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Isozyme analysis of primary trisomics in beet (*Beta vulgaris* L.). Genetical characterization and techniques for chromosomal assignment of two enzyme-coding loci: leucine aminopeptidase and glutamate oxaloacetate transaminase

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Abstract. Segregating families of beet (*Beta vulgaris*) were used to verify the monofactorial inheritance of two enzyme-coding loci, leucine aminopeptidase (*Lap1*) and glutamate oxaloacetate transaminase (*Got3*). With a series of primary trisomics and using three methods to discriminate between the critical trisomic (the locus is situated on the triplicated chromosome) and the non-critical ones, it was possible to allocate the two loci to beet chromosomes I and II, respectively. For the locus *Lap1* distorted segregation ratios were estimated, and the incorporation of three alleles into one plant was attempted. In the case of *Got3* the measurement of the allele dosage effect after electrophoresis was chosen as the major strategy. The output of laser densitometric scans were subjected to the non-parametrical Wilcoxon–Mann–Whitney test.

Key words: Primary trisomics – Beet – *Beta vulgaris* – Isozyme polymorphism – Chromosomal assignment – Distorted segregation – Dosage shift

Introduction

The association of linkage groups and genes with their respective chromosomes is an elementary step in developing a genetic map of a specific species. Plants with aberrant chromosome numbers, such as primary trisomics, telotrisomics, monosomics, nullisomics and alien chromosome additions, as well as plants with structural chromosome rearrangements, such as translocations, have already demonstrated their usefulness in this respect in a wide variety of species.

In the case of trisomics, various methods can be applied. (1) The presence of an additional chromosome modifies the expected Mendelian ratio for genes on that chromosome (Hermsen 1970). By monitoring the segregation ratios in progenies from crosses with trisomic individuals it is possible to deduce the chromosomal location of the locus in question. (2) Additional chromosomes also can change the dosage of gene products. In higher plants dosage mapping has been carried out for isozyme loci and DNA probes mostly by means of heterozygous trisomics. This method involves only one plant generation and requires fewer plants to be assayed than the first-mentioned classical scheme. (3) The marking of chromosomes with allozyme variants by the introduction of three allelic forms into a single trisomic plants. This is probably the most elegant method, and only one fully heterozygous plant is required to assign a marker to the appropriate chromosome.

Up to now isozyme markers have been used the most. Their universal character (easy availability combined with an independence of specific probes) and simple applicability, have been the principle reasons for this choice. An overview of the applications of isozymes in identifying chromosomes or chromosome parts is provided by Birchler (1983) and Tanksley (1983). Trisomics have been used in rice (Ranjhan et al. 1988; Wu et al. 1988), in soybean (Hedges and Palmer 1991), in maize (Nielsen and Scandalious 1974), in petunia (Wijsman and Van den Berg 1982) and in many other species. Most often the distorted segregations

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served as a primary source of evidence, but triallelic heterozygotes were also generated and dosage effects were observed. Isozymes have also been used to identify the chromosomes of the wild species of the *Procumbentes* section in monosomic additions of *B. vulgaris* carrying such chromosomes (Jung et al. 1986; Van Geyt et al. 1988). Restriction fragment length polymorphism (RFLP) markers, which have a large potential value in genetic mapping, have been demonstrated to be powerful tools in dosage mapping using trisomic plants (Young et al. 1987; Ellis and Cleary 1988). Also, in situ hybridization of low-copy DNA sequences or RFLPs is a technique that is available for similar purposes (Gustafson et al. 1990; Mouras et al. 1987; Huang et al. 1988).

In beet a lot of effort has been expended to obtain trisomic series (Levan 1942; Butterfass 1964; Kaltsikes and Evans 1967; Shimura 1972; Bormotov et al. 1973; Romagosa et al. 1986), but only two complete trisomic series have been established. Butterfass (1964) identified a trisomic series on the appearance and morphology of the trisomic plants. Another trisomic series was developed by Romagosa et al. (1986) from triploids obtained by crossing colchicine-induced autotetraploids (4x) with their original diploids (2x). The identification of the triplicated chromosome was accomplished by karyotype analysis in somatic cells (Romagosa et al. 1987).

