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Location of the Recessive Gene *ym* (Yellow Margin) on Chromosome 12 of Diploid *Solanum tuberosum* by Means of Trisomic Analysis

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Summary. Ten out of twelve primary trisomics of diploid *S. tuberosum* were crossed as females with a recessive mutant for yellow margin (*ym ym*) obtained from *S. phureja.* All primary trisomics used proved to be homozygous dominant. Trisomic plants from all ten F_1's were backcrossed with the mutant and trisomics from eight F_1's were crossed also with a disomic heterozygous F_1 plant from triplo 10 × mutant.

In both BC_1 and half sib progeny of each trisomic type the mutant plants were easily identified because of their typical small roundish leaflets with yellow or reddish margins. The observed segregation ratios for normal to mutant were tested against the expected non-critical ratios and against various expected critical ratios.

From the results of these tests it is concluded that the gene *ym* is located on chromosome 12 of the potato. A hypothesis of linkage between *ym* and a gene l_x for lethality is put forward. It is concluded that l_x is not identical with a previously detected recessive gene l_2 which is responsible for yellow cotyledons and lethality.

Key words: *Solanum tuberosum* – Trisomics – Gene location – Yellow margin – Lethality

Introduction

In the genus *Solanum* only a few cases of gene location have been reported. A gene *a* for albinism was located on the long arm of chromosome 12 of *S. chacoense* by Lam and Erickson (1971), who used a di-isotrisomic of that species. Hermsen et al. (1973) associated gene *v* for chlorophyl deficiency with chromosome 12 of *S. tuberosum.* The latter chromosome 12 was numbered according to the identification of Yeh and Peloquin (1965) and is different from the 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). Lee and Rowe (1975) reported the association of the genes *P* and *Ac* with unknown iso-chromosomes of *S. chacoense.*

The *P* locus controls the production of delphinidin in both flowers and tubers. *Ac* is concerned with the acylation of anthocyanins with p-coumaric acid (Harborne 1960). Lee and Rowe also located one of the two genes Gl_1 and Gl_2 on the long arm of chromosome 9.

The genes Gl_1 and Gl_2 were found to control the glucosylation of rutin (Harborne 1962). Gl_1 is linked with *Ac.* The gene *df* (deformed flower), which in sensitive cytoplasm (df^s) is expressed as the character "short anther" (Grun et al. 1962; Grun 1970), was associated with trisomic V 1682.3 by Lee and Ruhde (1976). The extra chromosome of this trisomic was not identified. As soon as 11 of the 12 possible types of primary trisomics were available (cf. Wagenvoort and Ramanna 1979) crosses between these trisomics and plants which carried several marker genes were made.

In this paper the location of a recessive gene *ym* (yellow margin) is reported and the possible linkage with a gene l_x for lethality is discussed.

Materials and Methods

Pedigrees of all *S. tuberosum* material (trisomics, dihaploids and inbred clones) used in this study have been described earlier (Wagenvoort and Ramanna 1979; Wagenvoort and Lange 1980). Triplo 10, which had an interspecific hybrid origin, was obtained from Dr. R. E. Hanneman Jr., Wisconsin, USA. The mutant for yellow margin was selected from the diploid species *S. phureja.* Seeds of this species were kindly supplied by Dr. B. Maris (SVP, Wageningen). A crossing scheme for the production of the BC_1's of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent, and for the production of the half sibs of crosses between F_1 trisomics and a heterozygous (*Ym ym*) F_1 plant from triplo 10, is presented in Figure 1. Trisomic F_1 plants of crosses between trisomics and the mutant were tentatively selected morphologically and their possible trisomy was checked in root tip cells.

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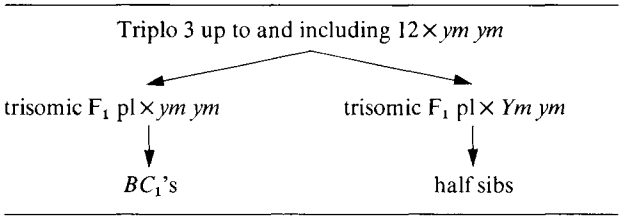

Fig. 1. Crossing scheme for the production of BC_1's and half sibs of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent and a heterozygous (*Ym ym*) F_1 plant from triplo 10 respectively

Both the BC_1 and half sib progenies were assessed for the proportion of mutant plants and the observed ratios were tested for goodness of fit to the expected critical and non-critical ratios. In general random chromosome association was assumed.

