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Cytomorphological studies of artificially induced tetraploids of *Catharanthus roseus*

By

Satendra S. RAGHUVANSHI and A. K. S. CHAUHAN *)

With 21 Figures

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Introduction

During the course of cytogenetical survey of family *Apocynaceae* along with the study of chromosome number and their behaviour during meiosis, efforts were made to artificially induce polyploids and heterosomics of different members of this family. This report presents the details of results of similar investigations on *Catharanthus roseus*. In India this genus is represented by *C. pusillus* which grows wild and *C. roseus*, an exotic species very well naturalised throughout the country. It is an ornamental herb commonly grown in the gardens and has a number of varieties which differ in their flower colour.

According to LAWRENCE 1959 three cultivars of *C. roseus* are recognised 'Alba' with white flowers 'Ocellata' with corolla white but eye rose pink to carmine red and 'Roseus' with uniformly red coloured flowers. However, this paper deals with six varieties of *C. roseus* identified on the basis of flower colour as reported by SIMMONDS 1960. In our collection we have five varieties which correspond with description of varieties given by SIMMONDS. We have adopted his classification and for further description we have used the basis of describing both eye colour and petal colour in distinguishing different varieties. They have

*) Dr. S. S. RAGHUVANSHI and A. K. S. CHAUHAN, Cytogenetics Laboratory, Botany Department, Lucknow University, Lucknow, India.

also been given serial numbers from 1 to 6. The variety no. 2 having light red eye colour and white petals, does not correspond to any of SIMMONDS' varieties.

Variety Number (1) Greenish white/white.
Variety Number (2) Light red eye/white.
Variety Number (3) Strong purple/white.
Variety Number (4) Strong purple/Shaded to white 'Flush'.
Variety Number (5) Strong purple/Light Violet purple.
Variety Number (6) Strong purple/Light violet.

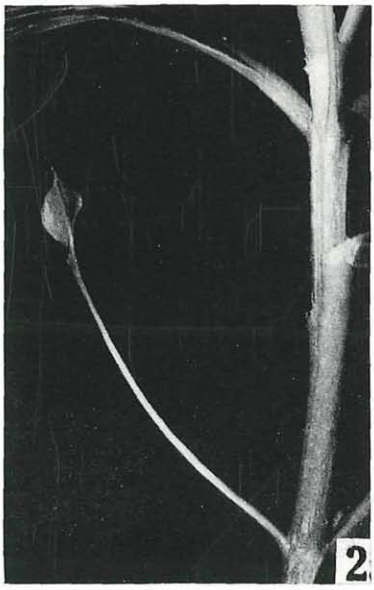
The flower colour in *C. roseus* is governed by four major genes. Two **A & R** are basic complementary genes the absence of which produces white flowers. These two genes are modified by the action of **W & B**. ww plants vave pigment confined to eye only, **B**, by copigmentation blues the pigments in an **ARWw** background and probably also in **ARww** genotypes (SIMMONDS 1960).

All the plant parts, particularly root bark contain alkaloids. These include three alkaloids of *Rauvolfia serpentina* group viz — ajamalicine, serpentine and reserpine. The concentration of first two alkaloids is greater in the roots of *C. roseus* than in the roots of *R. serpentina*. Alkaloids in *C. roseus* posses hypotensive, sedative and tranquillizing properties similar to but more marked than those of total alkaloids of *R. serpentina*. They also depress the central nervous system and cause relaxation of muscles. They also inhibit the growth of certain bacteria as *Vibrio cholerae* and *Micrococcus pyogenes* variety *aureus* but possess no antibacterial action for the enteric group of organisms (CHOPRA & al. 1959; KAMAT & al. 1958).

Materials and Methods

All the varieties of *C. roseus* under investigation are easily cultivated through cuttings. Stem cut into pieces of suitable size when planted in soil, sprout in a few weeks. The growing points of the shoots were treated with 0.2% aqueous colchicine solution intermittantly for six hours daily for four days. The other set was treated with 0.2% colchicine prepared in saturated solution of gammexane (RAGHUVANSHI & JOSHI 1965). Suitable controls were maintained for comparison.

Buds were fixed for cytological investigations in 1 : 3 acetic alcohol for 24 hours. A few drops of ferric acetate was added to the fixative for facilitating the staining. Acetocarmine squashes of dividing PMCs were made by teasing anther in a drop of acetocarmine. The slides were made permanent by ethanol-butanol schedule. Anthers from the same bud were not always found to be at the same stage of meiotic division. In some cases, certain, anthers showed PMCs at telophase II while other were at metaphase I and early prophase stages.



