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Genetical control and linkage relationships of isozyme markers in sugar beet (*B. vulgaris* L.)

1. Isocitrate dehydrogenase, adenylate kinase, phosphoglucomutase, glucose phosphate isomerase and cathodal peroxidase

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Summary. Five isozyme systems were genetically investigated. The different separation techniques, the developmental expression and the use as marker system in sugar beet genetics and breeding is discussed. Isocitrate dehydrogenase was controlled by two genes. The gene products form inter- as well as intralocus dimers, even with the gene products of the *Icd* gene in *B. procumbens* and *B. patellaris*. Adenylate kinase was controlled by one gene. Three different allelic forms were detected, which were active as monomeric proteins. Glucose phosphate isomerase showed two zones of activity. One zone was polymorphic. Three allelic variants, active as dimers, were found. Phosphoglucomutase also showed two major zones of activity. One zone was polymorphic and coded for monomeric enzymes. Two allelic forms were found in the accessions studied. The cathodal peroxidase system was controlled by two independent genes, of which only one was polymorphic. The gene products are active as monomers. Linkage was found between red hypocotyl color (*R*) and Icd_2. Pgm_1, Gpi_2, Ak_1 and the Icd_2-*R* linkage group segregated independently.

Key words: *Beta vulgaris* – Sugar beet – Isozymes – Genetics – Linkage

Introduction

In the past, breeders have mostly used morphological markers in the assessment of breeding programs. However in sugar beet, such markers are only available in limited numbers (Smith 1980). Also, the presence of such markers is often accompanied by deleterious effects or a decrease of economic potential. Their dominant/recessive way of inheritance may hamper the scoring of the distinct genotypes in a segregating cross. An additional drawback is the fact that some of these morphological markers are sensitive to environmental effects and can only be scored in specific stages of development.

Molecular markers are essential in the in vitro technology for controlling haploid induction, for the identification of non-sexual hybrids obtained by protoplast fusion, for the control of transfer of genetic information into receptor cells and for monitoring chromosome modifications in tissue culture. In classical breeding, they can be used in a variety of ways: genotype patenting, detection of non-controlled crossing, identification of sexual hybrids, estimation of genetic variability in a population and gene tagging by establishing linkage relationships with characters of agronomic value (Tanksley and Orton 1983). One of the most promising possibilities concerns the identification of chromosomes and the establishment of a genetic map of the sugar beet.

Isozymes are very useful for such purposes. This type of molecular marker does not normally decrease the genetic potential of the cultivar and is mostly expressed codominantly, in a way that allelic and/or epistatic interactions do not mask the phenotype. This study describes the analysis of the polymorphism and genetics of five isozyme systems. Linkage relationships between certain markers and red hypocotyl marker are reported. Their suitability as genetic markers is discussed.

Material and methods

Methods for extraction and gel electrophoresis were described previously (Van Geyt and Smed 1984; Van Geyt 1986). The

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Table 1. Staining procedures

Enzymes	Staining recipes	
AK	25 ml	0.06 *M* Tris HCl, pH 8.0
E.C. 2.7.4.3.	100 mg	glucose
	7 U	G6PDH (glucose-6-P-dehydrogenase from yeast)
	7 U	HK (hexokinase from yeast)
	100 mg	ADP
	2.5 mg	NADP
	2.5 mg	MTT or NBT
	1.25 mg	PMS
	25 ml	2% agar solution in $MgCl_2$ at 45 °C modified from Harris and Hopkinson (1976)
POD	100 ml	0.2 *M* acetate buffer pH 4.6
E.C. 1.11.1.7	100 mg	o-dianisidine (in ethanol)
	0.2 ml	H_2O_2 (30%)

staining recipes for ICD, GPI and PGM are described in Van Geyt and Smed 1984), and supplementary staining recipes are summarized in Table 1.

