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Genetic improvement of Egyptian henbane, *Hyoscyamus muticus* L. through induced tetraploidy*

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Summary. Induced autotetraploids with high pollen and seed fertility have been developed in a medicinally important Solanaceous plant: *Hyoscyamus muticus* (2n = 28). The colchiploids are vigorous and the increase in all the determinate parts – plant height, size and thickness of leaves, cell, pollen, seed and flower size – along with an increase in alkaloid content has been achieved. The overall improvement in the colchiploids has the advantage of enhancing nearly 1.5 times the production potential of the economic product – the total alkaloids. Although the seed is not the ultimate economic product in this species, it is still required for propagation. The seed fertility in the colchiploids improved because of reduction in quadrivalent frequency and subsequent balanced anaphase separation.

Key words: Colchiploidy – Genetic improvement – Fertility – Cytogenetic diploidisation – Alkaloid production – Breeding strategy

Introduction

Hyoscyamus muticus – the Egyptian Henbane is one of the most important medicinal herbs produced in Egypt and is a valuable source of the alkaloids hyoscyamine, and traces of hyoscine and atropine.

These alkaloids can be extracted from the entire aboveground plant parts. The drug obtained from this plant provides relief from the painful spasmodic condition of the nonstriated muscles, irritation of hysteria and irritable cough. A cataplasm of fresh leaves is used as a pain killer and dried leaves are smoked as cigarettes against asthma (Boulos 1983). Recently, this plant has been introduced in India for commercial cultivation by this Institute (Husain et al. 1979), and the development of relevant agrotechnologies and genetic improvement are underway.

Induced polyploidy is known to enhance the production potential of plants where vegetative organs and their biomass constitute the economic product. It is likely that the genome doubling attained through this approach may favourably affect the production of such secondary metabolites as alkaloids. However, when seed is needed for propagation, the improvement achieved through the polyploidy approach is fraught with limitations due to the seed sterility generally associated with chromosome doubling. Induced polyploidy has been successfully applied in Egyptian henbane to achieve improvement in vegetative vigour coupled with enhanced alkaloid content as well as the maintenance of seed fertility. The results of these investigations are described in the present communication.

Material and methods

The seeds obtained from open pollinated selected plants of *Hyoscyamus muticus* L. (2n = 28) grown at the experimental farm of this Institute were sown in earthen pots containing a mixture of soil and farm yard manure. Shoot tips of young seedlings (4–6 leaf stage) were treated continuously for 24 h with 0.2% aqueous solution of colchicine (Sigma) by the cotton plug method to raise the colchiploids. The selfed seeds from C_0 colchiploids were collected. The different genotypes revealed differential seed and pollen fertility. Based on meiotic anaphase separation, and pollen and seed fertility, two different colchiploid genotypes were isolated and carried through the C_1 and C_2 generations following selection for seed fertility. For comparison with the diploid control, the selfed seeds were collected from the lower lateral branches, which were not treated with colchicine.

For meiotic analysis flower buds of appropriate size were collected between 9.00–10.00 a.m. and fixed in Carnoy's

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This paper is dedicated to Professor A.K. Sharma on his decoration as golden jubilee professor of Indian National Science Academy fixative. Pollen fertility was examined following the usual acetocarmine staining. Data were recorded on meiotic behaviour, pollen and seed fertility, morphological characters, metric traits and total alkaloid content. For analyses of the frequency of multivalent association in autotetraploids, the analytical procedure suggested by Sybenga (1984) was applied. Leaf area was measured by a portable leaf area meter (model LICOR-3000). The data on alkaloid content were recorded in the C_1 generation on an individual plant basis and in the C_2 on a population basis, following the gravimetric method of Cromwell (1955). To evaluate the overall performance, relative yield data were recorded in the diploid control and on autotetraploids in the C_3 generation. The entire above ground parts were harvested at the mid-flowering stage 140 days after sowing.

