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Chromosomal localisation of a recessive gene *tp* controlling the pleiotropic character topiary in *Solanum*

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Summary. Seven out of twelve possible types of primary trisomics of dihaploid *S. tuberosum* were crossed as females with a disomic recessive mutant for topiary (*tp tp*) identified in *S. infundibuliforme*. All primary trisomics used proved to be homozygous dominant. Trisomic plants from the seven F1's were crossed with a disomic heterozygous F1 plant (supposed genotype *Tp tp*). In the half sib progeny of each trisomic type the mutant plants could be easily identified by the presence of typical lateral shoots, particularly at the cotyledonary nodes. The observed segregation ratios for normal to mutant were tested against the expected non-critical ratio 3 : 1 and against various critical ratios. It is concluded that the gene *tp* is located on chromosome 3 of the potato.

Key words: *Solanum tuberosum* – Potato – Trisomics – Gene location – Topiary

Introduction

There has been increasing interest and activity in the field of somatic cell genetics and tissue culture of the economically important potato crop. Recently, significant progress has been made in somatic hybridization, including both tuber-bearing and non-tuber-bearing *Solanum* species (Gressel et al. 1984; Austin et al. 1985; Helgeson et al. 1986; Puite et al. 1986; De Vries et al. 1987). For a demonstration of the hybrid character of fusion products, specific markers for either parent are of significant value. However, the number of available markers, i.e. genes mapped on chromosomes of the potato, is restricted to only a few cases. Furthermore, genetic transformation of the potato with the aid of *Agrobacterium tumefaciens* (Ooms and Lenton 1985; Burrell et al. 1985) and *Agrobacterium rhizogenes* (Ooms et al. 1985, 1986) has been successful. Therefore, the construction of a genetic map of the potato is a necessity in regard to gene transfer in the near future.

A gene for albinism (*a*) was located on the long arm of chromosome 12 of *S. chacoense* by Lam and Erickson (1971), who used a di-isotrisomic clone of that species. Hermsen et al. (1973) associated gene *v* for chlorophyll deficiency with chromosome 12 of *S. tuberosum*. The latter chromosome 12 was numbered according to the pachytene identification of Yeh and Peloquin (1965) and is different from chromosome 12 of *S. chacoense* reported by Lam and Erickson (1971). These authors used their own numbering of pachytene chromosomes of *S. chacoense* (Lam and Erickson 1968).

Genes *Gl1* and *Gl2* control the glucosylation of rutin (Harborne 1962). *Gl1* is linked with the gene *Ac*, which is involved in the acylation of anthocyanins with p-coumaric acid (Harborne 1960). Lee and Rowe (1975) located either *Gl1* or *Gl2* on the long arm of chromosome 9. For gene localisation, studies in potato trisomic analysis appeared to be useful.

Wagenvoort and Ramanna (1979) established a nearly complete series of primary trisomics in diploid *S. tuberosum*. Eleven of the twelve possible types of primary trisomics are available and crosses between these trisomics and plants with some marker genes were made. In a previous paper, the location of a recessive gene *ym*, responsible for yellow margin, on chromosome 12 of diploid *S. tuberosum* was described (Wagenvoort 1982). In this paper the location of a recessive gene *tp* (topiary) is reported.

Materials and methods

Pedigrees of the *S. tuberosum* material (trisomics Triplo 2 through 11, except Triplo 10) used in this study have been described earlier (Wagenvoort and Ramanna 1979; Wagenvoort

and Lange 1980). Triplo 10, which has an interspecific hybrid origin, was obtained from Dr. R. E. Hanneman Jr., Wisconsin, USA. The mutant for topiary was identified in the wild diploid species *S. infundibuliforme* by Den Nijs et al. (1980). It is a pleiotropic character, which can easily be recognized in the seedling stage by profuse branching at the cotyledonary nodes. Seeds of this species were kindly supplied by Dr. Ton den Nijs and originally came from the laboratory of Prof. S. J. Peloquin, Madison, Wisconsin (USA). Crosses were made between the 11 primary trisomics and the disomic mutants for topiary. Trisomic F1 plants of crosses between trisomics and the mutant were tentatively selected morphologically and their possible trisomy was checked in root tip cells. These F1 trisomics (*Tp tp* or *Tp Tp tp*) were crossed with a heterozygous (*Tp tp*) disomic F1 plant from Triplo 10 as male parent. Seven progenies from these crosses were checked for the character involved and in both groups of normal and mutant plants samples were taken and used for counting the number of chromosomes.