Figs. 1—3. *Catharanthus roseus.* — Fig. 1. Flowers of diploid and tetraploid of var. no. 4. — Fig. 2. Abnormal leaf in var. no. 5. — Fig. 3. Fringing of petals with red streaks.

Morphological Studies

Morphological changes resulting due to colchicine and colchicine — gammexane treatment corresponded with the quantitative change in the number of chromosomes. The size of the tetraploid plant was smaller but there had been general increase in different organs of tetraploids. The leaves which developed after the treatment were broader, thicker, more rough, distorted and deep green in colour. An abnormal leaf with a long petiole and a small blade was observed in variety no. 5 (Fig. 2). The polyploids had larger floral parts. The interesting feature of the flower was the increase in the width of petals resulting in overlapping of the petals in a twisting manner. The gap among the petals was filled due to increase in petal size imparting a more enchanting look to the flowers (Fig. 1). In some flowers of variety no. 5 among tetraploids, margins of petals showed fringing and the red streaks were prominent (Fig. 3). Fertile pollen grains were larger in size than those of controls. The tetraploids were almost sterile and seed setting was extremely poor.

As not much difference was observed with regard to the morphological characters of tetraploids of different varieties an average is being presented in Table 1.

Table 1

Comparison om morphological characters of diploids and polyploids

Sl.No.	Characters	Diploid	Polyploid
1.	Height of the plant in cm	83.03	61.8
2.	Length of the leaf petiole in cm	0.58	0.87
3.	Length of the leaf blade in cm	4.62	5.98
4.	Breadth of the leaf in cm	3.25	3.75
5.	Length of the guard cell of stomata (μ)	127.322	193.311
6.	Breath of the guard cell of stomata (μ)	35.922	37.682
7.	Length of the stomatal aperture (μ)	78.352	125.911
8.	No. of stomata per field.	23.83	15.53
9.	Pollen size (μ)	332.00	407.198
10.	Length of sepals (cm)	0.52	0.629
11.	Length of petals (cm)	1.97	1.98
12.	Breadth of petals (cm)	1.88	2.008
13.	Spread of corolla (cm)	4.082	4.102
14.	Length of corolla tube (cm)	2.18	2.68
15.	Width of eye (mm)	1.00	1.078
16.	Diameter of throat (mm)	1.27	1.40
17.	Length of stamen (mm)	2.5	2.708
18.	Length of ovary. (cm)	0.3	0.3
19.	Length of style (cm)	2.1	2.25
20.	Length of stigma (cm)	0.1	0.1

Cytological studies

While investigating meiotic behaviour cytomixis was observed in diploids as well as in tetraploids. In such cases nuclear material migrated from one PMC to its neighbour leaving donner cell devoid of any nucleus while the other cell became binucleate (Fig. 21). This nuclear transfer was observed at an early stage of prophase. The cell after nuclear transfer separated from each other as no joined PMCs were observed at the later stage of meiosis both is diploids and polyploids. Cytomixis has also been reported in *Torenia fournieri* (JOSHI & RAGHUVANSHI 1966) and *Citrus* (NAITHANI & RAGHUVANSHI 1958).

The chromosome number of controls was found to be 2n = 16 which confirms the findings of FURUSATO 1940. The meiotic behaviour of diploids of all the varieties was found to be normal. Mostly bivalents of ring and rod type were found (Figs. 4 & 5). In very rare cases two univalents were observed. After metaphase I univalents moved to opposite poles. They could have arisen due to precocious separation of bivalents.

The meiotic behaviour of polyploids of different varieties followed more or less the same pattern so it is being presented together. At diakinesis normally condensed chromosomes were observed in various configurations like quadrivalents, trivalents, and univalents (Figs. 6, 8 & 11).

Terminalization coefficient of tetraploids of different varieties is as follows:

Figs. 4—7. *Catharanthus roseus* var. no. 1. — Fig. 4. Diakinesis showing 6 ring + 2 rod bivalents. — Fig. 5. Metaphase showing 1 ring + 7 rod bivalents. — Fig. 6. Diakinesis showing different configurations. — Fig. 7. Metaphase showing 1 quadrivalent + 14 bivalents.