Observations

Although two different C_0 plants, representing varied appearances in plant morphology, pollen and seed fertility, were isolated, there were only parental 2n seeds available from one genotype to allow a reasonable comparison of diploids and autotetraploids. Coincidentally, this colchiploid genotype exhibited a very good seed set (i.e. more than 65%) in the well-developed capsules. The open pollinated seeds from the selfed progeny of parental diploids and selfed seeds from the colchiploids (selected for at least 75% seed set) were grown in C_1, C_2 and C_3 and compared with the respective controls. The data on determinate, reproductive and other qualitative characters are recorded in Tables 1 and 2, yield data in Table 3 and the comparison of meiotic data in autotetraploids is given in Table 4. The representative features are shown in Figs. 1–3. The anaphase separation as a rule was almost balanced in both diploid and tetraploid. Anaphase anomalies, such as precocious separation or lagging chromosomes, were only occasionally observed in a few cells.

In general, the induced tetraploids were vigorous with gigantism traits observed for almost all the determinate parts. This gigantism is also accompanied by the associated increase in alkaloid content – the most important requirement in this species. One of the unique, positive features is the uniform seed germination in tetraploids as against differential germination in diploids.

Discussion

Genetic improvement

The common effect of induced polyploidy in *Hyoscyamus muticus* is an increase in cell size as was apparent from the size of the guard cells and pollen grains. This increase seem to be pleiotropic, resulting into an increase in almost all the determinate organs: seeds, floral parts, leaf size and thickness, stem thickness and height. Additionally, an important feature of the tetraploids is the increase in alkaloid content compared to diploid parent, a character of immense practical advantage. Agronomically, there is nearly a 29.5% increase in the fresh herb yield (17.6% increase on a dry matter basis) in the autotetraploids over the diploids as recorded in the C_3 generation (Table 3). Likewise, an increase of 22.5% in dry matter alkaloid content was recorded in the C_2 at a population level (38% increase in alkaloid content was observed in C_1 on an individual plant basis), thus showing a 44% improvement in the tetraploids for the production potential of the economic product – the total alkaloids. Although the seed is not the economic product, it is necessary for propagation. Good seed fertility, so essential to maintain genetic stability and reproductive capacity of the induced tetraploids, is an added advantage. Generally, poor seed set is one of the major drawback of induced polyploids. These results clearly indicate the potential of polyploidy breeding in this important medicinal plant.

An increase in alkaloid content has earlier been reported in autopolyploids of the related species *Hyoscyamus niger* L. but those autoploids had limited applications due to an associated high seed sterility (Anonymous 1959). High seed fertility has been particularly encouraging in the autotetraploids of cross fertilized crops (Gottschalk 1978; Pal and Khoshoo 1977). These results, through a reduction in quadrivalent frequency owing to the inherent heterozygous make-up of the experimental material, permit a comparatively higher degree of bivalent pairing in autotetraploids. This advantage of heterozygosity is typically enjoyed by *Hyoscyamus muticus* due to open pollination and the heterostylous condition of its flowers.

A good seed fertility, nearly comparable to that found in diploids, has been attained in colchiploids following rigid selection. Nevertheless, all plants in the progeny do not maintain the same level of fertility. Genetic and cytogenetic factors contribute to this variation in fertility. One of the major factors responsible for good fertility is the significant reduction in multivalent formation. This trend is evident from the data of Table 4. Similar observations have been reported by Swaminathan and Sulba (1959) in *Brassica campestris* var. 'toria', Gilles and Randolf (1951) in maize, Pal and Pandey (1982) in grain Amaranths, Pantulu and Krishna Rao (1982) in pearl millet, and Zadoo (1986) in *Atylosia scarabaeoides*.

The present findings clearly indicate the scope of polyploid breeding in this species, in which there has been significant improvements both in biomass as well as in the content of active principles – the alkaloids.

Strategy for polyploid breeding

The author developed raw colchiploids of a good number of morphotypes of *Hyoscyamus muticus* but only a few of them exhibited good seed and pollen fertility. The C_0 colchiploids were screened for pollen fertility and meiotic anaphase separation and those with high

