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Mitotic crossing-over in a higher plant

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SUMMARY

This paper provides direct evidence for the occurrence of mitotic crossing-over in leaf tissues and *in vitro* cell cultures of *Nicotiana tabacum*. The genotypic composition of both segregating regions of twin spots was analysed by *in vitro* culture of the segregating tissue and by regenerating plants from this tissue. The frequency of mitotic crossing-over is influenced by several physical and chemical agents.

1. INTRODUCTION

Though there are reports of somatic crossing-over in higher plants (Vig & Paddock, 1970; Barrow, Chaudhari & Dunford, 1973), evidence for its occurrence is indirect and dependent upon recognition of phenotypically mosaic regions in genetically heterozygous tissue. Since mosaic patterns can be caused by a variety of genetic fluctuations in somatic cells of higher plants (e.g. Hirono & Rédei, 1965; Dulieu, de Boelpaepe & Déshayes, 1971; Jones, 1937, Greenblatt & Brink, 1962; Ross & Holm, 1960; Brink & Nilan, 1952; Imai & Kanna, 1935), twin spots may not represent homozygous sectors derived from a heterozygous cell. This work was undertaken to obtain more direct evidence for mitotic crossing-over in higher plants, and to initiate development of a genetic mapping system based on mitotic recombination formally analogous to that available in fungi (Pontecorvo & Kafer, 1958; Manny & Mortimer, 1964). *In vitro* cell culture was utilized to characterize each of the two segregants of a twin spot, and to regenerate plants from tissue of both components. Evidence is presented which demonstrates that mitotic crossing-over does occur in cells of a higher plant, and that the rate of mitotic crossing-over is influenced by physical and chemical agents.

2. MATERIALS AND METHODS

Plants and *in vitro* cell cultures of tobacco, *Nicotiana tabacum* cv. John Williams Broadleaf, heterozygous for two linked genetic markers located on chromosome *S* were used in this work. One marker, *Sulfur* (*Su*), is a dominant colour mutation which causes a yellow-green colour in heterozygous condition. Homozygous mutant tissue (*Su*/*Su*) is yellow in colour and inviable as a plant, while homozygous wild-type tissue is green (Burk & Menser, 1964). The second marker, *chimeral* (*cl*) is located 38 map units from *Sulfur* and is a recessive mutation causing regions of chlorosis in leaf tissue. The two markers were in repulsion so that the genetic composition of the tissue was *Su*, +/+, *cl*. Yellow and green twin spots occurring on a yellow-green background were isolated and analysed (see Fig. 1).

Media and techniques utilized to produce and maintain *in vitro* cell cultures, and to

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regenerate entire plants from *in vitro* cultures of *N. tabacum* have been previously described (Carlson, 1970). An effort was made to quickly regenerate plants from *in vitro* cultures to avoid chromosomal fluctuations arising in culture.

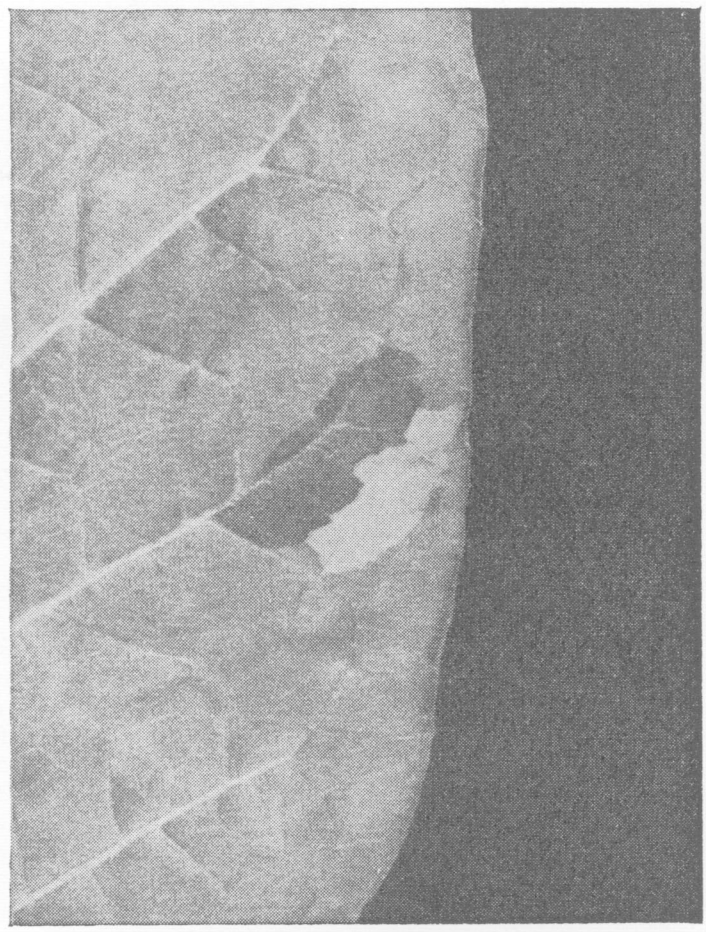

Fig. 1. A yellow and green twin spot induced by gamma irradiation on the leaf of a *Su*, +/+, *cl* plant.

