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Monosomic additions in beet (*Beta vulgaris*) carrying extra chromosomes of *Beta procumbens*

1. Identification of the alien chromosomes with the help of isozyme markers

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Summary. Eleven isozyme systems were used to identify the extra chromosomes, originating from *Beta procumbens,* in progenies of 33 monosomic additions in beet (*B. vulgaris*). Nine groups of monosomic additions could be distinguished, representing the nine different chromosome types of *B. procumbens.*

Key words: *Beta vulgaris* – *Beta procumbens* – Alien monosomic additions – Isozyme markers – Chromosome identification

Introduction

Alien monosomic additions can be used for transferring small segments of the alien chromosomes into the basic complement of the recipient species, often by applying ionising irradiation (for review see Khush 1973). In sugar beet, efforts have been concentrated on the transfer of genes for resistance to the beet cyst nematode (*Heterodera schachtii* Schm.) from *Beta* species of the section *Patellares* to cultivated beet. Savitsky (1975, 1978) was first to produce resistant monosomic additions in beet, carrying an extra chromosome of *B. procumbens* Chr. Sm., and from these additions she obtained resistant diploids. The same strategy was followed by Speckmann and De Bock (1982), Speckmann et al. (1985), Heijbroek et al. (1983) and Löptien (1984a). The co-operative between the two programmes in the Netherlands recently released breeding material of diploid homozygous resistant sugar beet (Heijbroek et al. 1988).

Alien additions also can be used for chromosome localisation of specific genes, especially if these genes cause effects specific to the donor species, or at least different from analogous traits in the recipient species. Thus, alien additions in cereals have been used to study genetic relationships between chromosomes of different genomes (e.g. Tang and Hart 1975; Bernard et al. 1977; Hart 1978; Cauderon et al. 1978; Salinas and Benito 1984). Such studies often concern isozyme markers. Due to their codominant expression, isozymes can be scored in addition to the pattern produced by the recipient genome. Especially in cases where important polymorphic differences exist between the two parents, as in different species, isozymes allow rapid identification of the extra chromosome. The obtained information can be used for tagging (economically important) genes and for studying chromosomal alterations or chromosome elimination, for example after sexual or somatic hybridisation, etc.

In the present study, differences in isozyme patterns between *Beta vulgaris* L. (recipient species) and *B. procumbens* were used to identify the chromosomes of the latter species in monosomic additions of these chromosomes to the recipient species.

Materials and methods

Plant material consisted mainly of alien monosomic additions ($2n = 19$) in diploid beet (*Beta vulgaris*, $2n = 18$), each carrying an extra chromosome originating from *B. procumbens* ($2n = 18$). The making of these monosomic additions involved the production of triploid hybrids between *B. vulgaris* ($4x$ and $2x$), var. *atriplicifolia* ($2x$) or var. *maritima* ($4x$) and *B. procumbens* ($2x$). In the case of a diploid female parent, it has been assumed that the female gamete had an unreduced number of chromosomes. The triploids were pollinated with *B. vulgaris*

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Table 1. Monosomic additions in diploid beet (*Beta vulgaris*), carrying an extra chromosome of *B. procumbens*, and the designation of the female parent in the original interspecific cross

Monosomic additions[a]	Original female parent	No. of plants[e]	Extra chromosome[g]
AU 5–1–2	*B. vulgaris* var. *atriplicifolia* (2*x*)[b]	2	2
AU 5–1–7		2	2
AU 6–1–4 (AN14)		1	7
AU 6–1–5		4	5
A 6–2–6 (AN101)	*B. vulgaris* var. *maritima* (4*x*), Gr5622[c]	4	7
B 6–2–2 (AN111)		?	7
D 1–2–13 (AN115)		?	1
D 2–2–27		3	2
D 2–2–185		2	3
D 2–2–211 (AN122)		4	7
D 3–2–13		2	8
D 3–2–17		4	3
D 3–2–35		2	6
E 3–1–3		2	9
F 2–2–1		2	6
I 3–2–1		2 (4)[f]	4
I 3–2–24		4	5
AU 4–1–1 (AN1)		?	1
C 6–1–3	*B. vulgaris* (2*x*), Swiss chard cv. Lucullus[b]	2	9
H 1–1–6	*B. vulgaris* (4*x*), Mangold (type Barres)[d]	2	3
N 2–2–13		1	5
J 4–1–1	*B. vulgaris* (4*x*), Mangold cv. Civarres[d]	3	9
J 4–1–12		4	5
J 5–1–1		2	2
K 3–1–1 (AN15)		?	7
K 3–1–17		3	6
L 1–1–30		1	3
L 1–1–32		1	2
AH 1–2–5	*B. vulgaris* (4*x*), Mangold cv. Buffalo[d]	4	3
AI 2–2–16		3	3
AI 2–2–30		1	2
AI 2–2–61		4	3

[a] Number in *brackets* is a new code for resistant plants
[b] From collections of SVP, Wageningen, the Netherlands
[c] Turkish wild beet, tetraploid cytotype made by Dr. W. Heijbroek, IRS, Bergen op Zoom, the Netherlands
[d] Tetraploid cytotypes made at SVP, Wageningen, the Netherlands
[e] ? = number of plants unknown, studied as a mixture of several plants
[f] Two out of four plants died in early stage
[g] Arbitrary numbering (see text)

(2*x*). From the progenies monosomic addition plants were selected on the basis of the number of chromosomes ($2n = 18$ versus $2n = 19$). Such families were the source of the monosomic additions of the present study (one or more extra backcrosses were carried out). The circumstances for the original crosses and backcrosses have been described by Speckmann and De Bock (1982).