The aim of the study reported here was the identification of the chromosomal location of two isozyme loci in beet: *Lap1* (leucine aminopeptidase) and *Got3* (glutamate oxaloacetate transaminase) using the trisomic series of Butterfass (1964). To this end a comparison was made between three approaches: estimation of the distorted segregation, evaluation of a dosage shift and the incorporation of three allelic forms into one plant.

Materials and methods

Plant materials and crosses

As starting material the complete trisomic series of beet made by Butterfass (1964) was used, and the same numbering strategy was applied. As this series proved to be highly homozygous for the isozyme loci involved, especially for *Got3*, two crossing cycles were often required: one cycle to introduce genetic heterozygosity and the second to obtain segregating progenies. Each trisomic plant of the original material was crossed with an appropriate pollinator. From the offspring trisomics and disomics were selected for isozyme analysis and, if needed, for the second cross. The pollinators, Ba (type barres, fodder beet), WB40 and WB42 (subsp. *maritima*), KGIII (type red garden beet, acc. III Greece), Bar (subsp. *maritima*, red) and BVM (diploid mixture), were chosen from beet material with a broad genetical variation.

Beet normally is a biennial species; the plant grows vegetatively in the first year and flowers in the second year after vernalization (cold treatment). Beet also is an outbreeder; self-fertilization is normally prevented through self-incompatibility. Therefore, the crosses were carried out as is practised by beet breeders. Two selected parental plants were vernalized and placed together in isolation during flowering and seed development. Isolation was achieved in specially designed cages or cabins, or was obtained through spatial isolation by growing the plants in clearings (4 m × 4 m) in a field of hemp.

Two sources of possible errors had to be taken into account: (1) self-incompatibility might be incomplete so that self-fertilization might occur, and (2) the spatial isolation might be insufficient so that air-borne pollen might participate in pollination, leading to unwanted cross-fertilization. Much care was taken to match the flowering periods of both plants of a pair as much as possible in order to ensure that the abundancy of the pollen of the pollinator could function as a shield against unwanted pollinations.

Selection of trisomics

As a result of the mode of transmission of the extra chromosomes through gametophytes and gametes, the progeny of a trisomic beet plant will contain 20–30% trisomic individuals among disomic sibs (Butterfass 1964). Trisomic and disomic plants were selected from among the offspring of the original trisomics and the crosses by counting the number of chromosomes. In most cases it was possible to make a preselection on the basis of plant morphology.

Chromosome numbers were determined in preparations of squashed leaflets or root tips of young seedlings. To make the preparations, the plant material was pretreated in aqueous 8-hydroxychinoline (0.002 *M*, 5 h), fixed in acetic ethanol (1:3), macerated in 1 *N* HCl at 60 °C and squashed in 45% aqueous acetic acid. The preparations were stained by carefully lifting the coverslip and adding a drop of 1% aqueous crystal violet and then studied by normal light microscopy.

Isoenzyme assays

Leaves from fresh plant material were used as the sample source. Extraction was performed principally as described by Van Geyt and Smed (1984) using a modified concentration of 1% β-mercaptoethanol and 10% glycerol Polyacrylamide gel electrophoresis (PAGE) was performed as described in the same article; the PAGE1 system was applied. In order to increase the resolution, the polyacrylamide concentration was reduced to 6%. The staining technique for LAP (leucine aminopepetidase) was as described in Van Geyt et al. (1988); the staining procedure for GOT (glutamate oxaloacetate transaminase) was carried out according to Vallejos (1983). (Gene symbols are written in small letters, e.g. *Lap1*, and gene products in capitals, e.g. LAP). Gels were fixed in 10% acetic acid. The addition of glycerol (10%) to the fixation medium allowed to obtain intact gels after drying.