Crosses were made by manual pollination of male sterile receptor plants. Subsequently, after pollination, the receptor plant was isolated in a porous and wet-strength paper bag. Alternatively, reciprocal crosses were done in an isolation greenhouse with a pollen-proof ventilator or by bagging two individuals. All crosses were controlled with markers with known genetic background. Crossing experiments showing a divergent mating system were not used for further analysis.

Haploids were isolated via gynogenesis, as described previously (Van Geyt et al. 1987).

Wild species, interspecific hybrids and alien monosomic addition lines (chromosome I) of *B. vulgaris* with *B. procumbens* and *B. patellaris* were put at our disposal by the Stichting voor Plantenveredeling, Wageningen (for a description of the lines, see Van Geyt et al. 1988; Lange et al. 1988). Conforming to the nomenclature used in the latter articles, allotriploids of *B. vulgaris* with *B. procumbens* were designated VVPro, allotetraploids with *B. patellaris* as VVPatPat and monosomic addition lines as $VV+Pro_1$ (chromosome 1 as extra chromosome).

The characteristics of the *R, r* (hypocotyl color) locus were originally described in Kajanus (1917).

Gene symbols are written in small caps (e.g. Icd_1), gene products in capitals (e.g. ICD_1^F).

Results and discussion

Isocitrate dehydrogenase [ICD; E.C. 1.1.1.42; Threo-D_s-isocitrate: $NADP^+$ oxidoreductase (decarboxylating)] was preferentially studied on starch gel electrophoresis (buffer system SGE 1 in Van Geyt and Smed 1984). The ICD system could also be studied on $PAGE_1$. This enzyme was expressed in all stages of development and the relative intensity of the different bands within one zymogram did not change markedly. Three different types of zymograms were detected in *Beta vulgaris* (Fig. 1). Two types expressed three bands (VV_1 and VV_2 in Fig. 1). The third type (VV_3) showed five bands at the corresponding positions. In zymograms of dry seeds, up to four additional, slower-migrating bands became visible after overstaining (not shown), but these have not yet been fully analyzed and are not considered here. The polymorphism was also studied in the species of the *Patellares* section. All species showed a single band (PP), faster-migrating than the activity zone in *Beta vulgaris*.

The array of progenies with regard to the different crossing experiments is presented in Table 2 A. Crosses of phenotypes showing the same pattern with three bands produced a progeny of similar patterns indicating fixed heterozygosity. Crosses of type VV_1 plants with type VV_2 plants revealed the VV_3 pattern. If type VV_1 or type VV_2 plants was crossed with type VV_3 plants, both types were detected in the progeny in equal amounts. Selfing or crossing type VV_3 plants resulted in a 1:2:1 segregation for type VV_1 : type VV_3 : type VV_2 individuals. Haploids regenerated from gynogenesis were compared with their corresponding mother plant. Haploids derived from mother plants with three bands conserved the pattern with three bands. On the other hand, a mother plant with five ICD bands gave two types of regenerants. Both corresponding types with three bands were found. These results indicate that isocitrate dehydrogenase was controlled by two genes. The gene products form inter- as well as intralocus dimers. The intralocus heterodimers migrated to an intermediate position between the homodimeric bands. Some bands overlap each other. One of the genes did not show any polymorphism in the accessions studied. The gene was named Icd_1. With regard to the other gene (Icd_2), two forms could be detected. The allelic forms will be considered as Icd_2F (fast) and Icd_2S (slow) (Fig. 1).

The hypothesis mentioned above was further confirmed by the study of interspecific hybrids. In hybrids between the two species (VVPro and VVPatPat) and alien monosomics with chromosome I as extra *B. procumbens* ($VV+Pro_1$) chromosome (Van Geyt et al. 1988), interspecific heterodimers were formed between the subunits encoded by the *Beta vulgaris* genes and the gene products formed by the gene(s) of the alien chromosome (Fig. 1).

