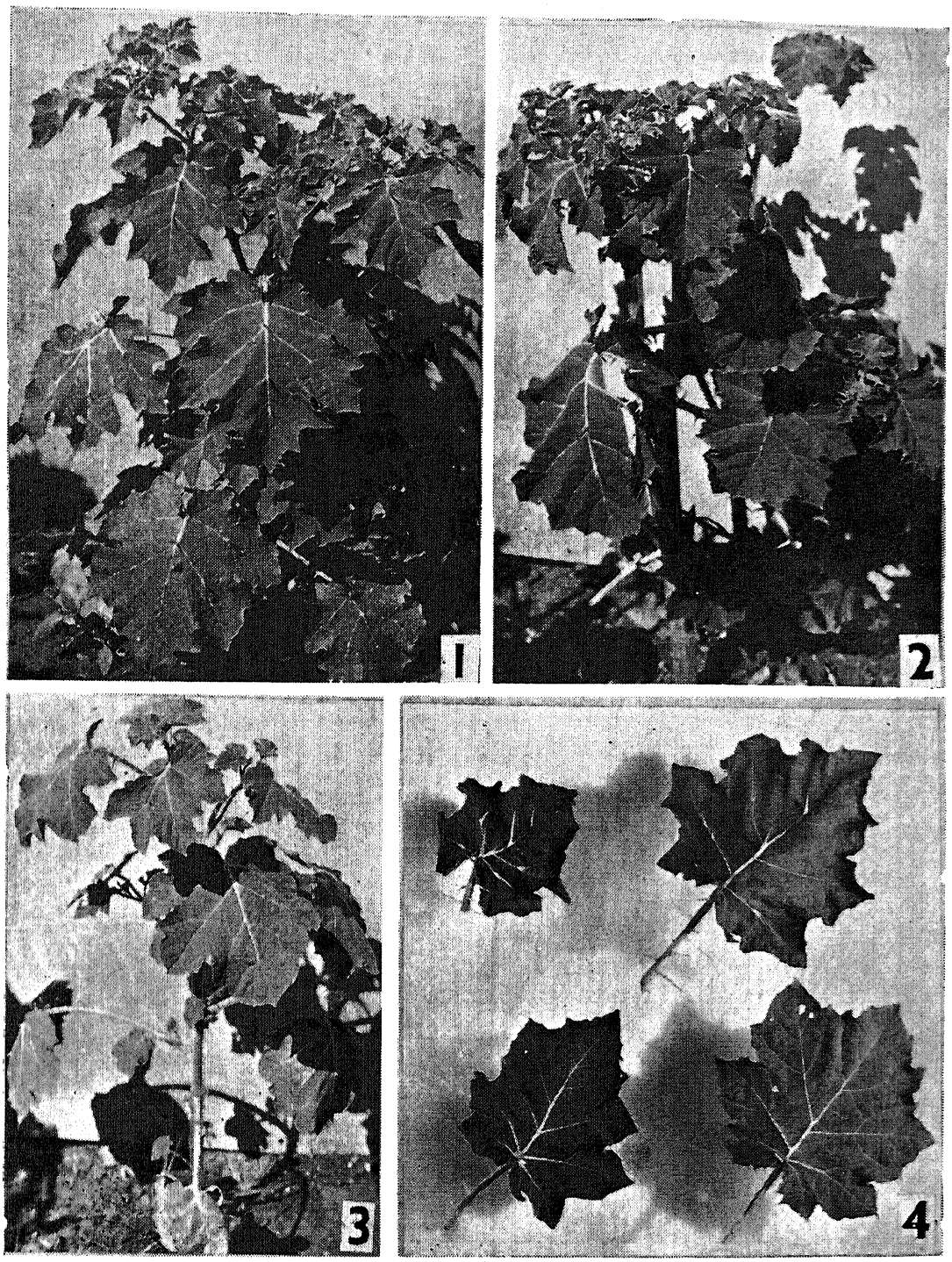
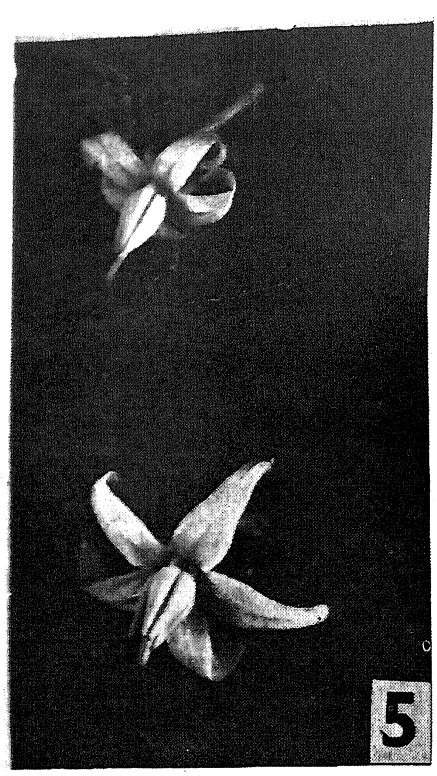
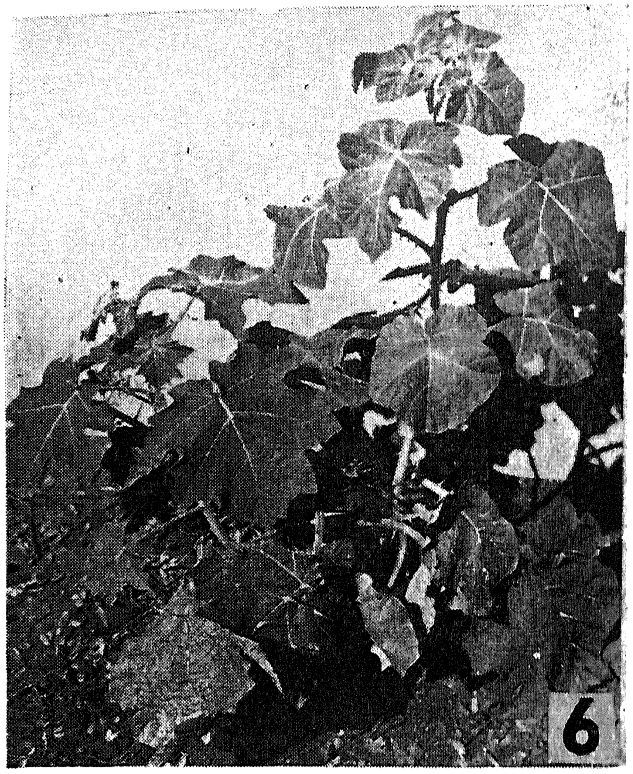
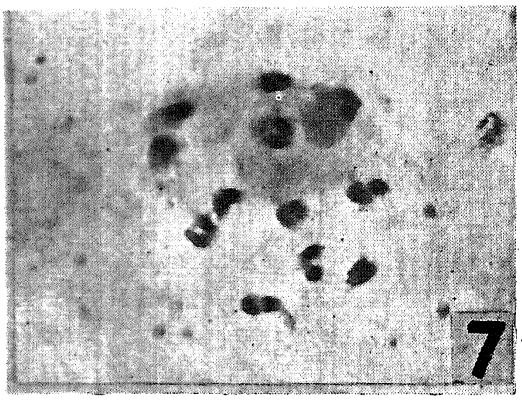
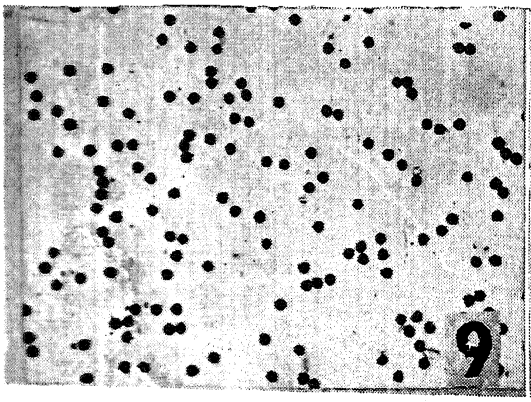


