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Chromosomal Instability in *Lathyrus sativus* L.

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Summary. In *Lathyrus sativus* (2n = 14), variety 'LSD-1' shows an instability of somatic chromosome number which can be observed in root tip and shoot tip mitoses. In this variety, approximately 54% of the seedlings showed intra-individual variation in chromosome number ranging from 2n = 14–3. This variability in chromosome number was recorded in approximately 60% of the dividing cells. Two seedlings were triploid with 21 chromosomes. Variation in chromosome number in somatic cells within individual plants is possibly controlled by genetic factors, which result in spindle abnormalities, chromosome degradation and minute chromosomes. The variation in chromosome number is probably responsible for the pollen polymorphism noted in this particular strain. The possible mechanism of intra-individual variability and the occurrence of the phenomenon vis-a-vis its applications are discussed.

Key words: *Lathyrus sativus* – Chromosomal instability – Chromosomal elimination – Somatic chromosomal variation

Introduction

Variability of chromosome-number and morphology in somatic plant and animal cells cultivated in vitro is a general fact, the primary cause of which remains unknown (Sacristan 1971; Sunderland 1977; Orton 1980). However, there are numerous reports on chromosomal chimerism in higher plants in vivo (Ogura 1976). A number of investigators have reported the phenomenon in somatic tissues. Sachs (1954) pointed out that not only can subdiploid variation be caused by environmentally controlled mitotic abnormalities but certain gene combinations may bring about this behaviour in somatic tissues. Later on, it was established in such important crop plants as wheat and tobacco that this chromosome chimerism and elimination in chromosome number may be genetically controlled in some biotypes (Watanabe 1962; Ogura 1976, 1978).

While working on pollen fertility and morphology, a wide range of pollen polymorphism for size and shape was noted in a variety of *Lathyrus sativus.* We decided to work out the genetical basis for pollen polymorphism in this variety. The original mother seed material and the selfed progeny was analysed for chromosome-behaviour and distribution. This cultivar exhibited some unusual chromosomal behaviour in somatic tissues, including chromosome variation up to the subdiploid level, the results of which are presented here. The possible use of this variety, which is otherwise a normal diploid, for future applied research is suggested.

Materials and Methods

Seeds of *Lathyrus sativus* var. 'LSD-1', obtained from IARI, New Delhi, were used in the present study. These seeds represented single plant selections for neurotoxin content for a number of generations from the variety 'Pusa 24' (Professor S. Ramanujam – personal communication). Root tips and shoot tips from young seedlings grown at 25±2 °C were collected, pretreated with saturated aqueous solution of para-dichlorobenzene for somatic chromosome analysis for 3½ h at 14 °C, fixed in Carnoy's fixative and squashed for somatic metaphase chromosome preparations after staining in 2% aceto-orcein N · HCl (9 : 1) mixture. In addition, observations were also recorded directly on fixed root tips (without pretreatment) to note the behaviour of chromosomes during mitotic cell division. Pollen grains were studied using the acetocarmine staining technique. Both root and shoot tip mitoses were analysed for variation in chromosome number. Observations for frequency and number of chromosomes were recorded only from well scattered metaphase plates with intact cell walls, where individual chromosomes could be easily counted.

Results

Somatic Cell Division

In this particular variety of *Lathyrus sativus,* some abnormalities in cell division were encountered, viz. lag-

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Table 1. Analysis of chromosome number from root tip cells (showing variation in somatic chromosome number)

S_1 no.	Frequency of cells in each chromosome number class (14 – 3)														
	14	13	12	11	10	9	8	7	6	5	4	3	Total		% cells abnormal
													Overall	Abnormal	
1)	23	15	10	4	4	1	–	1	–	1	–	–	59	36	61
2)	32	18	16	8	5	–	–	1	–	1	–	1	82	50	60.9
3)	25	15	12	5	5	–	–	1	–	–	1	–	64	39	60.9
4)	28	18	10	7	3	4	1	–	1	–	–	–	72	44	61.1
5)	24	11	12	9	3	1	2	–	–	–	–	–	62	38	61.29
6)	36	22	20	9	7	3	1	2	1	3	–	–	104	68	65.38
7)	30	19	15	7	5	1	–	2	–	–	–	–	79	49	62
8)	28	14	14	8	3	–	–	1	–	–	1	–	69	41	59.42
9)	32	20	12	6	7	4	3	3	–	–	2	–	89	57	64
10)	33	22	11	7	6	8	5	2	4	2	1	2	103	70	67.96
Total	291	174	132	70	48	22	12	13	6	7	5	3	783	492	62.81