This type of genetic control is not unusual for plant isocitrate dehydrogenases. A similar system was described in maize (Goodman et al. 1980; Stuber and Goodman 1980) and in barley (Brown and Munday 1982). In *Triticinae* (Mitra and Bhatia 1971), rice (Endo and Morishma 1984), *Pinus pondarosa* (O'Malley 1979), pitch pine (Guries and Ledig 1979), NADP ICD was found not be controlled by one single gene. The structural affinity of the protomers of both genes could point to a gene duplication of the ICD system. Indeed, in some of the wild relatives of *Beta* (section *Patellares*), a single gene system could be detected. Nevertheless, the affinity

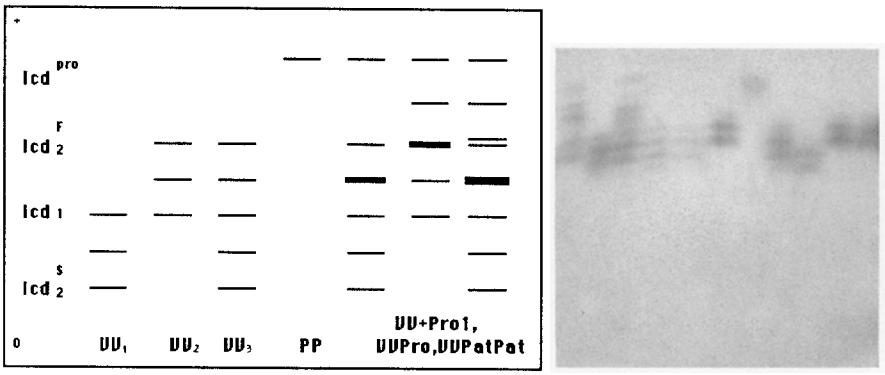

Fig. 1. Schematic representation and photographic example of the zymograms detected for *Icd*. VV_n – phenotypes detected in the accessions studied. PP – pattern as detected in the species of the *Patellares* section (*B. procumbens, B. webbiana* and *B. patellaris;* VVPro – allotriploid of *B. vulgaris* with *B. procumbens;* VVPatPat – allotetraploid of *B. vulgaris* with *B. patellaris;* VV + Pro1 – alien addition lines of *B. vulgaris* with chromosome 1 of *B. procumbens* as extra chromosome