Topiary seedlings were distinguished by the presence of excessive lateral branching, particularly at the cotyledonary nodes. Seedlings were judged weekly for this character over a period of several weeks, starting when the plants were four weeks old. The observed ratios for normal to mutant plants were tested for goodness of fit to the expected critical and non-critical ratios. In general, random chromosome association was assumed. For a reliable distinction between disomic and trisomic inheritance the size of the population was calculated with the formula:

$$n = \left[\frac{1+(\mu\lambda)^{1/2}}{\mu^{1/2}-\lambda^{1/2}}\right]^2 \cdot \chi_\alpha^2$$

where

n = the total number of plants
μ = the expected ratio dominant to recessive in the case of trisomic inheritance
λ = the expected ratio dominant to recessive in the case of disomic inheritance
χ_α^2 = Chi-square for $P = 0.05$

The method used to study the chromosomes in mitosis was the same as described by Wagenvoort and Lange (1975).

Results and discussion

The mutant for topiary found in the wild tuber bearing diploid species *S. infundibuliforme* develops lateral branches at nearly every node and shows a globular shape as it produces a dense growth of numerous slender stems. Stolons are absent or very short and the tubers are located in a tight cluster around the base of the stem. Figure 1 shows three seedlings: the one on the left and the one in the middle have mutant phenotypes with numerous stems originating from nearly every node, whereas the seedling on the right shows only one stem as found in normal plants. In addition to these characters, earlier tuberization in the field and the appearance of knobby tubers were described by Den Nijs et al. (1980). These authors suggested that the topiary character could be the result of an altered cytokinin activity. This study focused on the first character only, viz. the presence of lateral branches.

Although some older F1 plants from trisomics × mutant developed some lateral branches, they never showed the typical dense growth and globular shape of the mutant. Therefore it was concluded that the original trisomics were homozygous for the dominant allele *Tp*.

Table 1 shows the segregation ratios of normal versus mutant plants in seven half sib progenies of crosses between F1 trisomics and a male fertile F1 disomic heterozygous for topiary.

The observed ratios were tested against the non-critical ratio 3 : 1 and against the critical ratios 5.67 : 1 (if f = 0.10) and 9.91 : 1 (if f = 0.45), where f is the female transmission of the extra chromosome. The test against the non-critical ratio revealed that the ratios for Triplo 4, 6, 7, 9 and 10 fit the expected value. For Triplo 3 and 11 there was a significantly deviating ratio (Table 1). Both trisomics had an excess of normal plants, pointing to trisomic inheritance. However, a reliable distinction between disomic and trisomic inheritance can only be made if the population is sufficiently large. With f = 0.10 or f = 0.45, populations of at least 240 and 80 plants, respectively, are needed for a reliable distinction. (See "Materials and methods".)

Fig. 1. Three seedlings of the half sib progenies of crosses between F1 trisomics (*Tp tp* or *Tp Tp tp*) and a male fertile F1 disomic (supposed genotype *Tp tp*). Two mutants (*left* and *middle*) for topiary clearly show the numerous slender stems originating from the nodes in the leaf axes. The normal plant on the *right* has one stem only and already shows the development of long stolons which can be seen extending across the pot

Table 1. Segregation of normal (*Tp*) vs mutant (*tp tp*) plants in half sib progenies of crosses between F1 trisomics (*Tp tp* or *Tp Tp tp*) and a male fertile F1 disomic of Triplo 10 (supposed genotype *Tp tp*), as well as tests for goodness of fit to 3 : 1 (expected non-critical ratio), 5.67 : 1 (expected critical ratio if f = 0.10) and 9.91 : 1 (expected critical ratio if f = 0.45) where f is the female transmission of the extra chromosome

