Cytomorphological Studies on Induced Auto-octoploids of Egyptian Henbane \( (Hyoscyamus muticus \text{ L.}) \)

O. P. Dhawan and B. R. Tyagi

Central Institute of Medicinal and Aromatic Plants,
Post Bag No. 1, P. O. RSM Nagar, Lucknow-226016, India

Accepted June 6, 1988

Induction of polyploidy is known to be useful in plant species where total biological yield is an essential economic parameter. Autotetraploid plants can be easily obtained in most of the diploid species by colchicine treatment. However, it is difficult to produce autopolyploid plants with levels higher than \( 4n=4x \) chromosomes. On the other hand, if these plants could be produced, they are mostly not suited for detailed cytological investigations as only a few autopolyploid plants with higher ploidy levels have been found to produce flowers with normal sex organs (Gottschalk 1978).

Egyptian henbane \( (Hyoscyamus muticus \text{ L.}, 2n=2x=28) \) of the family Solanaceae is one of the most important commercial source of hyoscyamine—a major tropane alkaloid (Mittal and Saxena 1977, Husain \textit{et al.} 1979, Koul \textit{et al.} 1983, Tyagi \textit{et al.} 1984). This alkaloid is extracted from all aerial parts of the plant and drug thus obtained is used as a sedative, anticholinergic, antispasmodic and to control gripping pain in intestinal disorders. During the course of cytological investigation on a large number of colchicine treated seed raised plants of \( H. \text{ muticus} \), in addition to 21 autotetraploids, two auto-octoploids were identified. Cytomorphological characteristics of these auto-octoploid plants are presented in this paper.

Materials and methods

Two auto-octoploids (C-43 and C-65) identified in the colchicine treated seed raised (seeds soaked in 0.5\% aqueous solution of colchicine for 72 h) plants of an \textit{elite} inbred CIMAP/NP-41 developed at our institute (Tyagi 1986) were used in this study. Comparative data on various morphological traits (at mid flowering stage of plant growth), pollen and seed fertility, and meiotic behaviour were recorded on both the octoploids and their standard diploid counterparts (CIMAP/NP-41). Random five observations were recorded for all the morphological characters. Leaf area was measured by the Area Meter (LICOR Model-3100). Pollen stainability, an estimate of viability was determined using acetocarmine stain. For meiotic studies, fixation, preparation of slides and related techniques were the same as described earlier (Tyagi and Dhawan 1988).

Observations and discussion

The application of colchicine is still the most frequently used method to induce higher ploidy levels. Though, auto-tetraploidy can be rather easily achieved in most diploid species, higher ploidy levels are difficult to obtain. Induced octoploids have been reported earlier in \textit{Lycopersicon esculentum} (Gottschalk 1959), \textit{Brassica oleracea} var. \textit{Capitata} (Shchavinskaya 1973), \textit{Nicotaina longiflora} (Kostoff 1938), \textit{Bryophyllum diangremontianum} (Schwanitz 1961), \textit{Petunia axillaris} (Padmaja 1985) among others. However, very little information is available on the detailed meiotic chromosome behaviour of such octoploids. This information is im-
important in understanding the organization and distribution of genetic material during meiotic cycle at higher ploidy levels. The comparative morphological data on both the auto-octoploids (C-43 and C-65) along with their normal diploid counterpart (CIMAP/NP-41, Fig. 1) is presented in Table 1. Whereas, chromosome associations at diakinesis/metaphase I (MI) and their distribution at anaphase I (AI) and anaphase II (A II) are given in Tables 2 and 3, respectively.

The increase in cell size and decrease in fertility are the two near universal consequences of induced polyploid leading to larger but fewer plant parts (Eigsti and Dustin 1955). Induction of auto-octoploidy in H. muticus (Fig. 2) also led to the expected exophenotypic effects (=*gigas* characteristics) such as broader and thicker leaves, thicker stems, and larger flower parts, stomata and pollen grains. A significant increase in the thickness of the main shoot of both the auto-octoploids was accompanied with the reduction in plant height and number of branches (Table 1). However, relative increase in plant height and number of branches with the induction of auto-tetraploidy has earlier been reported in H. muticus (Lavania 1986). It seems, therefore, that in H. muticus the plant growth and development are inhibited beyond a certain optimal ploidy level and further duplications cause disharmonious growth.

Size of stomata showed an increase (918.70 μm² and 1008.90 μm² in C-43 and C-65, respectively) as compared to those of diploid (619.60 μm²) thus decreasing the frequency of stomata per unit area on both adaxial as well as abaxial sides of leaves. A similar increase in length and breadth of stomata in artificially induced octoploid *Petunia axillaris*, as compared to their respective diploids has been reported by Padmaja (1985).
In comparison to the diploid inbred CIMAP/NP-41 (Fig. 8) a wide variation in pollen grain size was observed in both the auto-octoploids (Fig. 9). The mean pollen grain size in auto-octoploids (2956.60 μm² and 2696.36 μm² in C-43 and C-65, respectively) was found to be more than double that of diploid CIMAP/NP-41 (1156.36 μm²).