The methods for studying the chromosomes in mitosis and meiosis were the same as described by Wagenvoort and Lange (1975) and Wagenvoort and Ramanna (1979). Male fertility was estimated by staining the pollen with lactophenol – acid fuchsin (Sass 1964).

Results and Discussion

The feature of yellow margin is generally characterized by small roundish leaflets with yellow or reddish margins. In some populations, however, the mutants showed variation with respect to the size of the leaflets. Figure 2 shows three leaves: two are of the mutant phenotype, but only the leaf at the right shows the typical small roundish leaflets in combination with the yellow leaf margin. Originally the mutant was found in two families of crosses between normal plants of *S. phureja,* whose families segregated 65:28 and 78:21 for normal

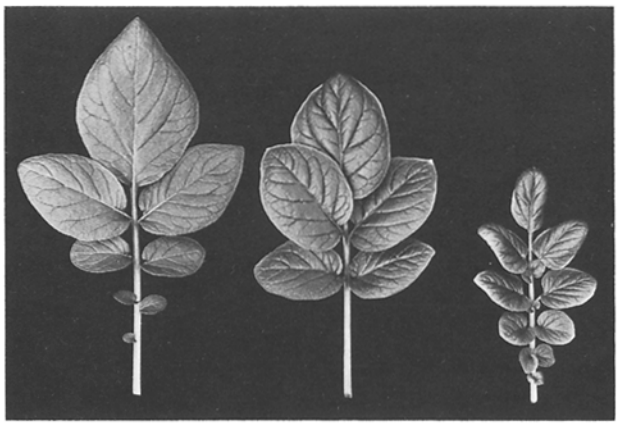

Fig. 2. Three leaves of BC_1 plants: normal phenotype for the yellow margin character (left), mutant type (middle) and mutant type showing the yellow leaf margin in combination with the typical small roundish leaflets

Table 1. Segregation of normal vs. mutant (*ym ym*) plants in ten BC_1 progenies of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent (*ym ym*), as well as tests for goodness of fit to 1 : 1 (expected non-critical ratio), to 2 : 1 (expected critical ratio, if f = 0.0) and to 3 : 1 (expected critical ratio if f = 0.25), where f is the female transmission of the extra chromosome

Triplo	Normal	Mutant	$X^2_{1:1}$	$X^2_{2:1}$	$X^2_{3:1}$
3	39	36	0.12	7.26*	21.16*
4	24	32	1.14	14.29*	30.86*
5	46	44	0.04	9.80*	27.39*
6	9	8	0.06	1.44	4.41*
7	72	75	0.06	20.69*	53.08*
8	35	28	0.78	3.50	12.70*
9	47	46	0.01	10.89*	29.68*
10	24	35	2.05	17.93*	37.07*
11	4	13	4.76*	14.24*	24.02*
12	22	20	0.09	3.86*	11.46*

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

Table 2. Segregation of normal vs. mutant (*ym ym*) plants in nine half sib progenies of crosses between eight F_1 trisomics (*Ym ym* or *Ym Ym ym*) and a male fertile F_1 trisomic of triplo 10 (supposed genotype *Ym ym*), as well as tests for goodness of fit to 3 : 1 (expected non-critical ratio), to 5 : 1 (expected critical ratio, if f = 0.0) and to 7 : 1 (expected critical ratio if f = 0.25), where f is the female transmission of the extra chromosome

Triplo	Normal	Mutant	$X^2_{3:1}$	$X^2_{5:1}$	$X^2_{7:1}$
3	107	28	1.31	1.61	8.38*
4	6	7	5.77*	12.94*	20.32*
5	21	8	0.10	2.49	6.03*
6	12	15	13.44*	29.40*	45.76*
7	200	64	0.08	10.91*	33.28*
9	13	8	1.92	6.94*	12.58*
11	8	4	0.44	2.40	4.76*
12	31	9	0.13	0.98	3.66
12	115	15	12.56*	2.67	0.07

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

to mutant. The observed ratios fitted the expected ratio 3 : 1, indicating that for both families the parental plants were heterozygous for the *ym* locus. A homozygous recessive plant was selected from one of these populations. This plant had a pollen stainability of 80–90%. Meiosis appeared to be regular and no 2n gametes were observed in second metaphase or anaphase.