Triplo	Normal	Mutant	χ^2 3 : 1	χ^2 5.67 : 1	χ^2 9.91 : 1
3	408	31	75.70*	21.84*	2.34
4	220	55	3.79	5.62*	39.60*
6	31	4	3.73	0.23	0.36
7	41	15	0.09	7.14*	21.96*
9	274	83	0.53	18.35*	83.48*
10	257	65	3.74	7.07*	45.03*
11	50	7	4.63*	0.52	0.88

* Significant at a probability level of $P = 0.05$

Table 2. Results of cytological analysis of parts of the groups of normal and mutant plants of five progenies of crosses between F1 trisomics (*Tp tp* or *Tp Tp tp*) and a male fertile F1 disomic of Triplo 10 (supposed genotype *Tp tp*)

Triplo	Normal		Mutant	
	Disomic	Trisomic	Disomic	Trisomic
3	23	22	25	0
4	19	16	19	14
6	19	3	2	0
7	20	7	10	0
11	25	15	4	0

The population sizes of Triplo 3, 4, 9 and 10 fulfilled these criterions. In the progenies of Triplo 9 and 10 the segregations for the topiary gene were in accordance with disomic inheritance. For both a low female transmission (f = 0.10) and a high female transmission (f = 0.45), the observed ratios deviated significantly from the expected critical ratios (Table 1). For this reason no chromosome counts were made in these progenies. The observed ratios for Triplo 3 deviated significantly from 5.67 : 1 and fit the expected value 9.91 : 1. In Triplo 11 the observed ratios were in accordance with both expected ratios, although the population size was insufficient for a reliable test. Therefore, it was necessary to split up the populations into trisomics and disomics in order to test for normal versus mutant ratios within these two groups. In the case of trisomic inheritance and if random chromosome segregation is assumed, all mutants will be disomic and consequently all trisomics will show the normal phenotype. With random complete chromatid segregation, however, 1 out of 15 trisomics will be mutant (Hermsen 1970).

The results of chromosome counts are presented in Table 2. For Triplo 3, 6, 7 and 11 no trisomics were found among the mutants (Table 2). All trisomics under investigation had trisomic plants among the normal phenotypes. Fifteen out of forty normal plants were trisomic for Triplo 11, in addition to no trisomics among the mutants. For this trisomic type a female transmission of 34% was estimated in this study. In the case of disomic inheritance, this transmission rate would lead to at least one of the four mutants being trisomic. However, possibly because of the small number of mutants investigated for Triplo 11, not a single trisomic was found among the mutants. The same probably holds true for Triplo 6 and 7. Therefore, it seems unlikely that the topiary gene is located on one of these three chromosomes. However, in Triplo 3 among 45 normal plants 22 trisomics were found and no trisomics were found among 25 mutants (Table 2). In the last group nearly eight trisomics would be expected based on disomic inheritance and the actual female transmission of 31%. Hence, it was concluded that the gene *tp* for topiary is located on chromosome 3 of the potato.

The F1 trisomics from Triplo 3 used for the production of the half sib progenies were derived from the trisomic coded GNA77-61-6. Pachytene analysis of this trisomic clearly revealed the presence of a complete chromosome 3 as the extra chromosome. Figure 2 shows a trisomic configuration of chromosome 3 in GNA77-61-6. This chromosome has three distinct chromomeres in the achromatic part of the short arm. Meiotic studies in some F1 trisomics revealed the presence of the short arm of chromosome 3, indicating that no univalent shift had taken place during transmission of the chromosome at meiosis. In the progenies studied at mitosis there were no indications of the occurrence of telos. For this reason, it seems justified to conclude that the extra chromosome in the F1 trisomics of Triplo 3 is indeed a complete chromosome 3, which carries the recessive gene for topiary.