For LAP the gels were scored visually and for GOT the gels were scanned using the LKB Ultroscan XL laser densitometer. In order to have a positive control the trisomic and disomic progenies of each cross were compared and run on the same gel. To compare the numerical values obtained with the densitometer, the peaks corresponding to the homomeric enzymes, the fastest and slowest peaks, were normalized towards the middle peak, which corresponds with the heteromeric enzymes. The ratio between the peak heights corresponding with the homomeric enzymes was used as value in the Wilcoxon–Mann–Whitney statistical test.

Statistical method: the Wilcoxon–Mann–Whitney test (from Sprent 1989)

This test is a non-parametrical test for which no normal distribution of the data must be assumed and the sample sizes can be very

small (from 5 up to 20). The principle of the test is to make a joint ranking of observations from the two tested samples and sum the ranks associated with one sample. If both samples originate from the same "population" a fair mix of observations in each sample is expected (null hypothesis). If the alternative to a null hypothesis is that the samples come from populations with distributions differing only in location, lower ranks dominating in one population and higher ranks in the other might reasonably be expected. For the calculations the following formulas were used:

$$Um = Sm - 1/2m\,(m + 1)$$

$$Un = Sn - 1/2n\,(n + 1)$$

and

$$Um + Un = mn$$

with Um and Un = U-value for m or n, m = number of observations in disomic group, n = number of observations in trisomic group and Sm and Sn = sum of ranks for m or n.

In tables in Sprent (1989, p 236 A6) the maximum of the lower Um and Un are given that apply to significance at a nominal 5% level in a two-tailed test. Statistical analysis of the data was performed with Statview 2.0 on a Macintosh PC.

Results

Enzyme genetics

Leucine aminopeptidase

The activity of this enzyme can be measured on fresh leaf material as well as on freeze-dried and frozen material (−70 °C). No activity was seen at the seed level, and the activity measured in the cotyledons was rather poor.

The electrophoresis pattern for LAP reveals three zones of activity (Van Geyt et al. 1988). The main zone, LAP1, consists of one or two bands. Three alleles were observed in the plant material investigated. These were characterized by a F (fast), a S (slow) or a M band (moving in between F and S) (Fig. 1). Crosses of individuals with distinct bands expressed both bands in their progeny. Backcrosses segregated in an equal ratio of single versus double-banded patterns. F_2 segregations showed a 1:2:1 ratio (Table 1). The data demonstrate that LAP1 activity is controlled by a single gene, *Lap1*, the enzymes being active as monomers. With respect to the different allelic forms, FS and FM phenotypes could be easily distinguished, but due to low band resolution the MM and SS types were hardly distinguishable from the MS types (Fig. 1). Polymorphism was detected in both the trisomic series and the pollinators (Table 2). The trisomic series revealed all existing phenotypes, but the pollinators proved to be less polymorphic, and the red garden beet (KGIII) showed no polymorphism at all.

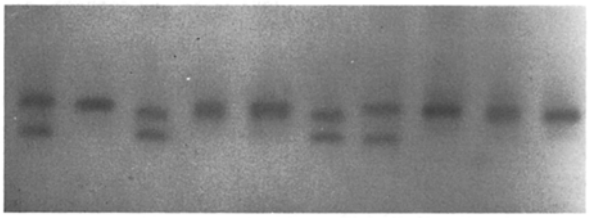

Fig. 1. LAP1 electropherogram showing different polymorphic forms. *FF* (not shown), *SS* and *MM* are banding patterns corresponding with the homomers consisting of the three different alleles. The heterozygotes, *FS*, *FM* and *MS* show two bands, as expected for a monomeric enzyme. Due to poor band resolution the *MM* and *SS* types are barely distinguishable from the *MS* types

Both the second and third activity zone appeared after overstaining. Genetical studies have not yet been carried out for these additional activity zones.