Table 1 presents a survey of the monosomic additions used in the present study, as well as the designation of their original female parents. The male parents in the first crosses, *B. procumbens* (2*x*), came from a population from a seed field in which accessions WB2 (from USDA, Beltsville, USA) and WB21 (from Max-Planck Institut, Rosenhof, Heidelberg, FRG) were grown in mixed stand. The material of *B. vulgaris*, which was used for backcrossing, consisted of a mixture of a wide variation of types, including sugar beet, fodder beet, mangold, Swiss chard, garden beet, etc. A monosomic addition made by Savitsky (1975) was also studied, in addition to the material mentioned in Table 1. This material also originated from the Turkish wild beet Gr 5622, and was made available through Dr. W. Heijbroek, IRS, Bergen op Zoom, the Netherlands (accession IRS 1719) and Dr. M.H. Yu, USDA, Salinas/Ca, USA (accessions USDA 2488, USDA 4469 and H 770). Finally, some monosomic additions, made available by Dr. C. Jung, University of Hannover, FRG, were included in this study. Two types of control plants were used: (1) sib plants of the monosomic additions without the extra chromosome; and (2) triploid interspecific hybrids carrying two genomes of *B. vulgaris* and one genome of *B. procumbens*.

Mature leaves were used as a sample source, except in the case of alcohol dehydrogenase (ADH), where dry seeds or young seedlings were used. The extraction procedure and sepa-

Table 2. Survey of the marker genes or isozyme bands located on the chromosomes of *B. procumbens* in monosomic additions of these chromosomes in diploid beet (*B. vulgaris*)

Chromosome no.	Marker genes or isozyme bands	Resistance to *H. schachtii*	No. of additions[a]
1	Icd^{Pro}	Yes	2
2	Gdh^{Pro}, $Mdh_1{}^{Pro}$, $Mdh_2{}^{Pro}$, $Pgm_2{}^{Pro}$	No	6
3	$Lap_1{}^{Pro}$	No	7
4	GOT Band 6^{Pro}	No	1
5	GOT Band 7^{Pro}	No	4
6	$Pod_1{}^{Pro}$	No	3
7	–	Yes	5
8	$Aco_1{}^{Pro}$, $Aco_2{}^{Pro}$, 6PGDH Zone 2^{Pro}, SOD (two bands)	No	1
9	Adh^{Pro}	No	3

[a] The numbers refer to the plant material produced at Wageningen only (Table 1)

ration techniques (polyacrylamide gel electrophoresis and horizontal starch gel electrophoresis) were carried out according to the techniques described before (Van Geyt and Smed 1984; Van Geyt 1986). Information on most staining techniques and on the genetics of the isozyme systems can be found in Van Geyt and Smed (1984). Van Geyt and Jacobs (1986), Smed et al. (in preparation) and Van Geyt et al. (in preparation). Leucine aminopeptidase (LAP) was stained in 100 ml 0.05 *M* sodium phosphate buffer pH 6, 30 mg L-leucine-β-naphthylamide and 100 mg Fast Garnet. Esterase (EST) was stained in 100 ml 0.1 *M* sodium phosphate buffer pH 6, 100 mg Fast blue RR salt and 30 mg α-naphthyl acetate (solubilized in 3 ml 50% acetone). The staining procedure for glutamate oxaloacetate transaminase (GOT) was carried out according to Vallejos (1983).

The results of the isozyme analyses are shown in a series of figures. The scheme on the left side of each figure summarizes most bands observed. However, because of allelic variation or other reasons mentioned in the text, complete patterns mostly cannot be visualized in one photograph. Thus, the photographs should be considered as examples in which often only parts of the total pattern are shown.

Results and discussion

The last column of Table 1 lists the identification number given to extra chromosomes on the basis of isozyme markers. The numbering of the chromosomes is arbitrary, as no cytological comparison with existing classifications was done (e.g. De Jong and De Bock 1978; De Jong and Blom 1981). There is also no link with the numbers given to the linkage groups of *B. vulgaris* (Smith 1980). Table 2 summarizes the markers localized on the distinct chromosomes. To avoid confusion with regard to the two species involved, the genes or proteins of *B. procumbens* have been marked with the superscript ^{Pro}.

Chromosome 1

The monosomic additions carrying this chromosome are characterized by an Icd^{Pro} marker gene. The patterns, observed for NADP specific isocitrate dehydrogenase (ICD), are summarized in Fig. 1. The ICD system in *B. vulgaris* is controlled by two genes forming intergenic and intragenic heterodimers (Smed et al., in preparation). One of the genes, Icd_1, is not polymorphic, while Icd_2 has two different alleles. In *B. procumbens,* only one gene is found (Icd^{Pro}), giving rise to one single band. In the interspecific hybrids as well as in the monosomic additions carrying chromosome 1, the gene products of both species were detected. Besides the expected species-specific protein band, interspecific heterodimers were formed between the protomers of *B. procumbens* and the gene products of both *B. vulgaris* genes. The interspecific heterodimers migrated to an intermediary position between the respective homodimers. The hybrid protein of Icd^{Pro} and the slowest migrating product of Icd_2 migrated to a similar position as the heterodimeric band formed between the Icd_2^{FF} and Icd_1 protomers. The hybrid protein of Icd_1 and Icd^{Pro} migrated to a similar position as the Icd_2^{FF} band. These bands fused and appeared as one more intensively stained zone. In high quality separations, however, it was possible to distinguish two faint bands instead of the more common broad zone.

