Indian J. Genet., **67**(1): 99-100 (2007)

Short Communication

Induced autotetraploids in Love-in-a-mist (*Nigella damascena* L.)

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(Received: April 2005; Revised: November 2006; Accepted: December 2006)

Colchiploidization widens amplitude of variation in the gene pool offering scope for the development of novel and superior plant type; although, high sterility associated to polyploids of sexually propagating species has been detrimental for their positive selection [1]. However, normalization of meiosis due to reduction of quadrivalent frequency per cell over the generations leading to improved fertility of colchiploids has been reported in some crops [2-5], which indicates that cytological behaviour of induced polyploids forms an integral part of polyploid research. In the present study, colchiploids induced in Love-in-a-mist (*Nigella damascena* L.) a garden ornamental plant of the family Ranunculaceae, were cytomorphologically evaluated for four subsequent generations (C_0-C_3) and compared to normal diploids in an attempt to explore the ornamental traits and to develop fertile tetraploids which would be helpful for polyploid-breeding in the species in addition to its importance in interspecific hybridization with black cumin (*Nigella sativa*) a spice of commerce, for genetic improvement.

Meristematic tips of young seedlings at cotyledonary leaf stage were given a 3h treatment for 1 day and 2/3 consecutive days and 5h treatment for ld with either 0.25% or 0.50% colchicine. Out of 80 treated plants (10 seedlings/treatment), 5 colchiploids could be raised at C_0 of which only 1 plant (0.25%, 5h) could set seeds (15 seeds) at maturity. Progenies of this plant were raised as C_1 (3 out of 15 seeds sown), C_2 (5 out of 25 seeds sown) and C_3 (6 out of 25 seeds sown) generations. The autotetraploids were first screened at vegetative stage of growth based on general morphological characteristics (thicker and stouter stem, bushy habit and thick dark green pinnae of leaves) and later confirmed through meiotic analysis. Morphological attributes of diploids and tetraploids have been compared in Table 1. Meiosis and pollen fertility were analysed in diploids and tetraploids in PMCs and pollen grains respectively, stained in 1% propionocarmine solution. The fully stained pollen grains were considered fertile. Data of diploids (12 plants) as well as tetraploids (19 plants) were pooled over the generations.

The most prominent changes among the tetraploids were increase in capsule sterility (capsules containing only abortive seeds), reduction in seed number and seed fertility (expressed as per cent of control); although, an increasing trend in seed fertility was noted over the generations. Although flower size in tetraploids (range : 7.95 to 14.36 cm^2, mean : 11.22 ± 0.58) did not vary significantly (t = 1.35, d.f. 143, p>0.05) than diploids (range : 8.40 to 18.48 cm^2, mean : 12.99 ± 1.21), number of flowers varied positively (4n - range: 16-76, mean: 45.11 ± 3.22; 2n-range : 7-40, mean : 20.36 ± 2.39; t = 5.19, d.f. 29, p < 0.001). The tetraploids were similar in height to normal diploids with significant reduction in primary branch number accompanied by enhancement of total branches imparting them a bushy appearance. Bushyness and spreading nature (2n-range : 432.0 - 1375.0 cm^2, mean : 918.3 ± 126.6; 4n - range : 460.0-1500.0 cm^2, mean : 1175.7 ± 81.0; t = 2.08, d.f. 29, p<0.05) along with significantly enhanced number of flowers and capsules per plant in tetraploids have resulted into more ornamental values which could be exploited in polyploid-breeding of the species.

The parental diploids (2n = 12) formed mean chromosomal association (Fig. 1A) of 5.88 II + 0.25 I per cell at metaphase I (bivalent and univalent frequency per cell was random among generations) whereas, the induced tetraploids (2n = 4X = 24) formed quadrivalents (0 to 4), bivalents (2 to 12) and univalents (0 to 16) in varying proportions at metaphase I (Fig. 1B-1D) and their mean configuration per cell over the generations are summarised in Table 2. Trivalents could not be observed in meiotic plates. Frequency of quadrivalents (p>0.001) and bivalent (p>0.01) per cell at metaphase I among tetraploids were inconsistent over the generations as evidenced from χ^2 test of heterogeneity; while, that of univalents were random (p>0.05). The coefficient of quadrivalent realization was low among tetraploids (0.91 to 1.54/cell) and it decreased over the generations (C_0 - 0.257, C_1 - 0.242, C_2 - 0.222 and C_3 -0.152) with concomitant increase in bivalent frequency per cell (7.78 to 9.19) leading to a normalized meiosis (Table 2) which may be useful for improvement of fertility in subsequent generations. Further, cytological examination of induced tetraploids of *N. damascena* over the generations suggested that fertility (pollen and seed) had a cytological basis and was dependent on bivalent and quadrivalent frequencies per cell rather

Table 1. Morphorlogical attributes of diploids and tetraploids of *Nigella damascena*

