RESEARCH ARTICLE

J. Sci. Soc. Thailand, 7 (1981) 25-32

AUTOPOLYPLOIDY IN *DENDROBIUM PHALAENOPSIS*

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(Received 28 November 1980)

Abstract

This study compares the growth rates, leaf and flower morphology and pollen germination of diploid and tetraploid produced by colchicine treatment in protocorms stage of the orchid Dendrobium phalaenopsis. *Protocorms of the orchid were treated in 0.05% sterile aqueous colchicine solution for 9 days. At the end of six months the seedlings were planted in the small pots and grown in the lathhouse. Chromosome counts were made from the root tips. About 50% of treated plants were tetraploid. The growth of tetraploid plants was slower than that of the diploids. The leaves of tetraploids were thicker, and they had larger guard cells, and flowers are rounder than the diploids. Meiotic division in microsporocytes of diploids and most tetraploids were normal. The germination of pollens of diploids was better than those of tetraploids.*

Introduction

Polyploidy is very important in orchid improvement, whether for exhibition or cut flower purposes[1]. Polyploid orchids, like many other polypliod plants, produce larger and stronger flowers than their diploid relatives. In cutflowers trade, large size and heavy substance are considered highly favorable characteristics due to longer shelf life. Polyploid flowers are usually flat and round, and have other features likely to catch the judges' critical eyes. Polyploidy also plays a very important role in orchid breeding[2]. Since naturally evolved polyploid strains are usually unavailable, it is necessary to produce them through some artificial process. Generally the first step in their production is to induce the desired genetic modification in diploid plants,

and most commonly this is accomplished by the treatment with colchicine. The standard methods of colchicine treatment in the field have been applied to orchids with only limited success[3-5]. In the studies cited, only Menninger reported actual chromosome counts. Wimber and Van Cott first reported successful treatment of *Cymbidium* hybrid seedling and calli from shootip culture by incorporation of colchicine in a sterile liquid nutrient solution. In 1973, Chaicharoen in Bangkok and independently Sanguthai and Sagawa as well as Sanguthai, Sanguthai and Kamemoto in Honolulu each report their success in colchicine treatment of *Dendrobium* and *Vanda* hybrids using the technique of Wimber and Van Cott with modifications[6-9]. This paper presents the results of a detailed study on autopolyploidy in *Dendrobium phalaenopsis.*

Materials and Methods

Plant material, the young pod of *Dendrobium phalaenopsis* ($2n = 38$) was kindly donated by Prof. Thavorn Vajrabhaya. The seeds from the young pod, aged 69 days, were germinated on agar nutrient. Illumination of about 3,000 lux was provided 14 hours per day, and temperature was about $25 \pm 2^{\circ}C$[6, 10].

About six weeks after the seeds had been placed on the nutrient agar, they developed into small protocorms. They were removed from agar nutrient to 0.05 % steriled aqueous colchicine solution for nine days. Then the treated protocorms were transferred onto nutrient agar. At the end of six months the seedlings were then removed from flasks and planted in small pots and kept in the lathhouse.