The monosomic additions with chromosome 1 of *B. procumbens* are resistant to the beet cyst nematode *Heterodera schachtii.* One of them, AU 4-1-1 (=AN 1), has been described in detail by Speckmann et al. (1985) and De Jong et al. (1985). Diploid offspring of AN 1 was included in the present study. In AN 1-89 the gene(s) for resistance are located on a chromosome fragment, which most likely is the short arm telosome of the alien chromosome (De Jong et al. 1986). In AN 1-65-2 the gene(s) for resistance have been transferred to the genome of *B. vulgaris* (W. Lange and Th. S. M. De Bock, unpublished). The Icd^{Pro} marker could not be detected in any of these materials, indicating that the gene(s) for resistance and the isozyme

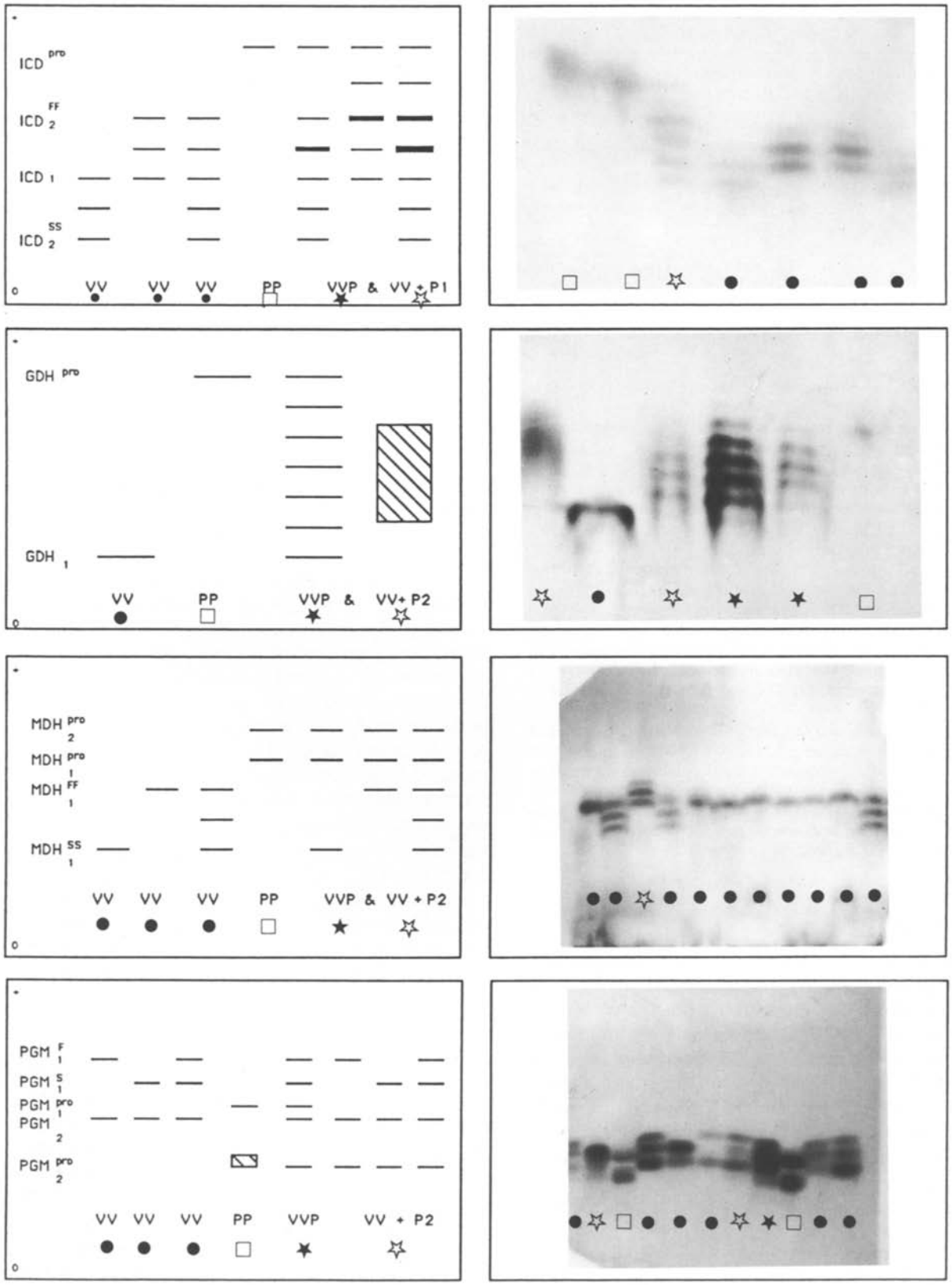

Fig. 1. Isozyme patterns (schematic representation and photographic example) of the markers characterizing chromosome 1 (ICD) and chromosome 2 (GDH, MDH, PGM). VV or ● = *B. vulgaris;* PP or □ = *B. procumbens;* VVP or ✱ = interspecific hybrid; VV + Pn or ☆ = monosomic addition (chromosome *n*)

marker are not closely linked. It seems most likely that Icd^{Pro} is located on the long arm of chromosome 1.