FIG. 1. A diploid plant of *Solanum khasianum* Clarke. FIG. 2. A tetraploid form of *Solanum khasianum* Clarke. FIG. 3. A tetraploid *S. khasianum* with few spines and less lobing of leaves. FIG. 4. Spine formation in diploid and tetraploid leaves of *S. khasianum*. Top, diploid; bottom, tetraploid.









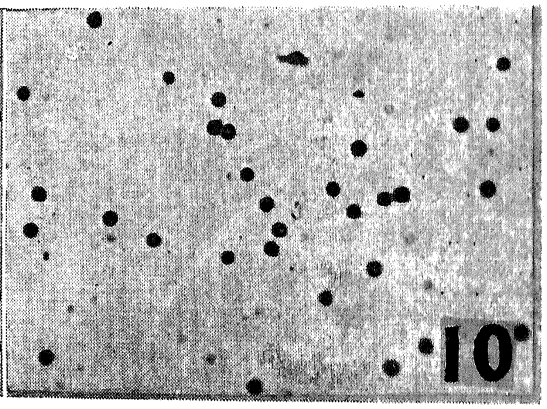


FIG. 5. Top, diploid flower, bottom; tetraploid flower in *S. khasianum*. FIG. 6. A chimera plant of *S. khasianum* with diploid and tetraploid branches. FIG. 7. Diakinesis in PMC of diploid *S. khasianum*. FIG. 8. Metaphase in PMC of tetraploid *S. khasianum*. FIG. 9. Pollen grains in diploid *S. khasianum*. FIG. 10. Pollen grains in tetraploid *S. khasianum*.

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