Table 1. Survey of the allele segregations from crossing experiments with diploid plants of beet to prove the monofactorial inheritance for two enzyme markers, *Lap1* and *Got3*

Marker	Crosses	Genotypes[a]					Total	Chi-square		Probability (*P*)
		FF	FS	SS	FM	MM		1:1	1:2:1	
Lap1	FF × FF	48	0	0	0	0	48	–	–	–
	SS × SS	0	0	44	0	0	44	–	–	–
	FS × SS	0	6	6	0	0	12	0	–	1
	FS × FF	12	6	0	0	0	18	2	–	$0.1 < P < 0.2$
	FS × FS	52	104	48	0	0	204	–	0.12	$0.90 < P < 0.95$
	FF × SS	0	54	0	0	0	54	–	–	–
	FM × FM	2	0	0	8	4	14	–	0.64	$0.3 < P < 0.5$
	FF × FM	5	0	0	7	0	12	0.4	–	$0.8 < P < 0.9$
Got3	SS × SS	0	0	276	0	0	276	–	–	–
	FS × SS	0	33	36	0	0	69	0.13	–	$0.7 < P < 0.8$
	FS × FS	13	38	19	0	0	70	–	1.5	$0.3 < P < 0.5$
	FF × SS	0	20	0	0	0	20	–	–	–

[a] F, S and M represent the different allelic forms present in the investigated material

Table 2. Genotypic frequencies of the original trisomic series (disomics and trisomics) of Butterfass (1964) and of the diploid accessions from which appropriate pollinators were chosen for the crossing experiments

Source	*Lap1*					*Got3*		
	FF	FS	SS	MM	FM	FF	FS	SS
Trisomics								
2n = 18	14	4	5	5	5	0	0	31
2n = 19	22	6	7	8	12	0	0	55
Pollinators								
Ba	1	1	7	0	1	10	2	7
WB40	7	3	0	0	0	0	0	15
WB42	3	1	2	1	3	0	0	10
KGIII	0	0	9	0	0	0	0	9
Bar	2	5	1	0	2	0	0	15
BVM	5	3	0	0	2	0	0	10

Glutamate oxaloacetate transaminase

The activity of this enzyme can be measured on fresh leaf material as well as on freeze-dried and frozen material (−70 °C). Enzyme activity measured at seed level is rather poor.

The GOT zymogram reveals three zones of activity (Van Geyt et al. 1988) (Fig. 2A). In zone 3, the most cathodal zone, polymorphism was introduced by the crosses. The pattern consisted of one band (a slow or a fast variant) or three bands. F_2 segregations revealed a 1:2:1 ratio (Table 1). The backcross of a FS phenotype with a SS phenotype segregated in a 1:1 ratio. Crosses of plants with one and the same band (SS type) showed an identical offspring. From these data it is clear that GOT3 is controlled by a single gene, *Got3*, and that the enzyme is active as a dimer. This hypothesis has already been formulated by Van Geyt et al. (1988). The trisomic series as well as the chosen pollinators proved to be rather homozygous for *Got3* (Table 2). Not one heterozygous plant was observed in the trisomic series and with respect to the pollinators, only the fodder beet (type barres) showed any polymorphism.

Data on the genetical control of zone 1 and 2 are not yet available, but earlier studies with monosomic additions in beet carrying extra chromosomes of *Beta procumbens* revealed that the enzymes present in zone 1 are also dimers (Van Geyt et al. 1988).