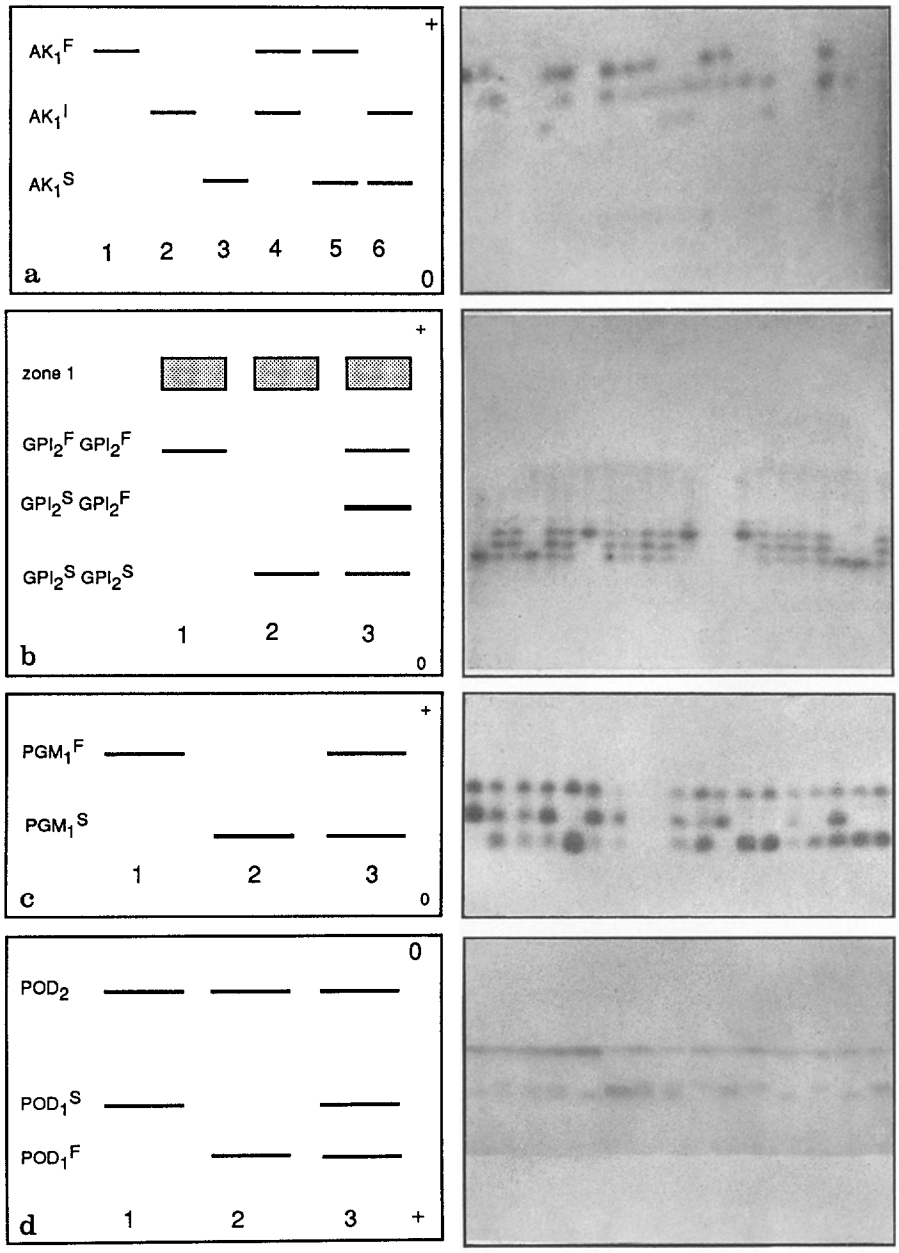

Fig. 2a–d. Schematic representation and photographic example of the zymograms of **a** *Ak*, **b** *Gpi*, **c** *Pgm* and **d** *Pod*. The numbers represent the different types of zymograms observed in *B. vulgaris* (arbitrary numbering)

Table 2. Survey of the different crossing experiments (receptor × pollinator) performed, respectively, with **A** – Icd_1; **B** – AK_1; **C** – Gpi_1; **D** – Pgm_1; **E** – Pod_1. Plants marked with an asterisk (*) were male sterile

Crosses	Phenotype			Expected ratio			Probability	
Icd	VV_1	VV_2	VV_3	VV_1	VV_2	VV_3	Chi^2	*P*
A								
$VV_1^* \times VV_1$	58	–	–	1	0	0	–	–
$VV_1 \times VV_1$	111	–	–	1	0	0	–	–
$VV_1^* \times VV_1$	97	–	–	1	0	0	–	–
$VV_2^* \times VV_2$	–	–	81	0	0	1	–	–
$VV_1^* \times VV_2$	–	46	–	0	1	0	–	–
$VV_1 \times VV_2$	–	113	–	0	1	0	–	–
$VV_2^* \times VV_1$	–	44	–	0	1	0	–	–
$VV_3 \times VV_1$	23	27	–	1	1	0	0.08	0.77
$VV_3 \times VV_1$	19	16	–	1	1	0	0.06	0.81
$VV_3^* \times VV_1$	32	36	–	1	1	0	0.13	0.75
$VV_2^* \times VV_3$		70	67	0	1	1	0.02	0.8
$VV_2^* \times VV_3$		75	55	0	1	1	0.77	0.37
$VV_3^* \times VV_2$		50	47	0	1	1	0.77	0.37
$VV_3^* \times VV_3$	42	89	38	1	2	1	0.20	0.90
$VV_3^* \times VV_3$	41	90	43	1	2	1	0.09	0.95
$VV_3 \times VV_3$	12	27	9	1	2	1	0.38	0.81