The meiotic behaviour in the diploid inbred line CIMAP/NP-41 was found to be almost normal showing 14 bivalents at diakinesis/MI in 92.00% of the pollen mother cells (PMC's). In the remaining 8.00% of the PMC's the presence of two univalents along with 13 bivalents (Fig. 3) was recorded at diakinesis/MI, which may be due to early separation of one of the bivalents. Frequency of meioocytes in the anthers of both the auto-octoploids was quite low. Data on chromosome associations at diakinesis/MI revealed that fairly high number of chain or ring octovalents were prevalent in both the octoploids ranging from 5 to 7 (C-43, Figs. 4 and 5) and 3 to 6 (C-65). Chain or ring quadrivalents (4 to 6 in C-43 and 5 to 7 in C-65) and bivalents (14 to 20 in C-43 and 13 to 14 in C-65) were the next predominant configurations. The other chromosome configurations like heptavalents, hexavalents, pentavalents, trivalents and univalents were also observed, though in low frequencies (Table 2). The data further revealed that only 40.30% and 33.14% of the chromosomes in auto-octoploids C-43 and C-65, respectively, were involved in octovalent formation. This might be due to increase in number of homologous chromosomes in a cell which might have affected the pairing process at octoploid level. Similar views have been expressed by other workers for the occurrence of lower frequencies of multivalents in induced polyploids (Padmaja1985, Morrison and Rajahathy 1960).
Figs. 1-9. Comparison of plant morphology and meiotic chromosome behaviour of diploid and auto-octoploid of _H. muticus_ L. 1, a diploid plant of inbred CIMAP/NP-41. 2, an induced auto-octoploid plant C-43. 3, metaphase I showing 13 I+2 I. 4, metaphase I showing 7 VIII+4 IV+20 II. 5, metaphase I showing 5 VIII+1 VII+2 VI+4 VI+14 II+9 I. 6, anaphase I showing 56: 56 distribution. 7, late anaphase II showing 59: 59:: 53: 48+5 L distribution. 8, pollen grains of diploid inbred CIMAP/NP-41. 9, pollen grains of auto-octoploid C-43.
At AI, regular separation of chromosomes (56:56, Fig. 6) was observed only in 29.62% and 21.05% of the PMCs in C-43 and C-65, respectively. Whereas in the remaining cells, lagging chromosomes (2 to 5) were recorded in varying frequencies (Table 3). At All, a normally expected 56:56::56:56 segregation was observed in only 12.90% and 14.28% of the cells in C-43 and C-65, respectively. While rest of the PMCs exhibited various irregular distributions with (Fig. 7) or without lagging chromosomes, thus resulting in the formation of variable size of pollen grains.

In the present study, a significant reduction in pollen fertility (66.05% and 57.08%) of the two auto-octoploids C-43 and C-65, respectively from 92.5% in their normal diploid (CIMAP/NP-41) was observed. The increased ploidy level is known to affect the normal meiotic chromosome behaviour. Darlington (1937) stated that the formation and irregular separation of multivalent associations are mainly responsible for sterility in autopolyploids. However, Randolph (1941) observed that sterility in autopolyploid maize was exclusively controlled by specific genes and gene combinations and is mainly physiologic in nature. On the other hand, Sparrow et al. (1942) and Sen and Chheda (1958) found no correlation between pollen sterility and multivalent formation but Sparrow et al. (1942) observed a positive correlation between pollen sterility and lagging of chromosomes at anaphase I. Self pollination of both the auto-octoploids resulted in the formation of five healthy seeds in C-43 only, whereas the other octoploid C-65 completely failed to set seed. Such an extremely poor or no seed setting in these induced auto-octoploids of *H. muticus* might be attributed to the observed irregular meiotic behaviour of chromosomes as well as other genetic and physiological factors.

**Summary**

Comparative cytomorphological studies between diploid (2n=28) inbred and colchicine induced auto-octoploids of medicinally important solanaceous plant *H. muticus* were made. In auto-octoploids the increase in most of the morphological characters such as thickness of the main shoot, leaf size, floral parts and size of stomata and pollen grains was accompanied by reduction in plant height and number of branches. The meiotic chromosome behaviour of the auto-octoploid plants was studied in detail. The high percentage of pollen sterility and very poor or no setting of seeds were attributed mainly to the chromosomal irregularities recorded during meiotic division.

**Acknowledgements**

The authors are grateful to Dr. Akhtar Husain, Director, Central Institute of Medicinal and Aromatic Plants, Lucknow for his keen interest in the study and for providing necessary facilities.

**References**