Chromosomal assignment

Triallelic approach

As three allelic forms were observed for *Lap1*, this marker could be used for introducing the three allelic forms into one trisomic plant. Six crosses, representing six of the nine chromosomes, were carried out in an attempt to introduce the three allelic forms into one trisomic individual in order to identify the critical chromosome for this marker. Segregation data revealed, however, that the two allelic forms, M and S, if present in one lane of the gel, migrated very close to each other. Homozygous MM or SS types were seldom distinguishable from heterozygous MS types. Due to this confusion of the three phenotypes, the phenotypes of some parents and offspring could not be interpreted with full certainty. As MM and SS types were not distinguishable from MS types, it is obvious that FS and FM will not be distinguishable from FMS types. Therefore, even if an appropriate cross would have been made, it is doubtful whether the triallelic forms would have been recognized.

Chromosomal assignment by measurement of a dosage shift

If the tested marker is situated on the triplicated chromosome, a dosage shift is expected. If the type of cross

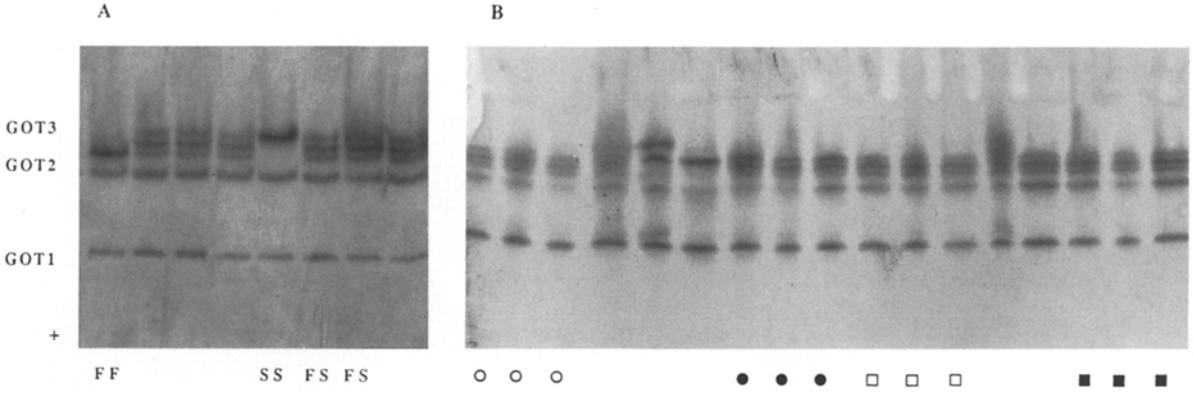

Fig. 2. **A** Electropherogram of GOT with three zones of activity. For *GOT3* one band, corresponding to a homozygous phenotype, *FF* or *SS*, or three bands, corresponding to a heterozygous phenotype, *FS*, are shown. **B** The banding pattern of heterozygous disomic (○) and trisomic (●) plants originating from a *critical* cross (chromosome II) and the banding pattern of heterozygous disomic (□) and trisomic (■) plants originating from a *non-critical cross* (chromosome VII)

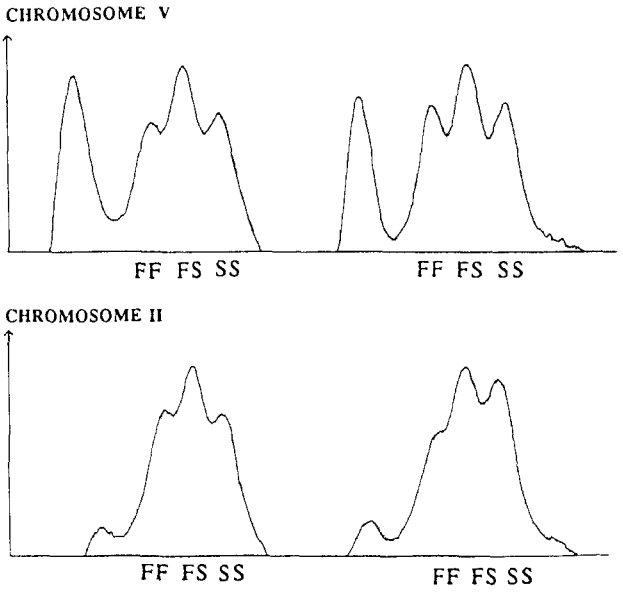

Fig. 3. The densitometric scans of *Got3* of a disomic (*left*) and trisomic (*right*) plant descending from a *non-critical* cross (chromosome V) and from a *critical* cross (chromosome II). The pattern of the latter is characterized by a dosage shift in favour of *SS*

is known, the sense of the dosage shift can be predicted. For instance, a cross of type FSS × FS has a heterozygous trisomic progeny containing FSS and FFS types in a proportion of three to two, respectively.