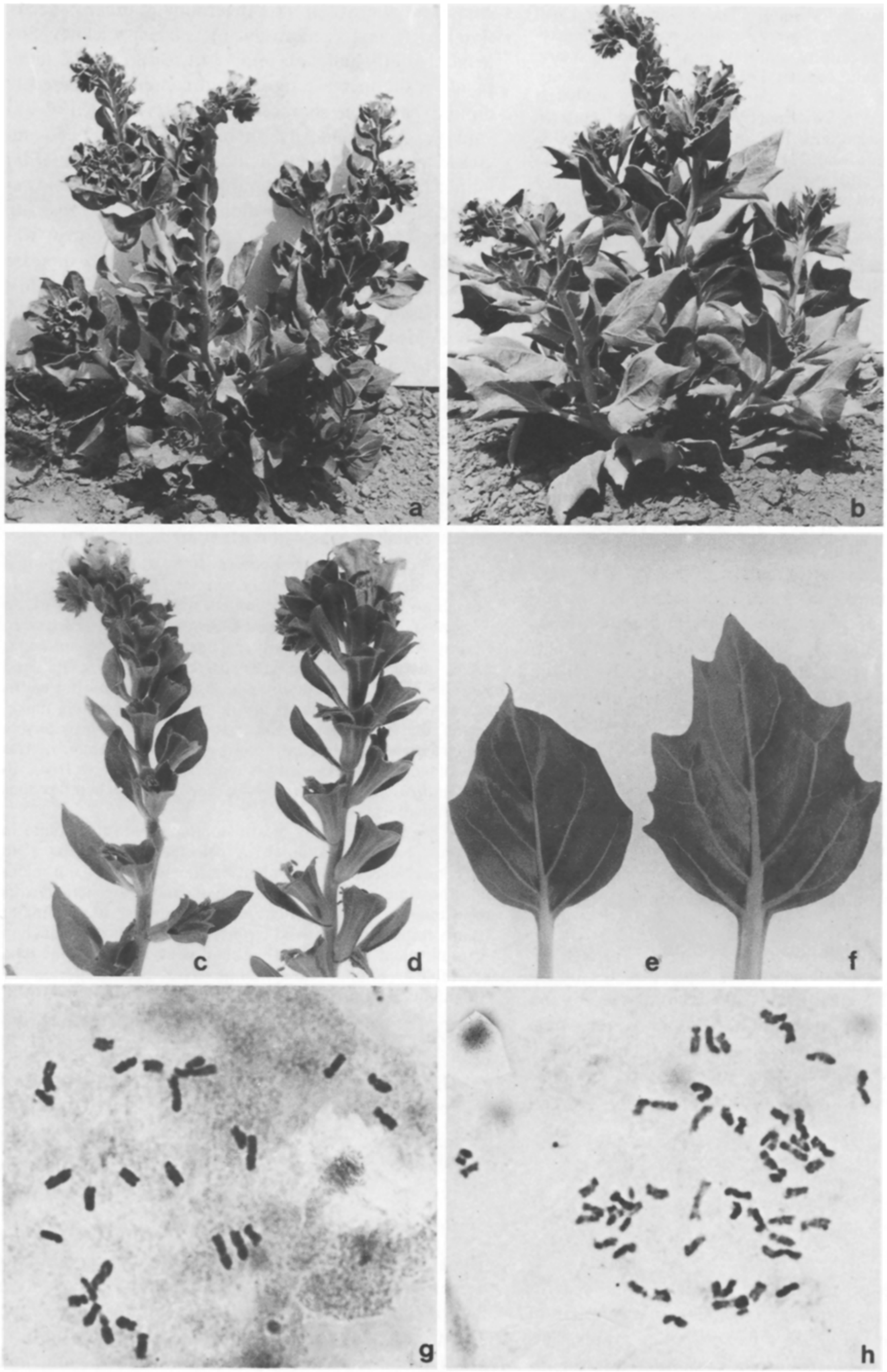
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Table 1. Comparison of determinate characteristics of diploid and autotetraploid *Hyoscyamus muticus*

S. no.	Characteristics	2n		4x		% increase in 4x
		Mean	Range	Mean	Range	
1.	Size of stomatal guard cell (μm²)	534.92	453 – 566	903.12	856 – 949	68.83
2.	Frequency of stomata/100² mm	484.00	449 – 495	334	315 – 350	−30.99
3.	Pollen grain size (μm²)	1,236.02	878 –1,323	1,845.71	1,423 –2,223	49.33
4.	Seed size (mm²)	4.16	3.51 – 4.43	5.36	4.62 – 5.73	28.84
5.	No. of seeds/capsule	228.12	129 – 313	95.43	68 – 121	−58.16
6.	1,000 seed weight (g)	0.5177	0.5028– 0.5292	1.0734	0.8351– 1.2885	107.34