From this study it can be concluded once more that a series of primary trisomics in potato is a very suitable tool for the localisation of recessive genes. Dominant genes also can be assigned to chromosomes with the aid of a series of primary trisomics. Such studies, however, take much more labour and time, compared with the location of a recessive gene. For the location of a dominant gene the backcross populations have to be divided into trisomics and disomics to allow for a reliable distinction between disomic and trisomic inheritance. A second approach would be to properly estimate the female transmission in each backcross progeny and to test the observed ratios based on the actual f values. In both cases it would be necessary to count the chromosome numbers of large groups of plants.

In the case of location of genes encoding for various isoenzymes, such as peroxidase (POD), glutamate oxaloacetate transaminase (GOT) and malate dehydrogenase (MDH), the schemes may be simpler because of the

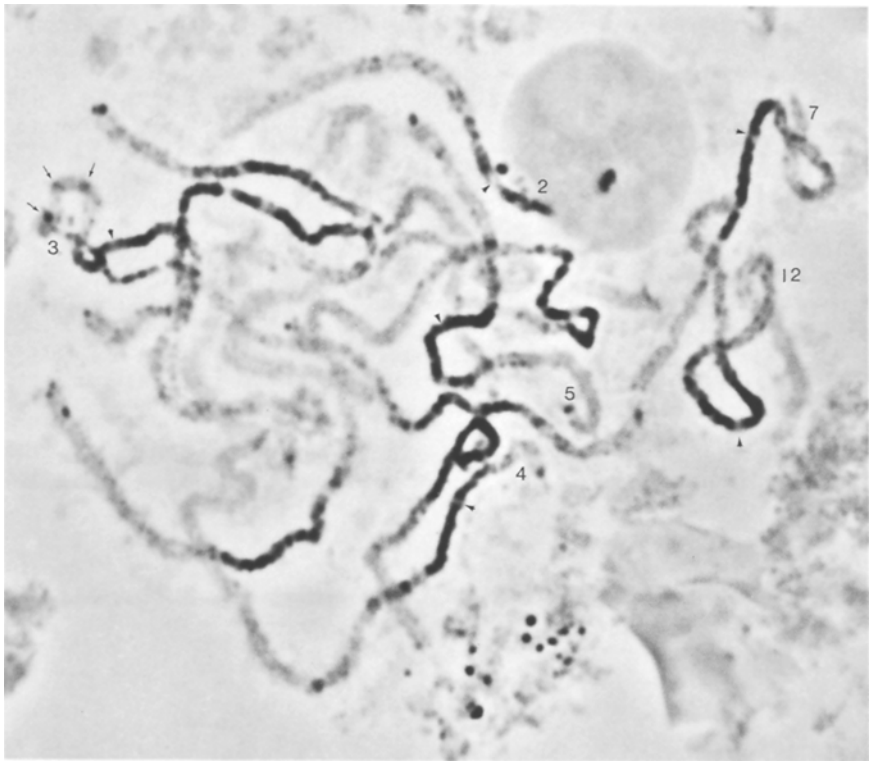

Fig. 2. A complete PMC of GNA 77-61-6 (Triplo 3) at mid-pachytene stage of meiosis. The PMC contains 1 trivalent and 11 bivalents, as expected for a trisomic with $2n = 24+1 = 25$. The trisomic configuration represents chromosome 3 and shows three chromomeres (*arrows*) on the achromatic part of the short arm. Some other bivalents, viz. chromosomes 2, 4, 5, 7 and 12, could also be identified with certainty. The centromeres are indicated by *arrow heads*

co-dominance of the alleles. This research is presently carried out at SVP. For many of these isozymes, however, the genetics still has to be elucidated. Therefore, the application of RFLP's (Restriction Fragment Length Polymorphisms) could become the most efficient method of gene localisation in potato. With respect to inheritance, the DNA markers have co-dominance in common with isozymes. A detailed linkage map of tomato and maize (Helentjaris et al. 1986) and lettuce (Landry et al. 1987) was constructed using many RFLP loci. Recently, the SVP started a programme using RFLP's in combination with our series of primary trisomics to construct a physical linkage map of the potato. This research is carried out in close cooperation with other research groups in the Netherlands and needs, because of the nearly complete lack of a gene map in potato, cooperation on an international level.

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