B														
AK_1	FF	FS	SS	FI	II	SI	FF	FS	SS	FI	II	SI	Chi^2	*P*
FF* × FF	202	–	–			–	1	0	0	0	0	0	–	–
II* × II	–	–	–	–	165	–	0	0	0	0	1	0	–	–
SS* × SS	–	–	155	–	–	–	0	0	1	0	0	0	–	–
FF* × SS	–	38	–	–	–	–	0	1	0	0	0	0	–	–
FF* × II	–	38	–	45	–	–	0	0	0	1	0	0	–	–
II% × SS	–	–	–	–		161	0	0	0	0	0	1	–	–
FI* × II	–	–	–	19	17	–	0	0	0	1	1	0	0.11	0.75
II* × FI	–	–	–	35	31	–	0	0	0	1	1	0	0.24	0.61
FF × FI	22	–	–	26	–	–	1	0	0	1	0	0	0.17	0.65
FI × II	–	–	–	49	74	–	0	0	0	1	1	0	0.20	0.65
FS* × FS	12	25	14	–	–	–	1	2	1	0	0	0	0.19	0.90
FI* × FI	17	–	–	35	11	–	1	0	0	2	1	0	0.58	0.74

C								
GPI_2	FF	FS	SS	FF	FS	SS	Chi^2	*P*
FF* × FF	34	–	–	1	0	0	–	–
FF × FF	133	–	–	1	0	0	–	–
SS* × SS	–	–	27	0	0	1	–	–
FF × SS	–	–	–	0	1	0	–	–
FF × SS	–	38	–	0	1	0	–	–
FS × SS	–	26	30	0	1	1	0.28	0.58
FS* × SS	–	24	22	0	1	1	0.09	0.76
FS* × FF	76	75	–	1	1	0	0.003	0.96
FS × FF	19	17	–	1	1	0	0.06	0.80
FS* × FS	18	35	15	1	2	1	0.28	0.86

D								
Pgm_1	FF	FS	SS	FF	FS	SS	Chi^2	*P*
FF* × FF	53	–	–	1	0	0	–	–
FF* × FF	67	–	–	1	0	0	–	–
FF* × FF	111	–	–	1	0	0	–	–
SS* × SS	–	–	41	0	0	1	–	–
FF* × SS	–	82	–	0	1	0	–	–
SS* × FF	–	168	–	0	1	0	–	–
SS* × FF	–	105	–	0	1	0	–	–
FS* × SS	–	43	47	0	1	1	0.18	0.65
FS* × FF	36	40	–	1	1	0	0.21	0.65
FS* × FF	116	128	–	1	1	0	0.15	0.70
FS* × FF	88	64	–	1	1	0	0.95	0.34
FS* × FS	30	58	25	1	2	1	0.56	0.75

Table 2 (continued)

Crosses	Phenotype			Expected ratio			Probability	
POD_1	FF	FS	SS	FF	FS	SS	Chi^2	P
E								
FF* × FF	44	–	–	1	0	0	–	–
SS* × SS	–	–	32	0	0	1	–	–
FF* × SS	–	64	–	0	1	0	–	–
FS* × SS	–	71	76	0	1	1	0.17	0.65
FS* × FF	87	83	–	1	1	0	0.09	0.76
F* × FS	42	80	39	1	2	1	0.12	0.95

of the subunits for the formation of heterodimeric forms remained. The ICD system appeared as a very suitable marker system. The enzyme was easily detectable on starch gel, is naturally polymorphic and was little influenced by the state of development and environmental factors.