Table 2. Reproductive and other qualitative characteristics of diploid and autotetraploid *H. muticus*

S. no.	Characteristics	2n		4x		% increase in 4x
		Mean	Range	Mean	Range	
1.	Pollen fertility (%)	97.68	93 –100	96.32	90 –100	−1.39
2.	Seed fertility (% filled seeds)	79.56	70 – 91	77.93	67 – 86	−2.05
3.	Germination of filled seeds (%)	56.00	48 – 64	86.00	74 – 96	53.57
4.	Leaf area (cm²)	61.50	16.76 –185.61	97.95	12.39 –234.42	59.27
5.	Fresh Leaf weight/100² cm (g)	7.66		10.51		37.22
6.	Dry matter alkaloid content (%)	0.688	0.667– 0.717	0.843	0.738– 0.939	22.53
7.	Plant height (cm)	67.30	59 – 76	79.81	69 – 95	18.59
8.	No. of primary branches	10.40	6 – 13	11.68	7 – 17	12.31

Table 3. Relative yield performance of diploid and autotetraploid of *Hyoscyamus muticus*

S. no.	Characteristics	2n Mean±SE	4x Mean±SE	% increase in 4x
1.	Total fresh weight/9² m (kg)	34.100±4.040	44.181±3.012	29.563
2.	Dry matter content (%)	7.38 ±0.081	6.7 ±0.024	−9.214
3.	Net dry weight/9² m (kg)	2.516	2.960	17.647
4.	Expected relative production potential for alkaloid content	100	144.117	44.117[a]

[a] Expected value is calculated considering 22.5% increase in autotetraploids observed for alkaloid content/unit dry matter

Table 4. Mean meiotic chromosome associations (±SE) in the Colchiploid (4x=56) of *Hyoscyamus muticus*

Colchiploid generation	IV		III	II		I	Multivalent pairing (f)
	Ring	Chain		Ring	Chain		
C_0	5.06 ±0.284	0.88 ±0.178	0.73 ±0.152	5.68 ±0.426	9.22 ±0.646	1.21 ±0.274	0.4552
*	**4.975**	**0.865**	**0.716**	**5.585**	**9.065**	**1.19**	**0.448**
C_2	3.468±0.223	0.492±0.061	0.552±0.079	6.008±0.453	12.736±0.274	1.348±0.209	0.3055
*	**3.449**	**0.489**	**0.549**	**5.972**	**12.660**	**1.340**	**0.303**

* The values in bold letters indicate the corrected figures calculated for 4x=56 from the actual data given

Fig. 1a–h. Comparison of vegetative morphology in diploid and colchiplid – whole plant, peduncle and middle leaf. **a, c, e** diploid, and **b, d, f** tetraploid. Note the difference in size. **g, h** somatic chromosomes of diploid and tetraploid, respectively