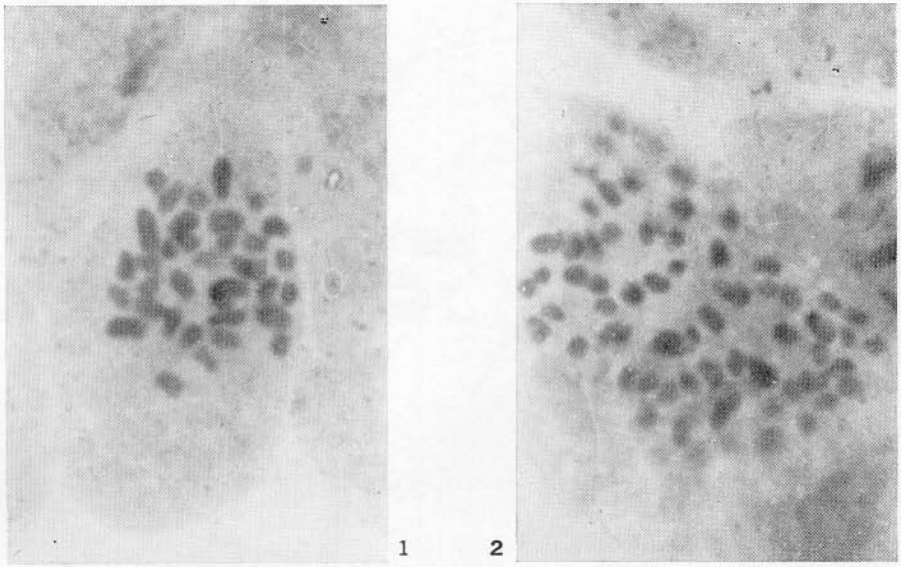
Chromosome numbers were determined from a number of treated seedlings. Actively growing root tips were removed from potted plants and treated in α-bromonaphthalene at $5^{\circ}C$ for 5–6 hours. Then the roots were fixed in 90% acetic acid for 20 minutes, rinsed with 70 % ethyl alcohol, and stored in 70 % ethyl alcohol. After two to seven days the roots were stained by the Feulgen reaction after 10 minutes of hydrolysis in 1 N HCl, 60 C[11]. Then the roots were squashed in 2 % aceto-orcein[6]. Chromosome counting of 10 somatic cells per plant was restricted to metaphase figures.

Thickness of leaves was measured with a screw micrometer. The leaves from five diploid and five tetraploid plants were measured at the middle of both sides of the mid rib[6].

Guard cell size was measured by peeling the epidermis from the under-surface of leaves. The width and the length of ten pairs guard cells per plant were measured from five diploid and five tetraploid plants under a microscope with an occular micrometer.

The following morphological data were recorded: number of flowers per inflorescence (five inflorescences of diploids and tetraploids each), the width and length of sepals and petals (10 flowers of diploids and tetraploids).

The pollinia from the diploid and tetraploid flowers were deposited in the stigmatic fluid. After three to five days, pollen were stained in 2% aceto-orcein for studying the germination of pollen tubes.



Figs. 1-2 Photomicrographs of somatic chromosomes from root-tips of diploid and tetraploid *D. phalaenopsis*. Magnification, × 1600.

Fig. 1, normal diploid (2n = 38). Fig. 2, tetraploid (2n = 76).