Adenylate kinase (AK; E.C. 2.7.4.3; ATP: AMP phosphotransferase) was separated on SGE_1. The pattern easily became blurred, with insufficient centrifugation of the homogenates. Six different AK phenotypes were encountered in the sugar beet and fodder beet lines studied. At present, bands could be visualized at three different positions (Fig. 2a). Crossing experiments (Table 2B) revealed that the progeny of crosses between parental plants, each characterized by a distinct single band pattern, always consisted of phenotypes showing both corresponding bands. Individuals with the same single band gave an identical band in the offspring. Crosses of plants with two bands with a type showing only one of the similar bands segregated in a one-to-one ratio. A cross between identical types with two bands segregated in a 1:2:1 manner. The results of the crosses fit in with a codominantly expressed Mendelian gene which codes for enzymes active as monomers. The gene controlling the adenylate kinase system was called Ak_1. At present, three different allelic forms were detected. They were designated Ak_1^F, AK_1^I and Ak_1^S in order of decreasing mobility.

AK showed a divergent level of expression of the allelic forms (results not shown). In heterozygotes, increased activity of one of the bands was detected in multiple cases. The difference could be detected in both forms.

The gene is a good marker but a disadvantage is the high cost of the staining solution (two coupling enzymes added).

Glucose phosphate isomerase (GPI; E.C. 5.3.1.9; D-Glucose-6-phosphate ketol isomerase) can be studied on SGE 1 as well as on PAGE 1. In the last case, however, contamination with the glucose-6-dehydrogenase pattern occurred when no agar overlay staining was used. Two zones of activity were found for GPI. One consisted of a diffuse pattern with some variation, but was too weak to allow a satisfactory distinction between bands. The other zone was polymorphic, expressing three different types of patterns (Fig. 2b).

At present, only the second zone could be studied genetically. Two single-band types and one type with three bands could be detected. Table 2C summarizes the different crossing experiments. Crosses of genotypes with different single bands resulted in phenotypes with three bands. The progeny of crosses of plants with three bands with plants with single band types proved to segregate in a 1:1 ratio. A 1:2:1 ratio was found in the progeny of crossed plants with zymograms with three bands. Segregation data of the second zone of glucose phosphate isomerase indicated that a single Mendelian gene controls this dimeric enzyme. The gene was named Gpi_2.

No hybrid formation could be detected with the isozyme bands of the first zone. The genetic control was in conformity with the one observed in other plants. Multiple gene control and dimeric enzymes were found in most plant species, e.g. maize (Goodman and Stuber 1983); barley (Brown 1983), tomato (Rick 1983), soybean (Kiang and Gorman 1983) and sunflower (Torres 1983). The first zone of glucose phosphate isomerase gave very diffuse bands and is not useful as a marker system. The other zone was easily identified as polymorphic and will be a useful genetic marker. The pattern appears to be constant during the development of the plant. The best separations, however, can be obtained using leaf extracts. The relative expression of the different bands in heterozygous patterns is not constant. As reported for AK, a divergent expression of both alleles in favour of both forms was also found for the polymorphic zone of GPI.

Phosphoglucomutase (PGM; E.C. 2.7.5.1; α-D-Glucose-1,6-biphosphate: α-D-glucose-1-phosphotransferase) was studied on starch gel electrophoresis (buffer systems

SGE_1 or SGE_2). On PAGE, the pattern was contaminated with glucose-6-P-dehydrogenase. PGM was detected as two zones of activity (Fig. 2c). In the most anodal zone, genetic variation was found, showing either a slow, a fast or two bands. The less anodal zone consisted of one or two weaker bands; no variation was detected (Fig. 2c). After overstaining, an even less anodal third zone was found with up to six more or less weak bands. As these patterns were usually very irregular, the zymogram was normally not overstained and this minor system was not considered. Table 2 D summarizes the crossing experiments with relation to the first activity zone. Crosses of plants with identical single bands gave identical bands in the offspring, while crosses of plants with distinct single bands showed a progeny of individuals with two bands. Crosses of heterozygote plants with plants showing one corresponding band segregated in a 1:1 ratio for the two parental types, while crosses of two heterozygotes segregated in a 1:2:1 ratio for the FF:FS:SS genotypes.