The monosomic additions of accessions USDA 2488, USDA 4469 and IRS 1719 all are characterized by the marker Icd^{Pro}, thus providing that the resistant monosomic addition described by Savitsky (1975) carried chromosome 1. As was the case for AN 1, the diploid offspring of Savitsky's monosomic addition (family H 770, Yu 1981; family 386, Heijbroek et al. 1988; both families kindly made available by the respective authors) did not show the Icd^{Pro} marker.

On the basis of plant morphological characters (Lange et al. 1988) it has been concluded that the resistant monosomic addition type a, as described by Löptien (1984 b), contains chromosome 1. According to Jung et al. (1986), some bands of esterase (EST) and of glutamate oxaloacetate transaminase (GOT) should be useful to mark this particular chromosome. This, however, could not be confirmed in the present study. For EST the separation on native PAGE gave no information at all. By using isoelectric focusing, the same technique as described by Jung et al. (1986), completely different polymorphisms were detected in *B. procumbens* and in the relevant monosomic additions. The broad genetic variability in *B. vulgaris* interfered with the unequivocal identification of the bands of *B. procumbens.* For most bands of *B. procumbens* an allelic form of *B. vulgaris* was migrating to a similar position. Thus the system needs further studies, both to clarify the genetics involved and to obtain a better visualisation of the bands with minor migration differences. A decreased pH gradient will possibly improve the separation of these bands. For the second enzyme mentioned by Jung et al. (1986), GOT, the present study did not reveal a band associated with resistance to the beet cyst nematode. Again, the polymorphism of the slowest migrating zone in *B. vulgaris* made it impossible to be sure of the bands of *B. procumbens* in the same region (see chromosomes 4 and 5). As was pointed out by Jung and Wricke (1987), none of the markers appeared to be located near the resistance gene(s). Therefore the markers have predictive value only with regard to the presence of the complete chromosome. Analysis of new marker systems, in casu RFLP markers, is envisaged in order to tag the resistance gene(s).

Chromosome 2

Six families carried this alien chromosome. Four different isozyme loci were located on chromosome 2, namely Gdh^{Pro}, Mdh_1^{Pro}, Mdh_2^{Pro} and Pgm_2^{Pro} (Fig. 1).

The pattern for the major zone of glutamate dehydrogenase (GDH) is characterized by a single band in both species, migrating at distinct positions. Hybrids and monosomic additions showed multiple hybrid bands. In several cases the bands were concentrated as a broad zone migrating to an intermediate position between the two parental bands. A similar effect of the running conditions on the resolution of GDH hybrid bands has been reported for maize (Pryor 1974). It is most likely that this phenomenon is due to an unresolved mixture of isozymes. Using low gel concentrations (4.5% acrylamide), seven bands could be visualised, suggesting a hexameric structure of the hybrid protein. The parental bands could hardly be visualised in the hybrids or addition plants. Indeed, considering a polymeric structure of the GDH hybrid molecule, the contribution of the parental bands should be, for example, only 8.8% (*B. vulgaris* homomer) and 0.14% (*B. procumbens* homomer) of the total GDH activity, assuming equal contributions of the two subunits to the activity of the polymeric molecule. No indications for the occurrence of modifier genes or preferential association of dissimilar subunits, as suggested by Pryor (1974), could be found. The second zone of GDH (Van Geyt and Smed 1984) could not be scored in *B. procumbens* or in the additions.

In *B. vulgaris,* NAD specific malate dehydrogenase (MDH) is characterized by two genes. Mdh_1 codes for two allelic forms, active as dimers. Mdh_2 (not shown in Fig. 1) is not polymorphic and migrates at an identical position as Mdh_1^{FF}. The gene is expressed only in seeds and juvenile material (Van Geyt et al., in preparation). In *B. procumbens* two zones were generally found. These bands migrated more anodal with regard to the bands of *B. vulgaris.* Normally, the fastest migrating system (Mdh_2^{Pro}) is not polymorphic. Although the slowest zone of *B. procumbens* (Mdh_1^{Pro}) can show polymorphism, only the fastest migrating band was scored in the material used. In hybrids as well as in the additions, the zymogram revealed the sum of the bands found in the two species. Although the different *Mdh* genes are known to code for dimeric enzymes, no hybrid bands were found.