Plant types	Plant height (cm)	No. of primary branches per plant	No. of flowers/ plant	Flower fertility (%)	No. of capsules/ plant	No. of abortive capsule	Seeds per capsule	Seed set/ plant	Seed fertility (percent of control)
Diploid	40.27±1.39	14.91±1.38	20.36±2.39	88.46	17.64±1.83	52.40	16.26±1.69	90.73±7.78	
Tetraploids:									
C_0	39.52±3.34	4.60***±0.83	34.20*±4.55	81.34	27.60±5.28	92.40	0.40***±0.29	3.2***±2.9	3.53
C_1	39.97±1.02	9.67*±0.72	45.67***±5.19	89.95	41.00***±4.50	94.51	0.24***±0.12	8.33***±3.54	9.18
C_2	36.94±1.91	8.80*±2.11	43.80**±7.85	89.08	37.40***±4.50	94.89	0.26***±0.03	9.00***±0.63	9.92
C_3	42.47±1.05	7.17**±0.28	55.00***±2.65	91.72	50.50***±3.01	96.16	0.19***±0.02	9.50***±0.77	10.47

*,** and ***Significant at 0.50, 0.01 and 0.001 probability levels respectively.

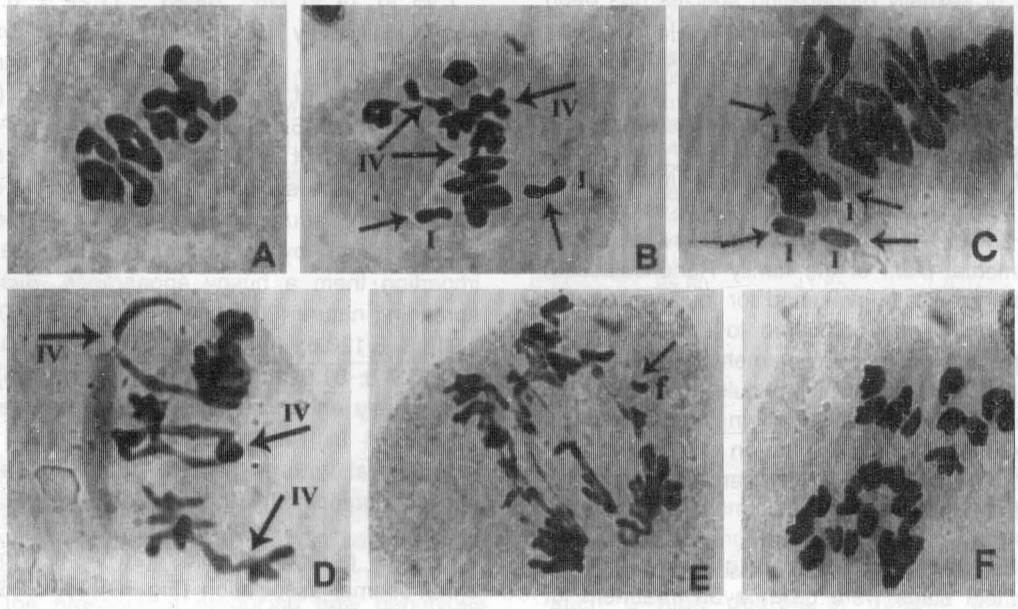

Fig. 1. Induced autotetraploids in *Nigella damascena* L.

Table 2. Chromosome configurations and pollen fertility in diploids and autotetraploids of *Nigella damascena*

Plant type	No. of MI cells scored	Mean per cell			No. of AI cells scored	Abnormal AI cells (%)	No. of pollens assessed	Pollen fertility (%)
		I	II	IV				
Diploid	120	0.25	5.88	0.00	74	13.51	297	83.83
Tetraploids :								
C_0	78	2.26	7.78	1.54	504	61.51	1525	34.75
C_1	84	2.00	8.12	1.45	131	55.80	828	46.50
C_2	116	2.17	8.26	1.33	252	50.00	1510	48.34
C_3	127	1.91	9.19	0.91	272	47.79	760	46.84

than univalent frequency per cell. The abnormal anaphase I cells (Fig. 1E-1F) demonstrated significant correlration with bivalent (r = -0.86, d.f 3, $p<0.05$) and quadrivalent (r = 0.86, d.f. 3, $p<0.05$) frequency per cell. The correlations between abnormal anaphase I and pollen fertility and seed fertility (r = -0.86, d.f. 3, $p<0.05$) were also significant.

References

1. **Gottschalk W.** 1978. Open problems in polyploidy research. Nucleus, **21**: 99-112.
2. **Sybenga J.** 1973. Allopolyploidization of autopolyploids II. Manipulation of the chromosome pairing system. Euphytica, **22**: 433-444.
3. **Bender K. and Gaul H.** 1966. Zur Frage der Diploidisierung autotetraploider Gerste. Z. Pflanzenzuchtung, **56**: 164-183.
4. **Reinbergs E.** 1963. Autotetraploids in barley. Proceeding of International Barley Genetics Symposium. Wageningen, 151.
5. **Jauhar P. P.** 1970. Chromosome behaviour and fertility of raw and evolved synthetic tetraploids of pearl millet, *Pennisetum typhoides* Stapf et Hubb. Genetica, **41**: 407-424.