The data point to a Mendelian gene (Pgm_1), which coded for enzymes active as monomers. Two allelic forms were found in the accessions studied. The zone is most actively expressed in green tissues, but other tissues mostly allow good visualization of the polymorphism. No polymorphism could be found in the other activity zones. If these genes should be used as a marker system, polyacrylamide gel electrophoresis should be preferred to starch gel electrophoresis. Monomeric activity and multiple gene control of the phosphoglucomutase system was found in most plants, for example, maize (Goodman and Stuber 1983), *Capsicum* spec. (McLeod et al. 1983), cole crops (Arus and Shields 1983), and soybean (Kiang and Gorman 1983), etc.

The major PGM system appeared to be useful as a marker system. This zone was stable during the development of the plant. The two other zones, however, were very faint, especially in non-green tissue.

Peroxidase (POD, E.C. 1.11.1.7; Donor: hydrogen peroxide oxidoreductase). The cathodal peroxidases gave a clear pattern on starch gel electrophoresis (SGE 1 system) and were characterized by two zones of activity (Fig. 2d). Both activity zones showed affinity for O-dianisidine, benzidine and guaiacol as electron donors. The slowest zone was characterized by one band and did not show any variation. The fastest migrating zone was characterized by one or two bands. Table 2 E summarizes the crossing experiments performed with phenotypes polymorphic for the fastest migrating zone. Crosses of plants with the same single band type revealed a progeny expressing the same pattern, indicating that no modifier genes act on the system. Crosses of an F type with an S type resulted in a progeny of types with both bands (FS). Crosses of FS types with F or S types gave an equal distribution of both types. Finally, crosses of two FS types segregated in the three phenotypes in the ratio 1(F):2(FS):1(S).

The segregation data of peroxidase fit in with a codominant expression of a Mendelian inherited trait. The gene products were active as monomeric enzymes. The gene was called Pod_1. A monomeric structure has been reported in most plant species, for example, maize (Brewbaker and Hasegawa 1975), alfalfa (Quiros 1983), *Petunia* (Wijsman 1983), etc. The slowest migrating zone was not polymorphic in *B. vulgaris*. In all crossing experiments, this zone was expressed as a single band, indicating independent inheritance of the first zone. The gene controlling this band was called Pod_2. The expression of the cathodal peroxidases was highly developmentally regulated. Both systems are only expressed in mature leaves. In young tissue and roots, only the POD_1 system can be visualized. The expression is more pronounced in root tissue. The cathodal peroxidases are barely expressed in dry seeds. Due to its developmental expression, the use of cathodal peroxidases as a marker system is limited to certain organs. In older leaves, POD_1 can be used as a suitable marker system.

The discrepancy in the relative activity of the allelic forms in the heterozygotes of AK and GPI raises the question as to whether it is linked to the state of development or is due to chromosomal changes. The phenomenon should be further studied to answer this question.

Linkage relationships

A linkage relationship of Icd_2 and the marker *R* (linkage group 1) could be detected. Table 3 summarizes the different double backcross segregations of the factor *R* with the isozyme gene Icd_2. From χ^2 analysis, it is clear that the null hypothesis can be rejected. The probability of a 1:1:1:1 ratio is not significant while the segregations of the single markers showed a high probability. The average distance of the two markers as measured in coupling is 16.98 c Morgan. A similar conclusion as presented for the measurement of linkage in coupling can be drawn in repulsion. None of the χ^2 values was significant. The segregations of the single markers are significant. The average recombination distance, as measured in repulsion is 16.76 c Morgan.