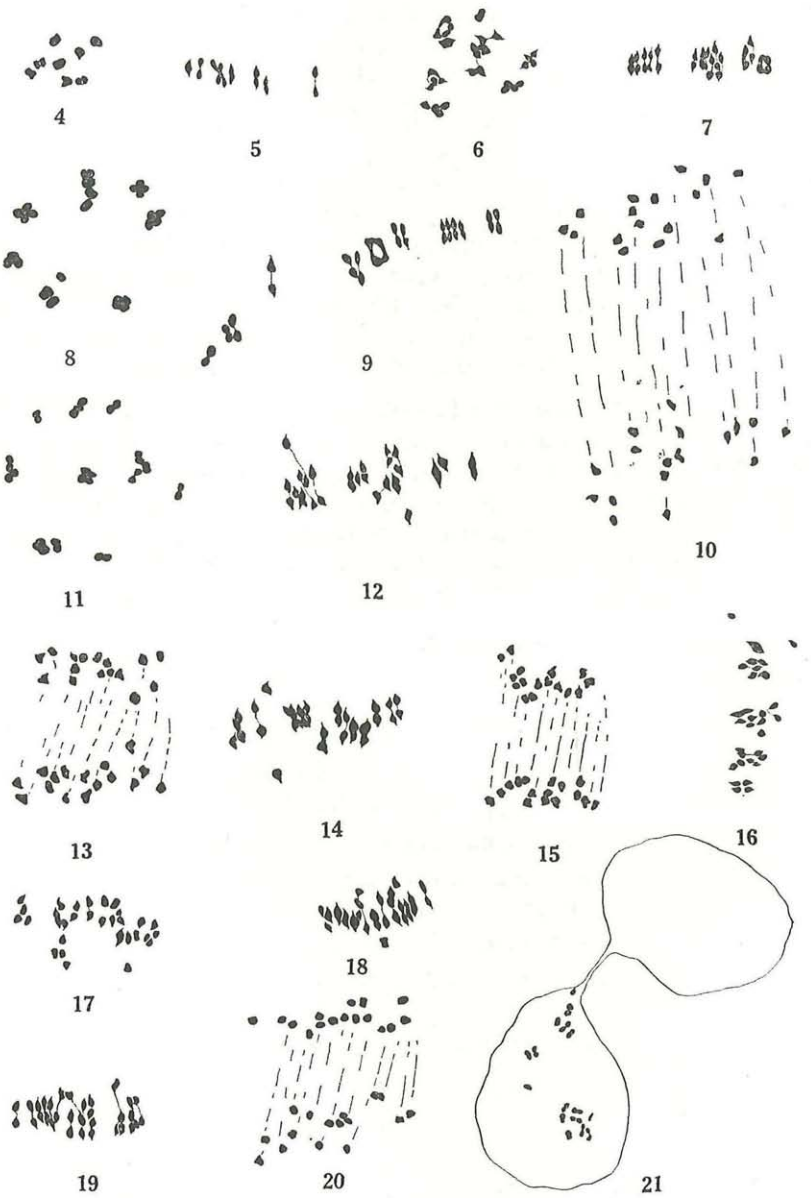
Figs. 8—10. *C. roseus* var. no. 2. — Fig. 8. Diakinesis (5 quadrivalents + 1 trivalent + 1 bivalents + 1 univalent). — Fig. 9. Metaphase (1 quadrivalent + 14 bivalents). — Fig. 10. Anaphase.

Figs. 11—15. *C. roseus* var. no. 5. — Fig. 11. Diakinesis (5 quadrivalents + 6 bivalents). — Fig. 12. Metaphase (6 ring + 10 rod bivalents). — Fig. 13. Anaphase. — Fig. 14. Metaphase (2 ring + 14 rod bivalents). — Fig. 15. Anaphase.

Figs. 16—18. *C. roseus* var. no. 4. — Fig. 16. Metaphase (1 ring + 14 rod + 2 univalents). — Fig. 17. Early anaphase. — Fig. 18. Metaphase (6 ring + 10 rod bivalents).

Figs. 19—20. *C. roseus* var. no. 6. — Fig. 19. Metaphase (16 rod bivalents). — Fig. 20. Anaphase.

Fig. 21. *C. roseus* var. no. 1. Cytomixis in diploid plant. The PMC's appear to be at diakinesis. Note the total transfer of the chromosomes from one PMC thus making the recipient cell binucleate.



Figs. 4—21. For explanation see opposite page.

Variety no. 1. Greenish White/White. = .56
Variety no. 2. Light red eye/White. = .54
Variety no. 4. Strong purple/Shaded to White = .53
Variety no. 5. Strong purple/Light Violet purple. = .57
Variety no. 6. Strong purple/Light Violet = .55

Metaphase I. Different configurations observed at M. I are summarised in table 2. The quadrivalents were very few and their number per cell varied from 0—2, only ring quadrivalents were observed (Figs. 7, 9). At this stage single bipolar spindle was always found and most of the chromosome were oriented on equater of this spindle. No multiple or multipolar spindle was observed. Number of ring bivalents varied from 0—8 and their average was 1.91 per PMC. Rod bivalents ranged from 6—16 (Figs. 12, 14, 16, 18, 19) and the average was 13.21 per cell. The average number of univalents was 0.88 per cell.