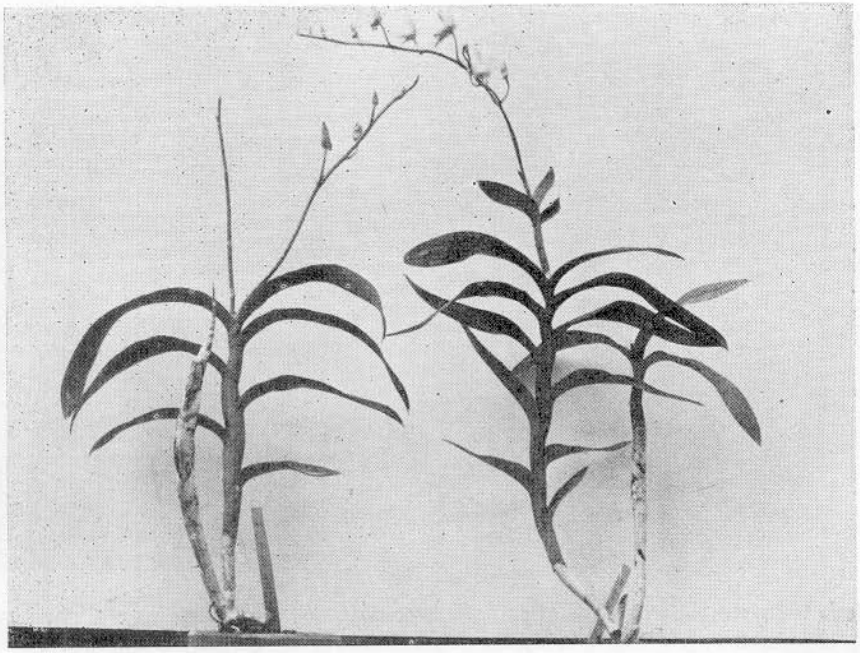


Fig. 3. Tetraploid (left) and Diploid (right) plant of *D. phalaenopsis*

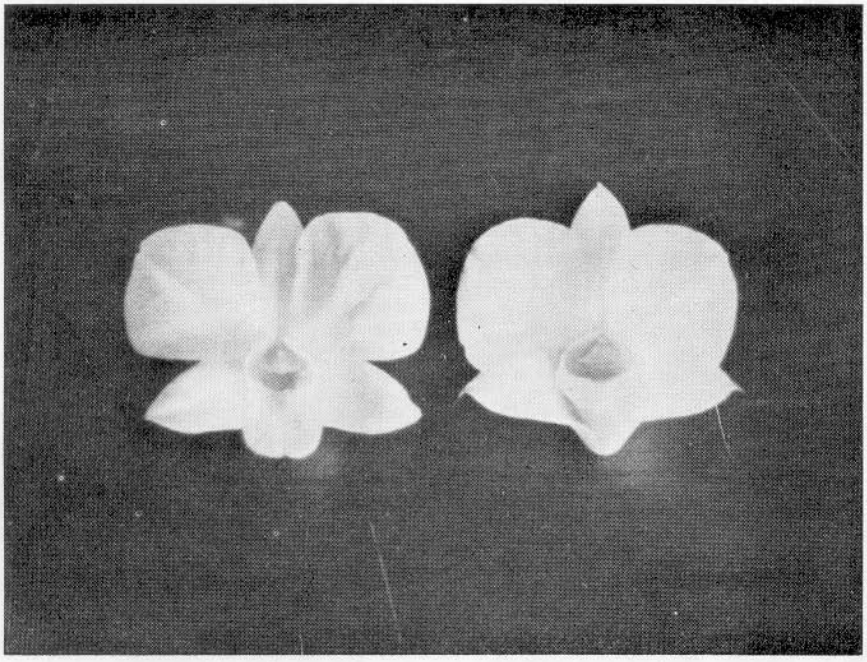
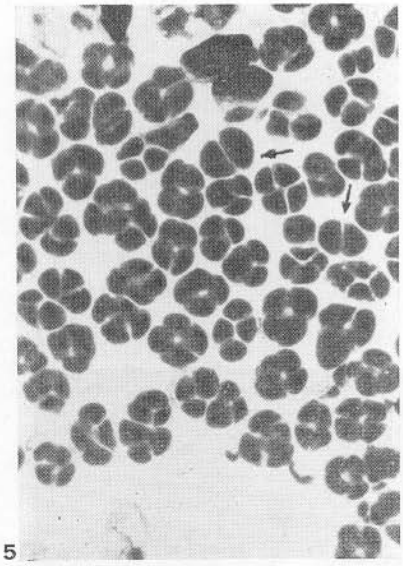


Fig. 4. Diploid (left) and Tetraploid (right) flower of *D. phalaenopsis*



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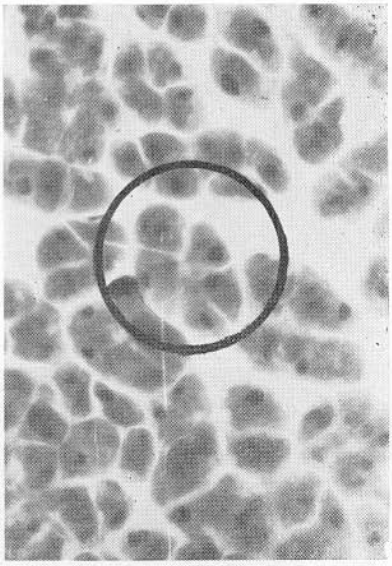

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Figs. 5-6 Microspores of tetraploid *Dendrobium phalaenopsis* showing abnormality. Arrows show 2-cell microspore in Fig. 5, × 80. Fig. 6 demonstrates a 5-celled microspore (circle), × 160

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Fig. 7. Diploid pollen tubes on the third day, × 80.

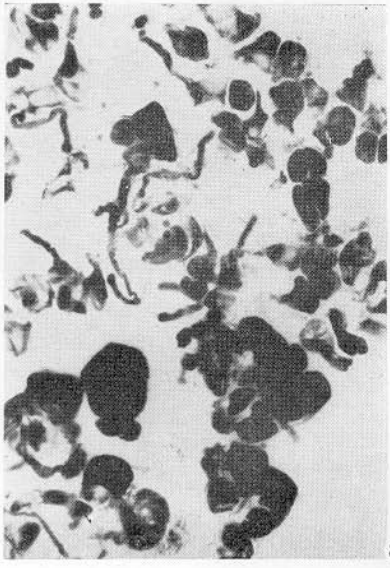

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Fig. 8. Tetraploid pollen tubes on the fifth day, × 160.