From each of ten progenies of crosses between trisomic plants, representing eight out of nine chromosome types, and disomic pollinators, two groups of plants were selected and analyzed for dosage shift. The selected plants were heterozygous for *Got3* and per progeny the two groups consisted of either trisomic or disomic individuals. The size of the groups varied from 6 to 15 plants.

Dosage shifts were barely perceptible on the gel with the naked eye (Fig. 2B). To obtain a non-biased idea of the dosage differences, densitometric scans were made (Fig. 3). In contrast to what is generally mentionned in the literature a high variability in the dosage pattern was seen between progenies and also between plants from one progeny. This variability might be attributed to the fact that the progenies of the crosses were analyzed throughout the year. Thus, the leaves were taken from plants at various growth stages, the plants of which had also been subjected to great differences in environmental conditions. Other sources of the observed variability might be the broad genetical background of the plant material or the more technical parameters, such as staining time and temperature.

To control some of the possible causes of variability, the trisomics and their disomic sibs were tested on the same gel. Surface calculations of peaks demonstrated that the theoretical values of 1:2:1 were reached rather often, but sincere deviations of these expected ratios were also measured. For instance, the cross for chromosome I deviated widely from this expected ratio, but the deviation occurred in both the trisomic and disomic fractions. The deviation was so high that, if no disomic control had been tested, a

Table 3. Representation of the results of the analyses of the Wilcoxon–Mann–Whitney U-statistics for *Got3*. The lesser U-value (Um or Un) is given for each pair of samples and is verified against the critical value (as found in tables in Sprent (1989) p 236 A6) for a two-tailed test at the 5% level. Two different crosses were studied for chromosomes IV and V

Chromosome number	2n	Number of plants	Sum of ranks	U-value	Two-tailed 5%	Critical
I	18	15	192	63	34	no
	19	9	108			
II	18	11	165	0	23	yes
	19	9	45			
III	18	8	82	34	17	no
	19	10	89			
IV	18	9	141	39	34	no
	19	15	159			
	18	8	73.5	26.5	13	no
	19	8	62.5			
V	18	15	239.5	60.5	49	no
	19	12	138.5			
	18	8	51	15	8	no
	19	6	54			
VII	18	10	73	18	17	no
	19	8	98			
VIII	18	8	97	27	19	no
	19	11	93			
IX	18	9	90.5	45.5	26	no
	19	12	140.5			

critical value would have been assumed. Though it would have been very interesting to trace the origins of these effects, no investigations on the influence of certain factors upon relative band intensities were carried out.

In Table 3 the results of the analyses are represented in statistical form. The lowest of the calculated U-values are given, and if this U-value is higher than the critical values from the tables the null hypothesis was accepted. On the other hand, if the U-value is lower than this value, a location shift exists between the disomics and trisomics of a certain progeny. This was certainly the case for chromosome II. Moreover, in this cross the U-value is very low, which means that the ranks of both samples are situated each on one end of the scale, indicating a severe location shift between both samples (disomics versus trisomics). All the other U-values are larger than the critical value, indicating that no dosage shift was perceptible. Thus the null hypothesis of identical populations for chromosomes I, III, IV, V, VII, VIII, IX may not be rejected. Note that both of the crosses carried out for chromosome IV and V showed no discrepancy.