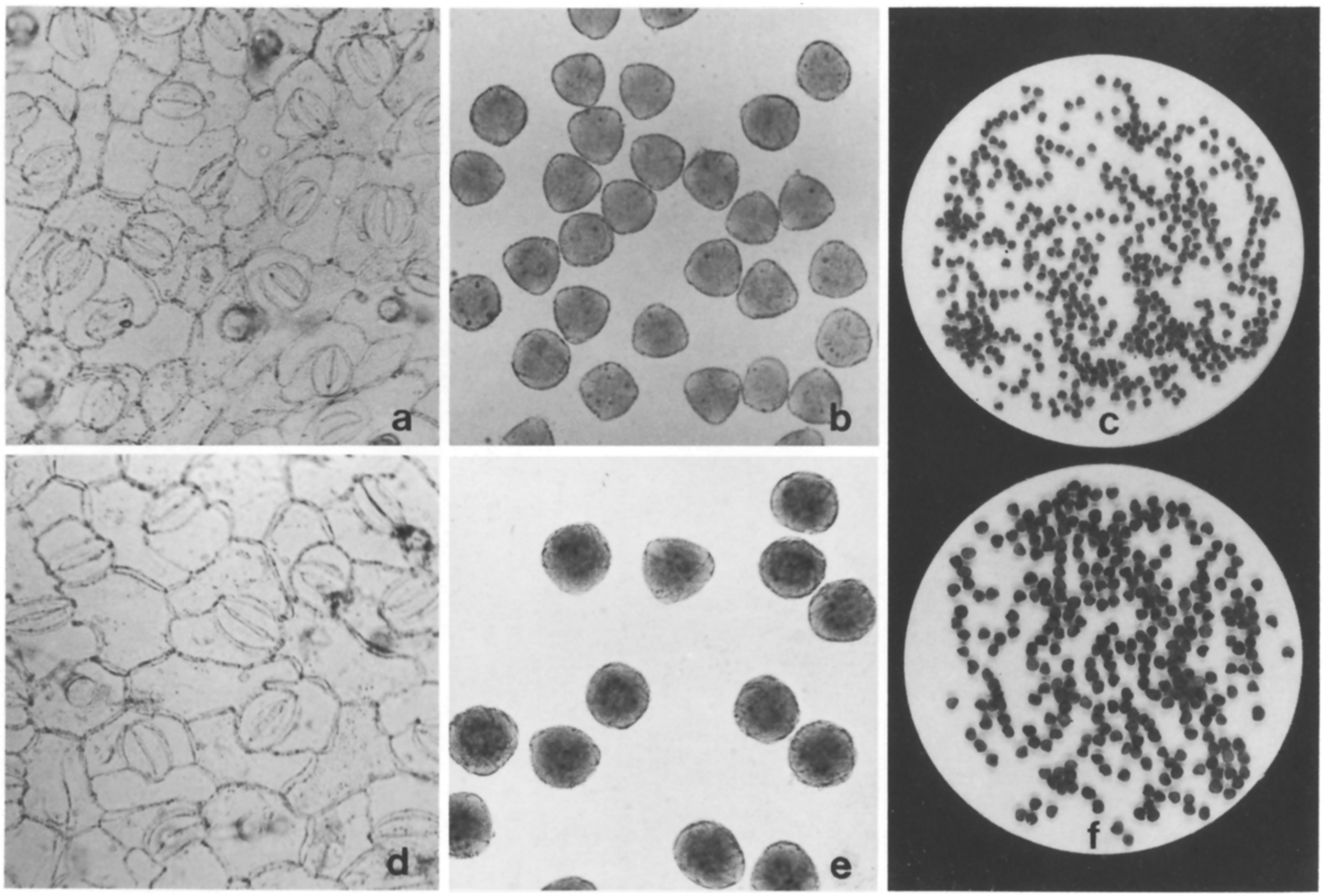

Fig. 2. Comparison of stomatal size, pollen grain and seed size. **a, b, c** in diploid and **d, e, f** in tetraploid