A gene of *B. procumbens* coding for the slowest migrating band of phosphoglucomutase (PGM) was located on chromosome 2. Similar systems were detected in both species, the two zones migrating to distinct positions (Fig. 1). In *B. vulgaris,* one zone was found to be polymorphic (Pgm_1) (Smed et al., in preparation). No polymorphism was found in accessions of *B. procumbens.* Although in *B. procumbens,* the slowest zone generally resolves as a smear, a single band could clearly be seen in the additions. Since the enzymes are monomers, no hybrid bands were expected. The resolution of starch gel electrophoresis was too low to map the fastest migrating zone of *B. procumbens,* its position being too closely located to one of the bands of *B. vulgaris.*

Chromosome 3

Seven monosomic additions carried chromosome 3 and are characterized by the expression of the major band regarding leucine aminopeptidase (Lap_1^{Pro}) of *B. procumbens* (Fig. 2). The LAP zymogram in *B. vulgaris* shows three zones of activity. The main, fastest migrating zone consists of two overlapping systems. One of them is characterized by a single band that is highly developmentally regulated (not shown). The other system, which has the highest activity on the gel and is active as a monomer, shows polymorphism. At present three allelic forms have been detected (results not shown). A second zone migrates slower and consists of four bands, which appear after overstaining. Their separation if not always clear and therefore the system was not considered. A third zone also appears after overstaining. At present, two different band positions have been found, whereas the genetic control remains unknown. *Beta procumbens* is characterized by similar activity zones, although at present no polymorphism has been detected. In the hybrids as well as in the monosomic additions, the activity of the minor systems was so low that no assigments could be given. The major systems, however, was found to be a marker for chromosome 3. The activity in the hybrid, as well as in the monosomic additions, was decreased compared to the expression in *B. procumbens*. As expected for monomers, no new type of band was found in hybrids or monosomic additions.

Chromosomes 4 and 5

In *B. procumbens* seven bands were found for glutamate oxaloacetate transaminase (GOT), which were numbered from 1–7 in order of increasing mobility. In *B. vulgaris* the glutamate oxaloacetate transaminase pattern consists of three zones of activity that are seldom polymorphic. GOT band 6^{Pro} characterized only one monosomic addition; the extra chromosome in this addition was numbered chromosome 4 (Fig. 2). No evidence for the formation of hybrid bands has been found.

Four monosomic additions were characterized by GOT band 7^{Pro}; the extra chromosome was identified as chromosome 5. The isozyme pattern in the hybrids as well as in the relevant monosomic additions showed an extra band, migrating at an intermediary position of the two parental bands. This could point to the formation of interspecific heterodimers, which would be in agreement with observations in maize (Scandalios et al. 1975), wheat (Hart and Langston 1977) and tomato (Rick 1983), where GOT is active as a dimeric enzyme.

Chromosome 6

Three monosomic additions were found to carry chromosome 6. They were characterized by a major band of the cathodal peroxidase (POD) system (Fig. 2). In *B. vulgaris*, this system, separated by starch gel electrophoresis, is characterized by two zones of activity. Only one of them, the fastest migrating system, is polymorphic in *B. vulgaris* and codes for monomeric enzymes (Smed et al., in preparation). The single band of *B. procumbens* proved to migrate to an identical position as the POD_1 product in sugar beet. An additional analysis of segregation in the addition was necessary to confirm the origin of the band in the monosomics. In most circumstances the patterns of a monosomic addition with chromosome 6 and a heterozygous genotype of *B. vulgaris* could also be distinguished by comparing the relative activity of the two bands. *Beta procumbens* also has a second zone for POD. However, this zone was barely expressed in the hybrids and could not be detected in the chromosome additions. It therefore is not shown in the figures.

Chromosome 7

Within the group of monosomic additions with resistance to *H. schachtii*, plants were found without the marker Icd^{Pro}. At present, no isozyme marker has been found to identify this group of plants. This is in agreement with the results of Jung et al. (1986), who reported no bands associated with resistant monosomic additions type b.

One of the monosomic additions carrying chromosome 7 (AU 6-1-4 = AN 14) has been reported to be only partly resistant to *H. schachtii* (Speckmann et al. 1985). Because the other monosomic additions with chromosome 7 show full resistance (Speckmann et al. 1985; W. Lange and Th. S. M. De Bock, unpublished), it is plausible that either more than one gene is active or that allelic forms of the gene for resistance exist. The differences in level of resistance could be useful to determine the genetic control of the particular resistance. However, at present the gene(s) of chromosome 7 have not yet been transferred stably into diploid sugar beet. Tightly linked markers would facilitate such transfer and would be necessary to control the segregation of the different genes or alleles in study. If no isozyme markers can be found, restriction fragment length polymorphism might be used as an alternative.

Chromosome 8

Several markers were localized on chromosome 8, namely two bands of both aconitase (ACO) and superoxide dismutase (SOD), and one band of 6-phosphogluconate dehydrogenase (6 PGDH) (Fig. 3). Only one of the monosomic additions carried this particular extra chromosome. In *B. vulgaris*, aconitase was controlled by two genes (Van Geyt et al., in preparation). The first gene (Aco_1) showed two allelic forms F and