The exact position of the *Icd* marker within the linkage group 1 could not yet be determined, or it is located near the bolting gene (*B*) either at the site of marker *Y*, which should be located at intermediary position of Icd_2 and *R*. In the first case, the *Icd* marker could be used as a tag for annual growth habit. However, it is likely that additional genes control the bolting characteristic of the plant, so that tagging of additional genes might be necessary in order to enable a marker-based control of bolting habit in beet.

Table 3. Chi^2 tests for linkage and deviation in Mendelian ratio of isozyme markers and the morphological marker Rr

Genes (X, Y)	Progeny						Total	Chi_x^2	Chi_I^2	Chi_L^2	P_x	P_I	P_L	Percent recombination	Type of cross
	XI^{FF}	XI^{FS}	xI^{FF}	xI^{FS}	XI^{SS}	xI^{SS}									
Rr-Icd_2^{FS}	87	12	26	80			205	0.119	1.076	83.566	0.65	0.31	ns	18.5	bag
	396	66	64	390			917	0.350	0.873	469.886	0.86	0.86	ns	14.2	ppg
	25	146	146	28			341	0.013	0.013	165.620	0.92	0.79	ns	15.5	bag
	98	15	30	85			228	0.008	1.720	86.982	0.93	0.19	ns	19.7	ppg
		7		38	40	5	90	0.089	0.000	48.578	0.76	1.00	ns	13.3	ppg
		130		24	28	126	308	0.104	0.000	135.325	0.75	1.00	ns	16.9	ppg
		12		70	76	20	178	0.011	0.550	74.135	0.92	0.45	ns	18.0	bag
		56		10	13	54	133	0.094	0.004	57.105	0.76	0.95	ns	17.3	bag
		15		119	118	38	290	0.993	0.834	120.400	0.75	0.37	ns	18.3	ppg
Rr-Pgm_2^{FS}	57	54	51	49			211	0.059	0.287	0.697	0.80	0.58	0.87	–	bag
		38		37	42	31	148	0.486	0.013	1.675	0.48	0.48	0.64	–	bag
	29	50	47	47			173	0.650	1.274	6.396	0.42	0.27	0.10	–	ppg
Rr-Gpi_2^{FS}	55	45	32	53			185	0.608	0.327	7.065	0.43	0.58	0.07	–	ppg
Rr-Ak_1^{FS}	43	37	26	26			132	2.970	0.136	6.485	0.08	0.73	0.10	–	ppg

ns: not significant;
bag: cross-produced by bagging two individuals;
ppg: cross-produced by isolation of two individuals in a pollen-proof greenhouse

Table 4. Chi^2 tests for independent segregation of various isozyme genes after backcrossing

Genes (I_1-I_2)	Allelic forms [Progeny (I1, I2)]						Total	Chi_{I1}^2	Chi_{I2}^2	Chi_L^2	P_{I1}	P_{I2}	P_L
	F, F	F, S	F, H	H, F	H, S	H, H							
Pgm_1-Gpi_2	39		46	53		45	183	0.462	0.003	2.158	0.50	0.95	0.53
Pgm_1-Icd_2	70		63	64		68	265	0.002	0.017	0.494	0.96	0.88	0.91
Pgm_1-Ak_1		64	58		50	50	222	1.090	0.081	2.505	0.31	0.77	0.47
Icd_2-Gpi_2	47		52	45		39	183	0.615	0.003	1.896	0.43	0.95	0.60
Ak_1-Gpi_2		40	31		30	27	128	0.765	0.562	2.940	0.39	0.81	0.40
Icd_2-Ak_1		47	43		35	33	158	1.531	0.114	3.316	0.22	0.75	0.35

The three other genes investigated (controlling, respectively, Pgm_1, Gpi_2 and Ak_1) proved to segregate independently with respect to the *R* gene. No linkage relationship could be found between the isozyme genes Pgm_1, Gpi_2, Icd_2 and Ak_1. As presented in Table 4, the null hypothesis can be accepted in all cases.

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