Application of chemical and physical agents was used in attempts to alter the frequency of mitotic recombination. Conditions of treatment were: irradiation with gamma rays, 200 rads/day from a ^{60}Co source to a final dose of 800 rads, irradiation with UV light 10 ergs/mm^2/sec to a final dose of approximately 1000 ergs/mm^2; psoralen at a concentration of 1 μM for 1 h followed by irradiation with 360 nm light at approximately 10 ergs/mm^2/sec for $\frac{1}{2}$ h; mitomycin C at a concentration of 0·1 mM for 1 h; and caffeine at a concentration of 0·5 mm for 1 h. Application of chemicals was carried out in liquid culture medium and followed by two rinse periods in fresh medium.

Table 1. *The effect of physical and chemical agents on mitotic crossing-over (i.e. occurrence of twin spots) in* in vitro *cell cultures of tobacco*

(Treatment protocol and dosages are described in Materials and Methods.)

Agent	Dose	Fold increase over the spontaneous rate
1. Irradiation with gamma rays	800 rads	3·1
2. Irradiation with UV light	1000 ergs/mm^2	4·3
3. Psoralen	1 μM	2·7
4. Mitomycin C	0·1 mM	4·7
5. Caffeine	0·5 mM	1·9

3. RESULTS AND DISCUSSION

Burk & Menser (1964), in their original description of the *Sulfur* mutation, noted the presence of both types of single spots and of twin spots on the leaves of heterozygous yellow-green plants. This report confirmed their findings (see Fig. 1), and attempted to analyse the genotypes of each of the two components of a twin spot via *in vitro* culture, and where possible to regenerate the tissue into entire plants. Since the genotype of the experimental plants was *Su*, +/+, *cl* a mitotic crossing-over event could produce several different types of twin spots depending on the relative location of the two markers. If *cl* and *Su* are located on different chromosomal arms, then a twin spot would be expected to be *Su*, +/*Su*, *cl* (yellow), and +, *cl*/+, + (green). If *cl* is distal to *Su* on the same chromosomal arm, then a twin spot would be expected to be *Su*, +/*Su*, + (yellow), and +, *cl*/+, *cl* (green). If *cl* is proximal to *Su* on the same chromosomal arm, then a twin spot would be expected to be a combination of these two possibilities. Twelve twin spots were chosen for further analysis. Of these 12, 3 were of spontaneous origin in leaf tissue, 2 arose spontaneously in *in vitro* cultures, 2 were found after gamma ray treatment of *in vitro* cultures, 2 were recovered after UV treatment of *in vitro* cultures, 2 arose after colchicine treatment of *in vitro* cultures, and 1 appeared after treatment of *in vitro* cultures with mitomycin C. Tissue excised from each of the two components of each of the 12 twin spots was further cultured *in vitro*. Entire plants were regenerated from the green tissue of each twin spot, and its genetic composition was determined by chromosome counts and by using it in a cross with a homozygous *chimeral* (+, *cl*/+, *cl*) plant. The yellow tissue was forced to regenerate a shoot. Its genotype was determined by chromosome counts, and by assaying for the expression of the *chimeral* phenotype. In 11 cases the green tissue displayed a diploid chromosome number ($2n = 48$), and was heterozygous for the *chimeral* marker. The F_1 generation segregated in approximately a 1:1 ratio for chimeral and wild type in crosses of these plants to homozygous *chimeral* individuals. The yellow tissue also had a diploid chromosome number and did not exhibit a *chimeral* phenotype in regenerated shoots. These 11 cases are interpreted to be the result of a mitotic crossing-over event. The results are not consistent with any other reasonable mechanism. There was no evidence that the event was not reciprocal. These findings further indicate that *chimeral* is proximal to *Sulfur* and may possibly be on the opposite arm of chromosome *S*. One unusual case was found in a twin spot induced by colchicine. In this case the green tissue had a chromosome number of 49 and the yellow tissue had 45. Shoots regenerated from the yellow tissue did not exhibit a *chimeral* phenotype. Using plants derived from the green tissue as the pollen parent in a cross to homozygous *chimeral* females yield the following F_1 progeny: 32 plants displayed a homozygous *chimeral* phenotype, 14 plants displayed a heterozygous *Sulfur* phenotype, 8 plants were of heterozygous *Sulfur* and homozygous *chimeral* phenotypes, and 11 plants were wild-type in phenotype. This case

is interpreted to be the result of a mitotic non-disjunction event which produced a twin spot where the green tissue was trisomic for chromosome *S*. Further reciprocal crosses using the green regenerated plants as the female parent confirmed this conclusion.

An attempt was made to quantitate the approximate rate of mitotic crossing-over by assuming that each twin spot represents a recombinational event and that each mitotic recombination event has a 50 % change of producing a twin spot. Tentative calculations indicate that mitotic crossing-over occurs spontaneously between the *Sulfur* marker and the centromere at a frequency of $6{\cdot}7 \times 10^{-5}$ per mitosis in leaf tissue, and at a spontaneous frequency of $4{\cdot}6 \times 10^{-5}$ per mitosis in *in vitro* cultured cells. The findings presented in Table 1 demonstrate that a number of physical and chemical agents increase the rate of mitotic recombination (i.e. occurrence of twin spots), over its spontaneously occurring level in *in vitro* cell cultures. Many of these agents have been found to increase the level of twin spot production in soybean (Vig, 1973) and of mitotic crossing over in fungi (e.g. Holliday, 1961, 1964). It is clear that mitotic crossing-over occurs in higher plants, and the phenomenon displays several of the features associated with mitotic crossing-over in fungi.

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