The range of chiasmata in different cells of tetraploid was from 12—24. The average number of chiasmata in diploids of all the varieties is 9.32 and in tetraploid is 17.30 per cell, or it may be expressed as .582 and .540 half chiasma per chromosome in diploid and tetraploid respectively. The data show that in tetraploid there is a decrease in the half chiasma per chromosome as compared to diploid. The difference may be expressed as reduction factor.

$$\frac{\text{Half chiasma per chromosome 4n}}{\text{Half chiasma per chromosome 2n}} = \frac{.540}{.582} = .92 \text{ (UPCOTT 1939)}$$

Reduction factor of different varieties of *C. roseus* is given below:

Var. no. 1 $\dfrac{\text{Half chiasma per chromosome 4n}}{\text{Half chiasma per chromosome 2n}} = \dfrac{.5}{.4} = 1.4$

Var. no. 2 $\dfrac{\text{Half chiasma per chromosome 4n}}{\text{Half chiasma per chromosome 2n}} = \dfrac{.34}{.53} = .76$

Var. no. 4 $\dfrac{\text{Half chiasma per chromosome 4n}}{\text{Half chiasma per chromosome 2n}} = \dfrac{.501}{.66} = .75$

Var. no. 5 $\dfrac{\text{Half chiasma per chromosome 4n}}{\text{Half chiasma per chromosome 2n}} = \dfrac{.58}{.57} = 1.0$

Var. no. 6 $\dfrac{\text{Half chiasma per chromosome 4n}}{\text{Half chiasma per chromosome 2n}} = \dfrac{.55}{.56} = .96$

Generally tetraploids have slow growth rate. It is stated that increase in chromosome number effects the rate of physiological process going on in the cell. But the time during which pairing takes place does not undergo any change with the result that all the homologous chromosomes will not pair. This explains the low chiasma-frequency in tetraploids. In the case of diploids the value of half chiasma varies

from .4 to .66 while in the case of tetraploids it ranges from .34 to .58. So in tetraploids the lower value is less then diploid and also the higher value does not reach that of diploid. At anaphase I the separation of chromosomes was regular. Although univalents were present at metaphase their movement to different poles at A I was not irregular (Figs. 10, 13, 15, 17, 20). Telophase I was also found to be normal. Metaphase II showed two spindles and chromosomes were normally arranged on two plates. At anaphase II four distinct groups were present. No anomalies were observed at A II. So the telophase II showed four nuclei. No laggards, strays or dividing univalents were observed.

Pollen. Tetraploids produced larger pollen grains and they were 62.82% fertile as observed on the basis of their stainability with aceto-carmine. The fertile pollen grains were full of cell contents and round in outline while sterile pollen grains were empty transparent and shrunken.

Table 2

Average frequency of different configurations per PMC at Metaphase I in tetraploids of different varieties

Plant	Quadrivalent				Bivalents				Univalents	
	Average per PMC			variation	Average per PMC			variation	Average per PMC	variation
	Ring	Rod	Total		Ring	Rod	Total			
Variety no. 1	.185	—	.185	0—1	1.814	13.77	15.584	Ring (0—8) Rod (9-16)	.074	0—7
Variety no. 2	.12	—	.12	0—1	1.64	13.57	15.21	Ring (0—8) Rod (8-16)	1	0—4
Variety no. 4	.11	—	.11	0—2	1.66	12.90	14.01	Ring (0—7) Rod (6-16)	1.82	0—4
Variety no. 5	.21	—	.21	0—2	2.57	12.85	15.42	Ring (0—5) Rod (8-16)	.32	0—4
Variety no. 6	.25	—	.25	0—1	1.9	13.00	14.90	Ring (0—5) Rod (10-15)	1.2	0—4

Discussion

Effect of increase in the level of ploidy on floral morphology resulting in enlarged petals certainly makes these 4n plants a novelty among ornamentals.

Certain interesting trends become apparent from comparative study of meiotic configurations of diploid and tetraploids of different varieties. In diploids of all the varieties rod bivalents are more frequent than rings and same trend appear to have been maintained at tetraploid level.