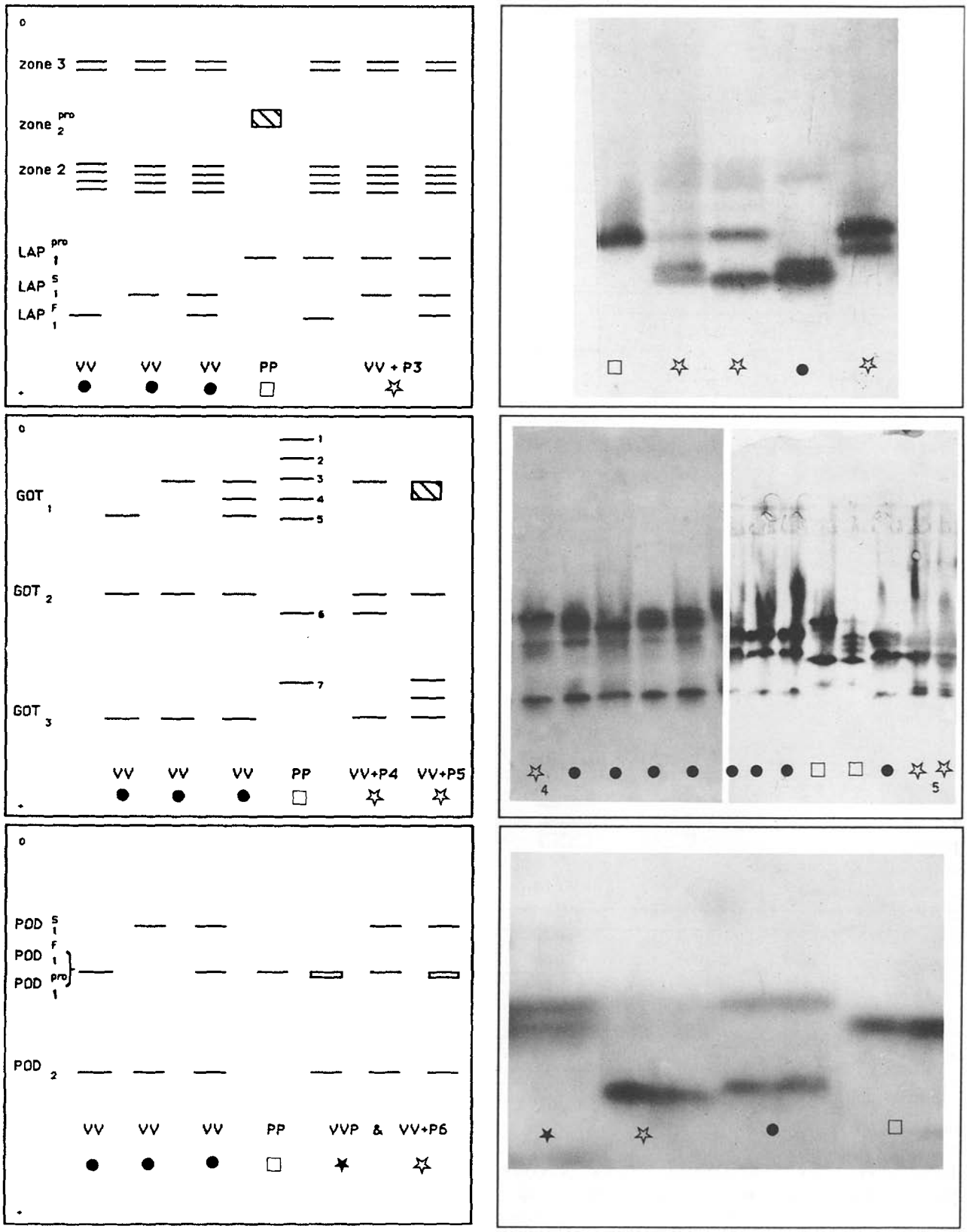

Fig. 2. Isozyme patterns (schematic representation and photographic example) of the markers characterizing chromosome 3 (LAP), chromosomes 4 and 5 (GOT) and chromosome 6 (POD). VV or ● = *B. vulgaris;* PP or □ = *B. procumbens;* VVP or ✶ = interspecific hybrid; VV + Pn or ☆ = monosomic addition (chromosome *n*)