F_1 plants from trisomics × mutant never showed the mutant character, indicating that the original trisomics are homozygous for the dominant allele *Ym*. The segregation ratios of normal vs. mutant plants in the BC_1 generation are summarized in Table 1. The observed ratios were tested against the non-critical ratio 1:1 and against the critical ratios 2:1 (if f = 0) and 3:1 (if f = 0.25), where

Table 3. Results of cytological analysis of parts of the groups of normal and mutant plants of both BC_1 and half sib progenies of crosses between F_1 trisomics (*Ym ym* or *Ym Ym ym*) and the mutant parent (*ym ym*) or a male fertile F_1 trisomic of triplo 10 (supposed genotype *Ym ym*), respectively

Triplo	BC_1				Half sib			
	Normal		Mutant		Normal		Mutant	
	Disomic	Trisomic	Disomic	Trisomic	Disomic	Trisomic	Disomic	Trisomic
3	2	3	8	3				
4			5	0				
5	3	1	10	8				
6	3	0	3	2				
7	1	0	0	3	129	51	44	17
8	1	2	15	4				
9	29	17	34	10	12	1	4	1
10			5	2				
11	1	2	8	2			1	1
12	16	6	19	0	(29)[a]	2	9	0
12					81	31	10	0

[a] This number is based on morphological selection only

f is the female transmission of the extra chromosome. For many trisomics a female transmission of 25% is a good estimate (cf. Wagenvoort and Lange 1980). The first test revealed that the observed ratios fitted the non-critical ratio 1:1 except in triplo 11 × *ym ym* (Table 1). But in this case the deviation from 1:1 was an excess of mutants, which does not point to trisomic inheritance at all. The second test is the most severe because with f=0 the difference between critical and noncritical ratio is the smallest. Populations of at least 131 plants are needed for a reliable distinction between 1:1 and 2:1. This number was reached only for triplo 7. It is expected that any significant deviation in this test will also be significant in tests to ratios that are based on higher f-values. The observed ratios in the F_1's involving triplo 6 and triplo 8 fitted both 1:1 and 2:1 but deviated significantly from 3:1, whereas all other F_1's fitted neither 2:1 nor 3:1. So with the assumption of f=25% none of the observed ratios were critical. Table 2 presents the ratios observed in the half sib progenies and the chi-squares calculated on the basis of 3:1, 5:1 and 7:1. The ratios observed in the progenies involving triplo 4 and 6, as well as that in one population from triplo 12, deviated significantly from the expected non-critical ratio 3:1. For triplo 4 and triplo 6, however, the deviation observed was an excess of mutants, as was the case in the BC_1 from triplo 11 (Table 1). These deviations cannot have been brought about by trisomic inheritance, for in that case a large surplus of normal plants would be expected. Therefore only the significance for triplo 12 may point to trisomic inheritance. This was corroborated by the results of testing against the two critical ratios. Only the two populations from triplo 12 showed non-significance both with f=0 and f=0.25. This result led to the tentative conclusion that the gene *ym* might be located on chromosome 12.

Additional evidence for this conclusion was obtained by counting the number of chromosomes of some normal as well as mutant plants from both the BC_1 and half sib progenies. The results of this analysis are presented in Table 3. The group of mutants will reveal the most relevant information: if the critical trisomic is involved and random chromosome assortment is assumed, all plants of this group will be disomic and consequently all trisomics in BC_1 as well as half sib progeny will show the normal phenotype; if, however, random complete chromatid assortment is assumed, one out of 15 trisomics will be a mutant (Hermsen 1970). In the case of disomic inheritance both groups of normal and mutant plants will show about equal proportions of trisomics, the size being dependent on the f-value. Table 3 shows that triplo 12, and perhaps triplo 4, fulfil the criteria of trisomic inheritance: only these trisomics revealed a complete absence of trisomics in the groups of mutants.