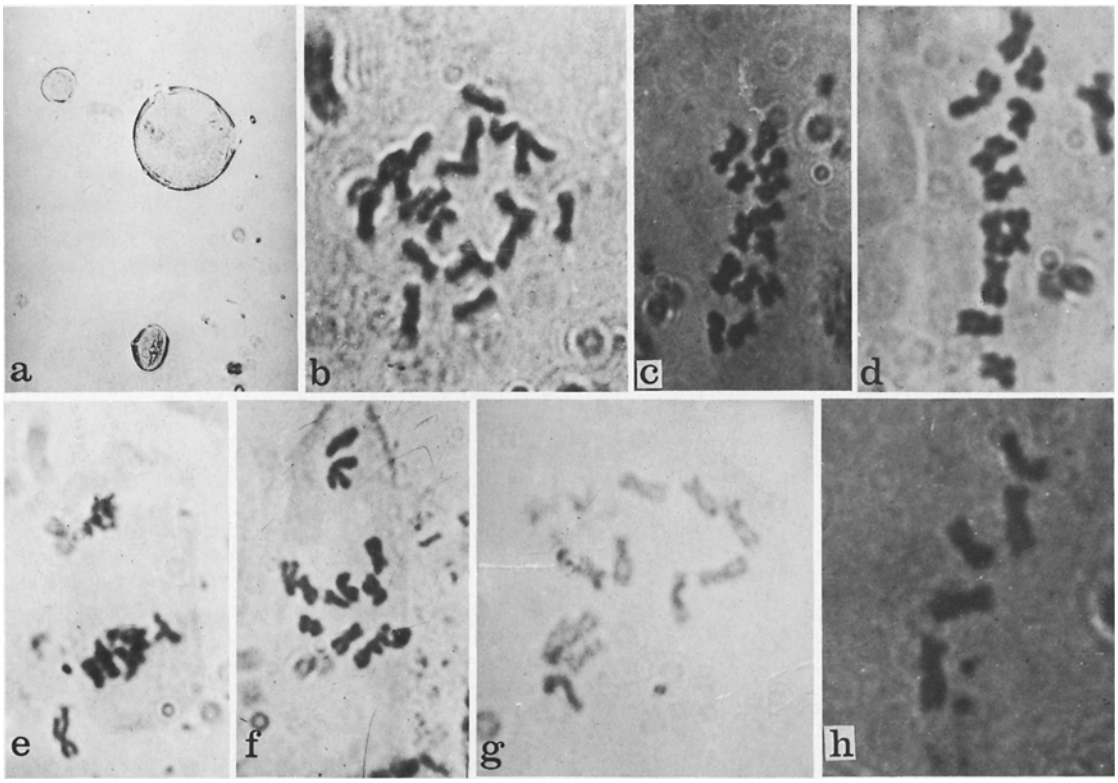

Fig. 1a–h. **a** Pollen grains (note the difference in size); **b** Triploid set – 21 chromosomes; **c** 13 chromosomes; **d** 11 chromosomes; **e, f** unequal separation and somatic reduction; **g** 11 somatic chromosomes +3 chromosomes showing chromosomal degradation; **h** 5 chromosomes +2 minute chromosomes in a cell

gards, unequal separation, tripolar anaphases and also somatic reduction. However, of these, unequal anaphase separation and the anaphase separation without chromatid separation were the most common, being observed in approximately 10–12% of the cells. In normal somatic mitosis (without any pretreatment for chromosome separation) up to 30–40% of the cells exhibited the countable separated chromosomes at metaphase, and to some extent at anaphase.

Variation in Somatic Chromosome Number

Eighty seedlings, selected at random, were scored for somatic chromosome numbers from root and shoot

tips, 43 of which showed variation in chromosome number (i.e., less than 14 chromosomes). The normal chromosome number in this species is 2n=14. The observations on variation in chromosome number from root tip metaphases of 10 seedlings exhibiting maximum variation are recorded in Table 1. The variation in frequency of cells with reduced chromosome number was noted to be slightly lower in shoot tips than in root tips of the same seedling.

Some cells having reduced chromosome numbers also showed signs of chromosomal degradation, terminal translocation resulting in end-to-end fusion and also the presence of minute dot-like chromosomes (probably B chromosomes). Such variations are shown in the Fig. 1. It is important to note that the mode of this chromosomal instability is also maintained with age, i.e. in secondary roots as well.

The plants in this variety of *Lathyrus sativus* produce pollen grains which exhibit polymorphism for shape and size (Fig. 1 a). However, all such pollen grains are well stained in acetocarmine. The seedlings obtained from selfing these abnormal plants also exhibit the pattern of chromosomal instability observed in mother plants. Two seedlings had the 3n chromosome number, i.e. 21 chromosomes (Fig. 1 b).

Discussion

Occurrence of Chromosomal Instability and its Possible Control

It becomes clear from the results that this unusual behaviour of chromosomes is genetically controlled since there is a tendency for this behaviour to be transmitted to the second selfed generation. The elimination of chromosomes is specific. The nucleolar chromosomes (which are easily distinguishable) are eliminated first followed by others. The majority of cells show the loss of one or two chromosomes and the frequency of cells which have lost a higher number of chromosomes is reduced. This may be accounted for by a tolerance limit of the cells depending upon the minimum chromosome requirement for cell survival. The slight variation observed for chromosomal variability in root and shoot tips of the same plant is possibly due to tissue specificity. This variation in chromosome number through microspore mother cells may bring about pollen polymorphism.

