The UV absorption spectrum of the purified virus was typical of the virus with the maximum at 256 nm and minimum at 240 nm. The A240/260 ratio was 0.86 and A260/280 was 1.64. Electron microscopic studies of the purified virus preparation showed spherical particles of 26 nm diameter (figure 1B).

Based on the information obtained from the host range, physical properties and particle morphology[2,3] the virus under study appeared to be identical to cucumber mosaic virus (CMV). However, this assumption could only be confirmed by the serological studies which are in progress. A perusal of the literature on plant virus diseases[4] indicates that there is no record of any virus disease on *H. muticus* from India.

The authors are thankful to Dr D. V. R. Reddy, Principal Virologist, ICRISAT, Hyderabad for facilities.

7 June 1983; Revised 17 December 1983

1. Waterworth, H. E. and Povish, W. R., *Phytopathology*, 1975, **65**, 728.
2. Orellana, R. G. and Antonio Quacquarelli, *Phytopathology*, 1968, **58**, 1439.
3. Kaper, J. M. and Waterworth, H. E., In *Hand book of plant virus infections and comparative diagnosis* (ed.) E. Kurstak, 1981, p. 258.
4. Sastry, K. S., *Plant virus and mycoplasmal diseases in India: A bibliography*. Bharati Publications, Delhi, 1980, p. 292.

INDUCED AUTOTETRAPLOIDY IN *CATHARANTHUS ROSEUS*— A PRELIMINARY REPORT

R. N. KULKARNI, R. S. CHANDRASHEKAR and B. P. DIMRI

Central Institute of Medicinal and Aromatic Plants, NAL Campus, Bangalore 560 037, India.

CATHARANTHUS ROSEUS, also known as *Vinca rosea*, derives its importance from the anti-cancer alkaloids, vincristine and vinblastine, found in its leaves. Its roots contain ajmalicine and serpentine alkaloids which are used in the treatment of hypertension. Several reports on the increase in chemical constituents of plants through polyploidy indicate the possibility of increasing the alkaloid content of *C. roseus* through autopolyploidy[1-3]. There are quite a few reports on induced autotetraploidy in *C. roseus*[4-10]. However, the effect of autotetraploidy on the alkaloid content in different parts of the plant and also, its effects on economically important characters such as, leaf and root yield have not been reported. Information on these aspects is necessary to evaluate the possibility of developing tetraploid varieties in *C. roseus*.

Tetraploids in *C. roseus* were induced by immersing the apical buds of seven-day old seedlings in 0.5% colchicine solution for 19 hr. Since no pure-lines were available, the treated seedlings were expected to be genetically heterogenous. The variability in the treated material would result in variability in the induced tetraploids which would, in turn, be useful for further improvement of tetraploids.

Several tetraploids were obtained through colchicine treatment of seedlings. Seeds of individual tetraploids were sown to raise C_2 generation. Seven diploid seedlings were obtained in the progeny of one tetraploid plant. These diploid seedlings and 10 tetraploid seedlings of the same plant were individually randomized in the field (in a plot of size 8.5 × 3.0 m.) along with the progeny of other tetraploids. When the plants were 9 months old, five diploid and five tetraploid plants (*i.e.* progeny of the same plant) were harvested and observations were recorded on different characters. (It was observed that defoliation due to senescence started at about this stage in the diploids). Rest of the plants were left for seed multiplication. The content of total alkaloids in different parts of the plant were estimated[12].

The differences between diploids and tetraploids were significant (at 5% level of significance) for only 4 of the characters studied, *viz* total number of branches per plant, average leaf weight, seeds per follicle and 50-seeds weight (table 1). Large standard errors associated with the mean of a majority of the characters studied appear to have rendered the differences between diploids and tetraploids non-significant. Comparisons based on replicated trials could be expected to reveal more significant differences between diploids and tetraploids.