Estimation of the distorted segregation

Progenies of crosses between trisomic and disomic individuals, both heterozygous for the enzyme marker, were analyzed for distorted segregations in order to identify the critical chromosome type carrying the marker locus. The progenies were first split up into groups of trisomic or disomic individuals. In the non-critical cross the segregations in both the trisomic and the disomic fractions were expected to be the same and not distorted, the segregation ratio being 1:2:1 for FF:FS:SS. In critical cross combinations, however, both the trisomic and the disomic fractions were expected to show distortion. If the trisomic parent was FFS the segregation ratios in the trisomic and disomic fractions were expected to be 1:5:0 for FFF:F*S:SSS

Table 4. Number of plants observed in three phenotypic classes (FF or FFF, FS or F*S, and SS or SSS) for two isozyme loci in disomic (2n = 18) and trisomic (2n = 19) offspring from crosses between heterozygous trisomic and disomic plants and the results of a comparison (chi-square test) with the non-critical segregation ratio (1:2:1)

Locus	Chromosome number	Number of crosses	2n	Observed			Chi-square (1:2:1)	P
				FF or FFF	FS or F*S	SS or SSS		
Lap1	I	2	18	5	14	6	0.4	$0.8 < P < 0.9$
			19	7	28	0	15.4	$0 < P < 0.001$
		1	18	4	9	5	0.1	$0.95 < P < 1.00$
			19	0	18	2	13.2	$0.001 < P < 0.01$
	II	2	18	6	11	9	1.3	$0.5 < P < 0.7$
			19	5	13	3	1.6	$0.3 < P < 0.5$
	IV	4	18	13	33	15	0.5	$0.7 < P < 0.8$
			19	13	32	17	0.8	$0.7 < P < 0.8$
	V	1	18	2	11	5	1.9	$0.3 < P < 0.5$
			19	3	9	7	1.7	$0.3 < P < 0.5$
	VI	1	18	3	8	4	0.2	0.9
			19	3	11	2	2.4	$0.3 < P < 0.5$
	VII	2	18	8	17	7	0.2	$0.9 < P < 0.95$
			19	11	13	7	1.8	$0.3 < P < 0.5$
	VIII	2	18	12	16	7	1.7	$0.3 < P < 0.5$
			19	13	19	9	1	$0.5 < P < 0.7$
	IX	1	18	8	8	1	5.8	$0.05 < P < 0.1$
			19	2	11	7	2.7	$0.2 < P < 0.3$
		Σ^a	18 + 19	95	212	100	0.8	$0.5 < P < 0.7$
Got3	IV	1	18	3	7	8	3.7	$0.1 < P < 0.2$
			19	7	10	3	1.6	$0.3 < P < 0.5$
	V	1	18	2	11	5	1.9	$0.3 < P < 0.5$
			19	4	12	3	1.4	$0.3 < P < 0.5$
	VII	1	18	2	10	4	1.5	$0.3 < P < 0.5$
			19	6	8	4	0.7	$0.7 < P < 0.8$
	VIII	1	18	6	10	2	2	$0.3 < P < 0.5$
			19	7	10	4	0.9	$0.5 < P < 0.7$
		Σ^a	18 + 19	37	73	38	0	$0.95 < P < 1.00$

F*S, Either FFS or FSS

[a] Sum of the complete offspring 2n = 18 + 2n = 19 for the non-critical segregations

and 2:3:1 for FF:FS:SS. In case the trisomic parent was FSS, the segregation ratios would have been 0:5:1 and 1:3:2. The asterisk in F*S means either F or S. Thus, the distortion in the trisomic fraction was the highest, and the absense of one of the homozygous genotypes in the critical trisomic fraction is especially strong evidence for the expected distortion.

Critical and non-critical trisomics were distinguished by comparing the number of observed phenotypes for the disomic and trisomic fraction of each cross with the expected values in the non-critical case for each enzyme by means of chi-square calculations. If more than one cross of the same type was carried out, the sum was made in order to increase the number of samples in each class.