Results and Discussion

Polyploidy can be expected to arise spontaneously in nature and during tissue culturing[12], but treatment with colchicine increases the number of polyploid plants considerably. Thirty treated seedlings regenerated from the diploid stock were examined and 15 were tetraploid or near tetraploid, 1 aneuploid, 2 octoploid and 12 diploid (Figs. 1 and 2).

The plants produced under colchicine treatment had considerably changed characteristics. The growth of tetraploid plants was slower and then generally had greener leaves than their diploid counterparts. Tetraploid plants had significantly thicker leaves. Similar results were found when the guard cell pairs of these leaves were compared (Table 1).

TABLE 1. AVERAGES OF THE WIDTH AND THE LENGTH OF LATERAL AND DORSAL SEPALS, PETALS, A PAIR OF GUARD CELLS AND LEAF THICKNESS.

Average measurements of 10 flowers, 50 guard cells and 20 leaves.

Characteristics	size (mm.)		Significance of difference
	Diploid	tetraploid	
The width of lateral sepal	17.6	20.5	ns
The length of lateral sepal	26.7	25.6	ns
The width of dorsal sepal	11.1	12.4	ns
The length of dorsal sepal	23.5	24.4	ns
The width of petal	22.1	23	ns
The length of petal	26.6	27	ns
The thickness of leaf	1	1.46	$P<.01$
The width of guard cell pair	0.29	0.36	$P<.01$
The length of guard cell pair	0.34	0.42	$P<.01$

Flowers of the autotetraploid plants differed from those of the diploids. The number of flowers per inflorescence of tetraploids averaged two less than that of diploids. The tetraploid plants had rounder flowers than the diploids but the average width and length of the sepals and petals of both diploids and tetraploids were not significantly different (Figs. 3, 4 and Table 1).

The meiotic division in microsporocytes of diploids and most tetraploids were normal except some microspores of tetraploid plants had two to five cells in one group resulting from abnormal division of microsporocytes (Figs. 5 and 6) The germination of pollen of diploids was better than that of tetraploids. The growth of pollen tubes of tetraploid was slow and many of them did not germinate after 3 days, whereas those of diploids were long and numerous by the third day. Pollen tubes of tetraploid in the fifth day were short and abnormal (Figs. 7 and 8).

The autooctoploid plant exhibited abnormalities in both growth and appearance; it grew very slowly and produced only a few roots, its leaves were thick and wrinkled and it died at the height of 2 cm.

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บทคัดย่อ

ได้ศึกษาเปรียบเทียบอัตราการเจริญเติบโต ลักษณะทางสัณฐานวิทยาของใบและดอก และการงอกของละอองเรณูของดิพพลอยด์ และเตตราพลอยด์ ซึ่งเกิดขึ้นโดยนำ protocorms ของ *D. phalaenopsis* มาแช่ในสารละลายโคลชิซีนความเข้มข้น 0.05% เป็นเวลา 9 วัน หลังจากนั้นประมาณ 6 เดือน นำต้นอ่อนปลูกลงในกระถางนำไปเลี้ยงไว้ในเรือนต้นไม้ จำนวนโครโมโซมนับจากเซลล์ของปลายราก พบเป็นเตตราพลอยด์ประมาณ 50% การเจริญของต้นเตตราพลอยด์ช้ากว่าของดิพพลอยด์ ใบของต้นเตตราพลอยด์หนากว่า และขนาดของเซลล์คุมใหญ่กว่าพวกดิพพลอยด์ ดอกของเตตราพลอยด์กลมกว่า การแบ่งตัวของ microsporocyte ของดิพพลอยด์ และของเตตราพลอยด์ ส่วนใหญ่จะปกติ ส่วนการงอกของละอองเรณูของดิพพลอยด์ดีกว่าของเตตราพลอยด์มาก