The differences between diploids and tetraploids for other characters (other than the four mentioned above) were tested at 10% level of significance assuming that differences for the characters found to be significant at 10% level of significance would be likely to be found significant at higher levels of significance when comparisons are made from data obtained from replicated experiments. It was found that tetraploids had significantly larger leaf yield/plant yield ratio than the diploids. The diploids had significantly more number

Table 1 *Effect of autotetraploidy on different characters in C. roseus*

Character	Tetraploid Mean ± SE	Diplod Mean ± SE	t-value
1. No of primary branches per plant	9.3 ± 1.1	7.0 ± 0.6	1.84
2. No. of branches per plant	32.3 ± 4.4	82.7 ± 13.9	3.44
3. No. of leaves per plant	560.4 ± 94.8	1169.3 ± 245.1	2.32
4. Weight of single leaf (g)	0.250 ± 0.021	0.143 ± 0.012	4.46
5. Leaf yield per plant (g)	136.9 ± 19.5	166.7 ± 19.5	1.08
6. Stem yield per plant (g)	179.3 ± 27.4	280.4 ± 46.0	1.89
7. Root yield per plant (g)	34.3 ± 10.1	48.5 ± 5.4	1.24
8. *Leaf yield* plant yield ratio	0.384 ± 0.020	0.333 ± 0.009	2.32
9. Seed per follicle	8.2 ± 1.8	17.6 ± 2.1	3.40
10. Weight of 50 seeds (g)	0.080 ± 0.0034	0.067 ± 0.0022	3.25
11. *Alkaloid content* (%) *in*			
i. Leaf	1.98 ± 0.052	2.12 ± 0.067	1.65
ii. Stem	0.61 ± 0.045	0.61 ± 0.098	0
iii. Root	1.65 ± 0.13	2.07 ± 0.21	1.68

Table value of 't' at 5% level of significance = 2.776
Table value of 't' at 10% level of significance = 2.132

of leaves per plant than the tetraploids. However, there were no significant differences between diploids and tetraploids for leaf and root yield. Larger leaf yield/plant yield ratio combined with lesser number of leaves and branches per plant in tetraploids compared with diploids suggested that it may, perhaps, be possible to increase leaf yields of tetraploids by increasing number of plants per unit area. Since diploids and tetraploids did not significantly differ in alkaloid content, increase in plant yield if obtained by increasing number of tetraploid plants per unit area, would also increase alkaloid yield.

It has been reported that the alkaloid content in leaves of diploids varies with the age of the plant and that it was maximum when the plants were 10 months old (under agro-climatic conditions at New Delhi)[11]. The age of the plant at which the alkaloid content is maximum in the case of tetraploids needs to be experimentally determined. The comparisons between diploids and tetraploids for alkaloid content should, therefore, be made taking this point into consideration. Further, visual observations indicated that diploids and tetraploids differ in their habit and growth rate. Therefore, the cultural and nutritional requirements (such as plant spacing, nitrogen requirement etc) for the production of the maximum alkaloid yield may, perhaps, be different for diploids and tetraploids. These requirements for diploids and tetraploids need to be first determined before any valid comparisons between diploids and tetraploids could be made for total alkaloid yield. Experiments are being taken up to determine differential response, if any, of diploids and tetraploids to plant spacing and nitrogen levels.

The authors thank Dr M. R. Narayana, Scientist-in-Charge of the Bangalore Centre and Dr Akhtar Husain, Director of the Institute for providing necessary facilities.

10 January 1984

1. Dijkstra, H. and Speckman, G. J., *Euphytica*, 1980, **29**, 86.
2. Janaki Ammal, E. K. and Sobti, S. M., *Curr. Sci.*, 1962, **31**, 387.
3. Ramanujam, S. and Parthasarathy, N., *Indian J Genet.*, 1953, **13**, 53.
4. Chauhan, A. K. S. and Rhaghuvanshi, S. S., *Proc. Natl. Seminar on Medicinal and Aromatic Plants*, 1982, p. 49.
5. Dnynansagar, V. R. and Sudhakaran, I. V., *Cytologia*, 1970, **35**, 228.
6. Dnynansagar, V. R. and Sudhakaran, I. V., *Proc. Indian Natl. Sci. Acad.*, 1977, **B43**, 133.
7. Furusato, K., *Bot. Zool.*, 1940, **8**, 1303.
8. Janaki Ammal, E. K. and Bezbaruah, H. P., *Proc. Indian Acad. Sci.*, 1963, **B57**, 339.
9. Krishanan, R. and Naragund, V. R., *Proc. Natl. Seminar on Medicinal and Aromatic Plants*, 1982, p. 14.
10. Schnell, L., *Am. J. Bot.*, 1941, **28**, 55.
11. Mandal, S., Maheshwari, M. C., Srivastava, V. K., Sarbjit Singh, Pareek, S. K. and Gupta, R., *Proc.*

Natl. Seminar on Medicinal and Aromatic Plants, 1982, p. 37.
12. Higuchi, T. and Bodin, J. I., In: *Pharmaceutical Analysis* (Eds, T. Higuchi, and E. B. Hanseen), Interscience, New York, 1961, p. 391.