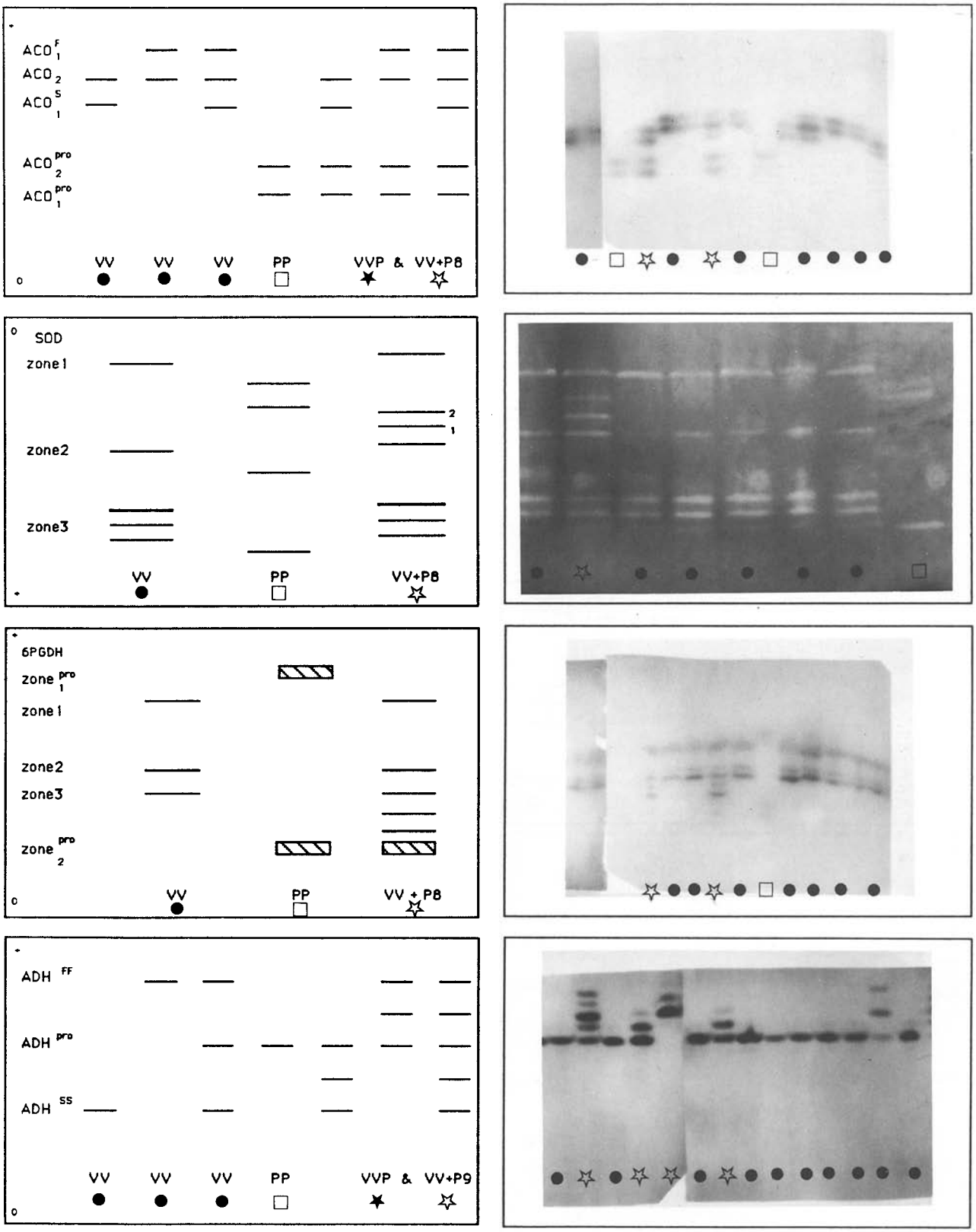

Fig. 3. Isozyme patterns (schematic representation and photographic example) of the markers characterizing chromosome 8 (ACO, SOD, 6PGDH) and chromosome 9 (ADH). VV or ● = *B. vulgaris;* PP or □ = *B. procumbens;* VVP or ✶ = interspecific hybrid; VV + Pn or ☆ = monosomic addition (chromosome *n*)

S. The second gene (Aco_2) was not polymorphic. The gene product of Aco_2 migrates to an intermediary position with regard to the allelic forms of Aco_1. In *B. procumbens* two bands have always been observed. In the accessions studied, no polymorphism was found, but one might assume a similar genetic system as in *B. vulgaris*. Both bands of *B. procumbens* were expressed in the hybrids as well as in the monosomic addition, suggesting that in *B. procumbens* the two aconitase genes are located on the same chromosome. Jung et al. (1986) reported that in *B. webbiana* the two bands of ACO were associated with resistance to *H. schachtii*. The present study reveals that such a correlation does not appear to exist for *B. procumbens*. In this light, the correlation between bands of ACO and resistance, as well as the existance of a third type of resistance (type c), should be carefully reconsidered.

The monosomic addition carrying chromosome 8 also was characterized by the expression of two SOD bands that do not occur in the patterns of both species. It seems plausible to assume that they are the result of interaction between the genes of *B. vulgaris* and one or two genes of *B. procumbens*. As the genetic control of SOD is not yet understood, it is not known how many genes are involved.

The 6PGDH system of *B. vulgaris* showes three zones of activity (only two of them can be visualized by PAGE, Fig. 3). In *B. procumbens* two zones can be visualized, migrating respectively slower and faster with regard to the isozymes of sugar beet. The slowest migrating band of *B. procumbens* and two new bands were expressed in the zymogram of the monosomic additions. At present, no information on the fast system could be gained, as the resolution of the gel was too low to distinguish the band in the hybrid.

Chromosome 9

Three different monosomic additions showed expression of the alcohol dehydrogenase (ADH) system of *B. procumbens* (Fig. 3). In sugar beet, ADH is controlled by a single gene, which codes for a dimeric enzyme (Van Geyt and Jacobs 1986). In the present material the F and S alleles were commonly scored. In *B. procumbens*, a single band was found, migrating at an intermediate position. The gene products of Adh^{Pro} formed active heterodimers with both allelic forms of the *B. vulgaris* ADH subunits, reflecting a pattern with three or five bands with respectively homozygous or heterozygous *B. vulgaris*. In *B. vulgaris* an additional allelic form Adh_M exists, migrating at an identical position as the *B. procumbens* bands (unpublished). This form, however, was not scored in the investigated lines.

Conclusions

Isozyme techniques proved to be excellent for the identification and tagging of chromosomes. Nine groups of monosomic additions of chromosomes of *B. procumbens* in *B. vulgaris* could be identified, obviously representing the nine different chromosome types. A Chi-square test revealed that the frequency distribution of the additions over the nine groups was not significantly different ($P > 0.2$) from hypothesized equal frequencies in all the groups. Although certain isozyme markers of *B. procumbens* showed decreased expression in the hybrid or monosomic additions, chromosome localisation of most genes was possible. Except for chromosome 7, at least one marker could be identified for each chromosome. For ideal chromosome marking, however, at least one marker should be available per chromosome arm. For this purpose additional markers will have to be identified. Besides other isozyme systems (for example, esterases), restriction fragment length polymorphism could be envisaged for future use. Mapping of the different genes with regard to gene tagging of economically important disease resistances present in species of the section *Patellares* requires further linkage analysis. In this respect, the natural variation of the markers and the economically important characters within the section needs further exploration.