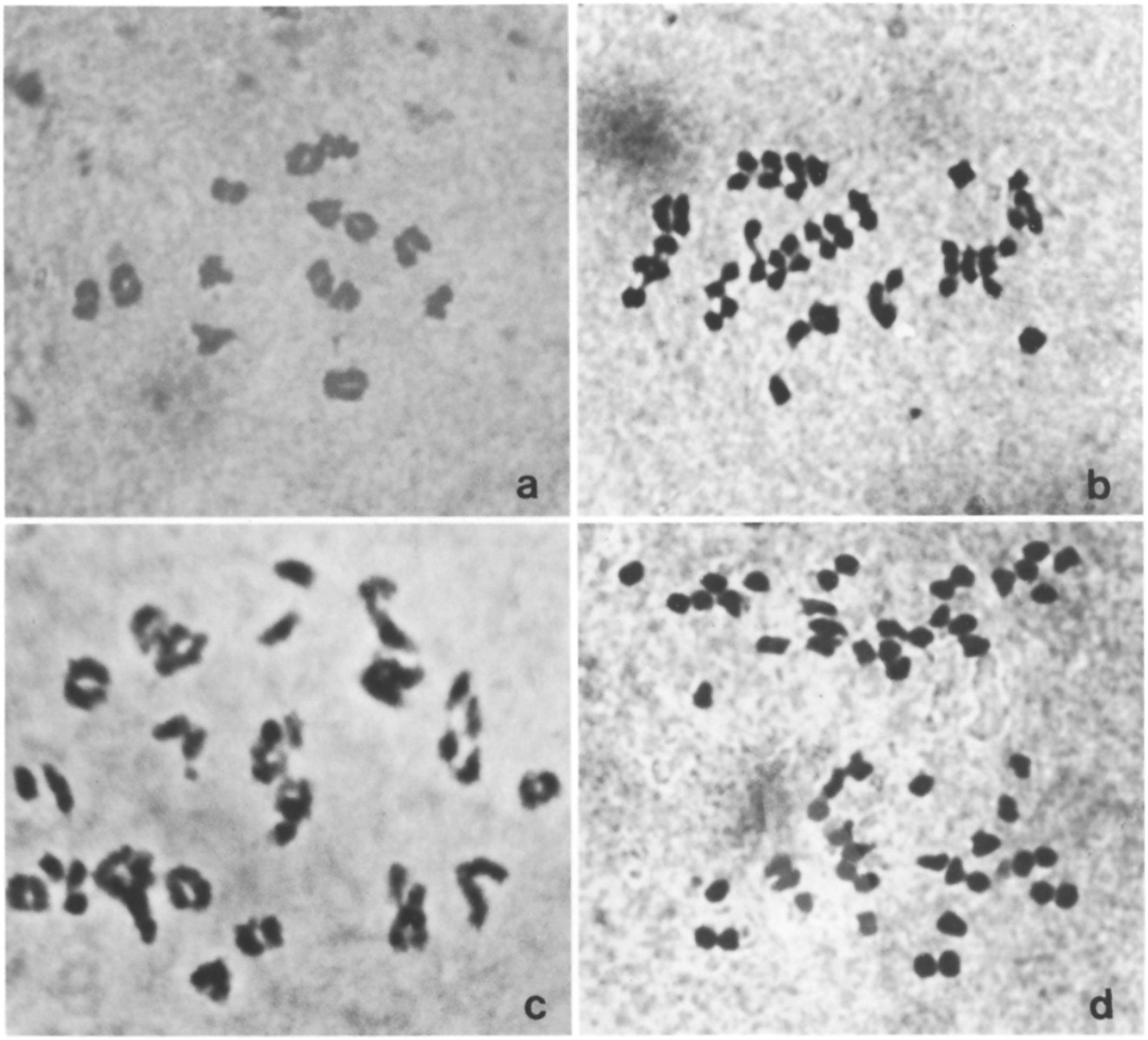

Fig. 3a–d. Meiotic chromosome associations in diploid and colchiploid in *H. muticus*. **a** diakinesis in diploid: note the formation of 14 ring II with distal chiasmata only. **b–d** Meiosis in colchiploid; **b** diploidisation of colchiploid: 25 rod II + 1 ring II + 4 I; **c** diplotene showing 3 ring IV + 1 chain IV + 2 III + 7 ring II + 7 rod II + 6 I; **d** anaphase I (28:28) separation

pollen fertility and balanced anaphase separation were isolated. Such genotypes also had good seed fertility (above 65% seed set). The detailed meiotic analysis of diploid parental control material revealed the occurrence of bivalents with distal chiasmata only, which on colchiploidisation result into predominance of rod bivalents and a substantial reduction in expected quardrivalent frequency. The chiasma initiation at telomeric sites and possibly an interdependence of chiasmata in a bivalent (as evident from predominance of rod bivalents in autotetraploids) reduces the chances of quadrivalent formation in tetraploids leading to cytogenetic diploidisation of colchiploids (Lavania 1985, 1986).

Keeping the above observations in mind a suitable strategy for polyploid breeding was envisaged. The starting material should have small chromosomes, preferably with a genetic system for distal chiasma initiation and interdependence of chiasma in a bivalent. An open pollinated material would be better suited since its heterozygous nature would have negative impact on quadrivalent formation. The raw tetraploids and their progeny should be selectively scored for segregation for fertility for 2–3 generations. This fertility could be further improved by intercrossing between the selected colchiploid genotypes followed by sharp selection for fertility and also for improvement in other agronomic characters. In fact, such an approach is underway in *Hyoscyamus muticus* to achieve further enhancement in seed fertility and also to secure heterotic advantage by intervarietal hybridization amongst the colchiploids.

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