EFFECT OF VINBLASTINE SULPHATE ON SISTER CHROMATID EXCHANGES IN HUMAN CHROMOSOMES

RACHEL THOMAS-JACOB and O. S. REDDI
Institute of Genetics and Hospital for Genetic Diseases, Osmania University, Hyderabad 500 016, India.

IN recent years, the reciprocal exchanges between sister chromatids occurring in somatic cells have been the subject of intensive study, particularly in human cells. The incorporation of bromodeoxyuridine results in differential staining of the sister chromatids after two cell cycles[1,2]. In the present communication, we report our observations on sister chromatid exchanges [SCE] frequency with vinblastine sulphate, an anti-neoplastic agent used in the treatment of a wide variety of carcinomas.

Human lymphocyte cultures were set up with peripheral blood samples obtained from ten healthy donors following the method of Moorhead *et al*[3]. 5-Bromodeoxyuridine (BrdU) (0.1 ml) at a final concentration of 30 μg/ml was added after 24 hr and all cultures were maintained in the dark to avoid photolysis. Vinblastine sulphate (VLB) (Sigma) was added to the cultures under dim light, 24 hr before termination at concentrations of 1.25, 2.50, 3.75 and 5 μg/ml respectively. Air-dried preparations of the chromosomes were further processed for SCE studies after a minimum period of three days by the method of Perry and Wolff[2]. The number of SCEs/cell was counted in randomly selected, well scattered metaphase plates.

Table 1 shows the frequency of SCEs *in vitro* in PHA stimulated lymphocytes treated with VLB. A concentration-dependent increase in the frequency of SCEs was observed in VLB-treated cells.

VLB produced a dose- and time-dependent increase in various chromosomal aberrations, including chromatid breaks and SCEs in Don lung cells from Chinese hamsters[4]. One large dose of cyclophosphamide (1000 mg) and vinblastine (10 mg) administered at weekly intervals resulted in a dramatic increase in the number of SCEs/cell. The frequency of SCEs returned to the initial level about ten days after termination of treatment[5]. Kucerova and Polivkova[6] using the fluorescence plus Giemsa technique noted that at 10^{-7} mg/ml of VLB, the frequency of SCEs in the cultured lymphocytes did not increase.

There are indications that a close relationship may exist between DNA synthesis, DNA repair processes and SCE[7–11]. The frequency of SCEs may be increased by mutagenic agents, either because of impaired DNA repair function or because the extent of DNA damage (*e.g.* cross-links) exceeds the capacity of the repair enzymes to remove them from DNA during subsequent cell cycles. The increase in the number of SCEs in VLB-treated cells may be due to the inhibition of DNA replication by VLB.

Financial assistance received from CSIR, New Delhi, is gratefully acknowledged.

1 December 1983; Revised 13 January 1984

1. Latt, S. A., *Proc. Natl. Acad. Sci.*, 1973, **70**, 3395.
2. Perry, P. and Wolff, S., *Nature* (*London*), 1974, **251**, 156.
3. Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M. and Hungerford, D. A., *Exp. Cell Res.*, 1960, **20**, 613.
4. Segawa, M., Nadamitsu, S., Konda, K. and

Table 1 *Frequency of sister chromatid exchanges in PHA stimulated lymphocytes after treatment with vinblastine sulfate*

Cytostatic drug	Concentration (μg/ml)	No. of cells observed	No. of SCEs observed	Mean SCEs/cell (Mean ± S.E.)
Control (only BrdU)	30.0	500	5250	10.5 ± 0.3
Vinblastine Sulphate	1.25	50	1110	22.2 ± 0.3
	2.50	50	1170	23.4 ± 0.3
	3.75	50	1210	24.2 ± 0.2
	5.00	50	1250	25.0 ± 0.2