Another most striking point of the meiotic behaviour of the 4n plants of all the varieties is extremely low frequency of quadrivalents despite presence of four homologous chromosomes of every type. The average number of quadrivalents varies from 1.1 per PMC in var. no. 4 to .25 in var. no. 6. Thus low multivalent frequency appears to be characteristic of genomic constitution of members of this species. The univalents are few which again is not very common feature of autoploids. The maximum number of 1.82 is observed in var. no. 4. The autoploids are generally marked by multivalent associations but this multivalent frequency depends both on cytological and genetical factors. If the chromosomes are small then there is less time available for pairing between the homologous chromosomes, this result in low multivalent frequency despite presence of more than two chromosomes of each type while in other case high bivalent frequency in autoploids appears to be the result of genic control of chromosomal pairing as reported by RIELY & CHAPMAN 1958 in the case of hexaploid wheat. Similar high bivalent frequency in autoploids have been reported in *Foeniculum vulgare, Anethum graveolens* and *Coriandrum sativum* (RAGHUVANSHI & JOSHI 1966, 1967, JOSHI & RAGHUVANSHI 1965).

In the present material the chromosome size is certainly very small and this may be the main reason for high bivalent frequency. But what is more interesting is general lack of anomalies at different meiotic stages — a characteristic feature of allopolyploids. Autopolyploids are more labile and prone to different meiotic anomalies. But in the present material 4n plants have almost negligible anomalies and they appear to behave as balanced individuals. No doubt certain plants are more unstable than others but predisposition of a plant to cytological instability could be traced to many factors. In present material fairly regular bivalent formation appears to forestall at least those anomalies which generally arise due to unequal multivalent separation or unpredictable behaviour of univalents that are left unpaired. Thus two main cytological sources of meiotic instability in autoploid, multivalents and univalents, are missing and it is mostly bivalents which predominate and behave normally during metaphasic orientation and anaphasic separation. No

doubt the spindle apparatus appear to be uneffected by change in ploidy level while in polyploids of *Capsicum frutescens* & *Coriandrum sativum* (RAGHUVANSHI & JOSHI 1964; JOSHI & RAGHUVANSHI 1965) spindle breakdown, multiple and multipolar spindle were quite frequent.

The pollen fertility of autopolyploids is fairly high. Obviously it is the outcome of quite regular meiotic separation of chromosomes without any marked anomaly at any stage whatsoever. One the basis of so high pollen fertility such tetraploids will be expected to have fairly normal seed setting but surprisingly enough very little seed setting has been obtained in tetraploids of any of the varieties under investigation. However, it may be mentioned that in some 4n plants of practically every variety fruit formation was observed but when the fully formed fruits were opened then either they did not have any seeds or had very small shrivelled seeds which were totally inviable. A few viable seeds of tetraploids were obtained in rare cases. Even these fruits had only few seeds in comparison to the larger number of seeds in a fruit of control plants.

The fertility of autotetraploids show great variability with genotypes of the plants. On one extreme are instances like autotetraploids of certain varieties of maize which have 80 to 90% seed setting (RANDOLPH 1935—1941) while autotetraploids of *Gossypium herbaceum* are completely sterile (BEASLEY 1940).

In earlier stages of study of causes of autoploid sterility, the multivalent formation and their unequal separation was considered to be the main reason, however, later studies (RANDOLPH 1941) have indicated that it may be the result of specific gene or gene combinations and is chiefly physiological in nature. Strong support for this view is being offered by investigations of autotetraploids of different varieties of *C. roseus,* which despite having very low multivalent frequency and almost normal meiosis are seed sterile. The pollen sterility in all the varieties varies from 35 to 41%. Even this much sterility does not appear to be in proportion to few meiotic abnormalities which have been observed.

The fertility of autoploids is known to increase in subsequent generations. Along with other factors which may improve fertility the frequency of multivalents also decreases in subsequent generation. The autoploid under study already have extremely low multivalent frequency so it is possible that in future generations almost complete bivalent formation may be obtained. Efforts in this direction are underway.

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Summary

Polyploids of six varieties of *Catharanthus roseus* were artificially induced by colchicine and colchicine-gammexane solutions. They show general increase in different plant parts. The significant increase in the width of petals of 4n plants imparts more beautiful look to the flowers.

The meiotic behaviour of tetraploids was found to be normal. A few univalents were observed but their movement to different poles was regular. It is interesting to note that quadrivalent frequency in polyploids was very low. This low frequency of multivalents in *C. roseus* may be due the smaller size of the chromosomes which provides less time for pairing. The pollen fertility was fairly high still there had been very poor seed setting. In some cases shrivelled seeds were produced and most of the fruits formed were seedless. The causes of sterility are discussed.

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