A situation comparable to the present findings on chromosomal instability has been recorded in other plants also: *Ribies nigram* (Vaarama 1949), apple (Hegwood and Hough 1958), *Pennisetum dubium* (Gildenhuys and Brix 1958), wheat (Watanabe 1962), *Claytonia virginica* (Lewis et al. 1971) *Orobanche gracilis* (Greilhuber and Weber 1975), Tobacco (Ogura 1976) and many others cited therein. However, most of these examples are either of the auto-or allopolyploid order. In all these cases the cause of chromosome instability was attributed to the disturbed spindle activity. The present situation in *Lathyrus sativus* is interesting since it deals with chromosome variation and elimination in a normal diploid.

In plants reproducing by sexual means, variation in chromosome number and gradual elimination of chromosomes is on record, particularly in interspecific hybrids. Interestingly a selective elimination of one genome with respect to the other was observed in such cases. The elimination of chromosomes was specific and gradual during the early development of the embryo of the inter-specific hybrids from various species of *Hordeum*. The balance between the genetic factors of the two parents was observed to regulate the stability or elimination of chromosomes (Kasha et al. 1970; Lange 1971; Subrahmanyam and Kasha 1973; Subrahmanyam 1977; Humphreys 1978). In such hybrids conclusive evidence was provided by Kasha et al. (1972) and Barclay et al. (1972) on the cause of chromosome elimination due to genetic factors present on the specific chromsomes. A similar situation may partly exist in the present case also where certain genes might by exercising their influence on the unusual behaviour of chromosomes controlling the various means for chromosomal variation. Further experimentation may be required to establish the genetic factors responsible for this behaviour by crossing with normally behaving genotypes in this species.

Mechanism of Chromosomal Instability

It is obvious from the results that these possible genetic factors influence the cell division, causing chromosome variation and elimination due to nondisjunction of chromosomes, disturbed polarity, disturbed spindle operation resulting in somatic reduction, unequal separation, chromating degradation and/or extrusion of the degraded material.

The possible mechanism underlying the observed chromosomal instability in *Lathyrus sativus* could be explained by comparing the phenomenon with other related examples. Bennett et al. (1976), from their studies on interspecific *Hordeum* hybrids, suggested that chromosome elimination may be caused by disturbed control of protein metabolism which in turn affects spindle organisation, possibly through genetic control. Treatments of *Hordeum vulgare* and *H. bulbosum* root tip cells with bacterial restriction endonucleases results in chromosome degradation (Subrahmanyam et al. 1976), which is similar to the occasional degradation of whole chromosomes in *H. vulgare* × *H. bulbosum* hybrid cells (Subrahmanyam and Kasha 1973) and also in the present case (Fig. 1 g). Thus, it is quite probable that selective endogenous nuclease activity may cause degradation of lagging chromosomes. Humphreys (1978) reported that although there is gradual elimination of chromosomes of one genome in *Hordeum* interspecific hybrids, some cells were also observed with less than the haploid number of chromosomes, showing thereby that the chromosomes of the stable genome in the hybrid are also lost. A comparable situation is also observed in the present variety of *Lathyrus sativus*, though in a low frequency of cells were a chromosome number less than the haploid number = 7 was observed.

Rhoades et al. (1967) reported elimination of certain segments with knobs in maize induced by supernumerary B chromosome. Subsequently Rhoades and Dempsey (1972) have shown genetic evidence of such losses and advanced the argument that faulty replication of heterochromatic segments in the presence of two or more B chromosomes leads to nondisjunction. A similar condition might operate in the present case as evidenced by the occasional presence of B chromosomes in hypoploid cells (Fig. 1 h), which might originate de novo or from the shedding of heterochromatic segments from the somatic chromosomes. The occurrence of triploid seeds (somatic chromosome number = 21, Fig. 1 b) is probably the result of the fertilization of unreduced 2n gamete with reduced n gametes.

Possible Use of Present Material and Further Suggestions

From the foregoing discussion it is apparent that the mechanism underlying the chromosome elimination appears to be a complex one. It is possible that genetic manifestation of certain genes may bring about this chromosome variation and elimination by operating through the various ways mentioned in the discussion. The mechanism of chromosome elimination could be elucidated from the behaviour of hybrid material produced upon crossing with normally behaving varieties in *L. sativus*. The application of Giemsa banding and N-banding techniques, which are helpful in chromosome identification, may provide an insight as to whether this gradual elimination is specific in relation to specific chromosomes. Also, this particular variety may be useful in the production of aneuploid series and in association of characteristics with specific chromosomes in this plant, which in turn may be utilized for gene or chromosome substitution.

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