Although only five mutants were investigated cytologically from triplo 4 and these five appeared to be disomic, it seems rather unlikely that this trisomic is critical for the *ym* locus because it was already rejected on the basis of segregation ratios in BC_1 and half sib progeny. Therefore it can be concluded that the gene *ym* is located on chromosome 12 of the potato. This chromosome is equal to the one on which Hermsen et al. (1973) located the gene *v* for virescens. The results in the BC_1 of triplo 12 need further discussion because two observed ratios were not in agreement with the

above mentioned conclusion. First, the ratio 22:20 for normal to mutant deviated significantly ($X^2 = 7.46$) from the expected critical ratio 2.5:1, calculated on the basis of the observed value of f=0.15. Second, the observed ratio 16 normal to 19 mutant in the group of disomics deviated significantly from the expected critical ratio 2:1 ($X^2 = 6.21$). To explain this phenomenon it can be hypothesized that in some populations the ratios were disturbed because of the activity of lethality genes. Dodds and Paxman (1962) suggested that the gene *ym* is linked to a recessive lethal in the repulsion phase.

In the progeny of a cross between two normal plants of *S. phureja* these authors found a segregation ratio for yellow margin which deviated significantly from the expected ratio 3:1. Hermsen et al. (1978) described three lethal genes viz. l_1 (seeds non-emerging from the soil), l_2 (yellow cotyledons) and l_3 (tiny dwarf) in a dihaploid plant (G 254) of cultivar 'Gineke'. These genes affect the germination rate of the seeds and may be present and segregating in the trisomics used in this study, as G 254 was the male parent in the original 3X×2X crosses, except for triplo 10, which has another origin.

Indeed, in seven BC_1 populations, as well as six half sib progenies, mutants for l_2 occurred and were readily observable by their yellow cotyledons, segregation for l_3 was not observed in any of the populations, and the occurrence of l_1 in the same populations was indistinct. The three populations of triplo 12 did not segregate for l_2, but nevertheless in BC_1 only 42 plants out of 126 germinated seeds could be reliably assessed for the yellow margin character. This loss of seedlings was not due to the action of l_2. Consequently it may be hypothesized that an unknown recessive gene for lethality (l_x) is involved that is linked with *ym*. In the original population of *S. phureja*, from which the homozygous recessive *ym ym* clone was selected, no seedlings died. Therefore it can be presumed, that one of the parents of this cross was heterozygous for l_x, the cross being $\frac{Lx\ Ym}{Lx\ ym} \times \frac{Lx\ Ym}{lx\ ym}$. The genotype of the homozygous yellow margin clone, which was used to incorporate the gene *ym* into the original trisomics as well as to produce the BC_1 could have been $\frac{Lx\ ym}{lx\ ym}$. If it is further assumed that the original trisomic of triplo 12 was duplex for l_x the genotype of the trisomic of the F_1 progeny of triplo 12 could have been $\frac{Lx\ Ym}{\frac{lx\ Ym}{Lx\ ym}}$. The cross for the production of the BC_1 then can be reproduced as $\frac{Lx\ Ym}{\frac{lx\ Ym}{Lx\ ym}} \times \frac{Lx\ ym}{lx\ ym}$. This situation will lead to segregation of l_x in the BC_1 and consequently will disturb the segregation ratio of *ym*. The ratio in the group of trisomics however will not be influenced and thus remains 1:0 for normal to mutant because the F_1 trisomic is assumed to be duplex for l_x, which means that all the gametes with an extra chromosome contain at least one dominant allele.

With this hypothesis the deviating ratios in the BC_1 of triplo 12 and the group of mutants can be explained satisfactorily. Since the size of the groups of plants was limited and the stage at which the seedlings died was not clearly established, the possible relationship between *ym* and l_x should be studied more extensively.

Conclusions

(i) The recessive gene *ym* is located on chromosome 12 of the potato and presumably linked to a recessive lethal gene l_x.
(ii) The gene l_x is not identical with l_2.

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