For *Lap1* 16 crosses were considered to be appropriate for investigating segregations that included eight out of nine different chromosomes (Table 4). Three different crosses carried out for chromosome I showed a distorted segregation. In all of these three crosses the three phenotypic classes were present in the disomic fraction. One class was missing in the trisomic fraction as would be expected in the critical case. Chi-square calculations also revealed that the observed values differed significantly from non-critical values. The observed values for chromosome II, IV, V, VI, VII, VIII and IX did not deviate significantly from the expected non-critical values.

The segregation data of *Got3* for chromosomes IV, V, VII and VIII (Table 4) showed no distortion, confirming the dosage data that *Got3* is not situated on one of these chromosomes.

In order to verify the origin of the detected distorted segregations, the sums of all classes, disomics + trisomics except for the critical ones, were made, and verified against the expected 1:2:1 with a chi-square test (Table 4). This confirms that the segregation distortions perceived in certain trisomic fractions are most certainly caused by the presence of the critical chromosome.

Discussion

The monofactorial inheritance of *Lap1* and the monomeric structure of the enzyme were confirmed by the analysis of the segregating progenies. Of the three allelic forms counted thus far in the accessions studied, *Lap1* could be a good candidate for assignment to a chromosome by introducing the three allelic forms into one trisomic plant. This goal, however, could not be reached as it proved to be impossible to discern the three allelic forms in one lane of the gel, probably due to the poor resolution of the electrophoresis system used.

Counting the segregations, though more time-consuming, proved to be a better choice. The trisomic offspring of the crosses between heterozygous trisomics and disomics was distorted if the tested marker was situated on the triplicated chromosome. Correct segregation estimates could be made even with very small samples. However, with such small sample sizes it is safer to verify the validity of the cross by analysing several non-segregating markers. With this approach, the marker *Lap1* could be allocated to chromosome I. So far no linkage data are available with this marker.

The genetical studies confirmed that *Got3* is controlled in a simple Mendelian way and that the enzyme is active as a dimer. This enzyme showed very little polymorphism, and different allelic forms were seen only in fodder beet (type barres), which restricts the applicability of this enzyme as a marker system in genetical studies.

A dosage shift in the banding pattern of the trisomics compared to the disomics revealed that chromosome II is the critical chromosome for *Got3*. No dosage shift could be detected in the controls consisting of seven of the eight remaining trisomic types. The main advantage of dosage studies is the small number of plants required and that only one generation of crosses (in order to obtain heterozygotes) is needed. It was demonstrated, however, that densitometry is an essential step in this procedure, as shifts were hardly perceptible with the naked eye. The application of the non-parametric Wilcoxon–Mann–Whitney test upon the data obtained revealed a statistical survey of the location of the compared samples. The choice of this test is prompted by the advantage that no assumption of normal distribution of samples is required and that sample sizes can be very small (between 5 and 20). The comparison of the disomic and the trisomic fraction of each chromosome has shown its usefulness when in non-critical situations severe dosage shifts were sometimes measured. Fortunately, these shifts were equally as large in both the trisomic and disomic fraction.

Segregation data on chromosomes IV, V, VII and VIII also confirmed the null hypothesis for these chromosomes. Lange et al. (1993) were able to show by means of segregation experiments in trisomics that *Icd1* (for isocitrate dehydrogenase) is also situated on chromosome II, whereas Butterfass (1968) found that the gene R (standing for red hypocotyl) is situated on this chromosome (the same trisomic series was used in both experiments). More confirmation of the value of the dosage approach was provided with linkage data. Wagner (1990) and Wagner et al. (1992) mention the linkage of *Icd1* and *Got3*. Moreover, linkage between *R* and *Icd1* has been confirmed by data from Smed et al. (1989) and Wagner et al. (1990). Linkage data proving linkage between *R* and *Got3* (Abe and Tsuda 1987; Wagner 1990) complete the circle.

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