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References

Bernard M, Autran JC, Joudrier P (1977) Possibilités d'identification de certains chromosomes de seigle à l'aide de marqueurs biochimiques. Ann Amél Plant 27:355–362

Cauderon Y, Autran JC, Joudrier P, Kobrehel K (1978) Identification de chromosomes d'*Agropyron intermedium* impliqués dans la synthèse des gliadines, des β-amylases et des peroxydases à l'aide de lignées d'addition Blé×*Agropyron*. Ann Amél Plant 28:257–267

Geyt JPC Van (1986) The use of an isozyme marker system in sugarbeet genetics and breeding. PhD thesis, Vrije Universiteit, Brussel, Belgium

Geyt JPC Van, Jacobs M (1986) Mode of inheritance and some general characteristics of sugarbeet alcohol dehydrogenase. Plant Sci 46: 143–149

Geyt JPC Van, Smed E (1984) Polymorphism of some marker enzymes of the sugarbeet (*Beta vulgaris* L.) investigated by polyacrylamide gel electrophoresis and starch gel electrophoresis. Z Pflanzenzücht 92: 295–308

Hart GE (1978) Chromosomal arm locations of *Adh-R1* and an acid phosphatase structural gene in Imperial rye. Cereal Res Commun 6: 123–133

Hart GE, Langston PJ (1977) Glutamate oxaloacetate transaminase isozymes of the *Triticinae:* Dissociation and recombination of subunits. Theor Appl Genet 50: 47–51

Heijbroek W, Roelands AJ, Jong JH De (1983) Transfer of resistance to beet cyst nematode from *Beta patellaris* to sugar beet. Euphytica 32:287–298

Heijbroek W, Roelands AJ, Jong JH De, Hulst C Van, Schoone AHL, Munning RG (1988) Sugar beets homozygous for resistance to beet cyst nematode (*Heterodera schachtii* Schm.), developed from monosomic additions of *Beta procumbens* to *B. vulgaris*. Euphytica 38:121–131

Jong JH De, Blom GH (1981) The pachytene chromosomes of *Beta procumbens* Chr. Sm. In: De Jong JH (ed) Investigation into chromosome morphology of sugar beet and related wild species. PhD thesis, University of Amsterdam, the Netherlands, pp 33–39

Jong JH De, Bock ThSM De (1978) Use of haploids of *Beta vulgaris* L. for the study of orcein and Giemsa stained chromosomes. Euphytica 27:41–47

Jong JH De, Speckmann GJ, Bock ThSM De, Voorst A Van (1985) Monosomic additions with resistance to beet cyst nematode obtained from hybrids of *Beta vulgaris* and wild *Beta* species of the section *Patellares*. II. Comparative analysis of the alien chromosomes. Z Pflanzenzücht 95: 84–94

Jong JH De, Speckmann GJ, Bock ThSM De, Lange W, Voorst A Van (1986) Alien chromosome fragments conditioning resistance to beet cyst nematode in diploid descendants from monosomic additions of *Beta procumbens* to *B. vulgaris*. Can J Genet Cytol 28:439–443

Jung C, Wricke G (1987) Selection of diploid nematode-resistant sugar beet from monosomic addition lines. Plant Breed 98:205–214

Jung C, Wehling P, Löptien H (1986) Electrophoretic investigations on nematode resistant sugar beets. Plant Breed 97:39–45

Khush GS (1973) Cytogenetics of aneuploids. Academic Press, New York, 301 pp

Lange W, Bock ThSM De, Geyt JPC Van, Oléo M (1988) Monosomic additions in beet (*Beta vulgaris*), carrying extra chromosomes of *B. procumbens*. 2. Effects of the alien chromosomes on in vivo and in vitro plant development. Theor Appl Genet (in press)

Löptien H (1984a) Breeding nematode-resistant beets. I. Development of resistant alien additions by crosses between *Beta vulgaris* L. and wild species of the section *Patellares*. Z Pflanzenzücht 92:208–220

Löptien H (1984b) Breeding nematode-resistant beets. II. Investigations into the inheritance to *Heterodera schachtii* Schm. in wild species of the section *Patellares*. Z Pflanzenzücht 93:237–245

Pryor AJ (1974) Allelic glutamic dehydrogenase isozymes in maize. A single hybrid isozyme in heterozygotes? Heredity 32:397–401

Rick CM (1983) Tomato. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding. Elsevier, Amsterdam, pp 147–166

Salinas J, Benito C (1984) Chromosomal location of peroxydase structural genes in rye (*Secale cereale* L.). Z Pflanzenzücht 93:291–308

Savitsky H (1975) Hybridization between *Beta vulgaris* and *B. procumbens* and transmission of nematode (*Heterodera schachtii*) resistance to sugar beet. Can J Genet Cytol 17:197–209

Savitsky H (1978) Nematode (*Heterodera schachtii*) resistance and meiosis in diploid plants from interspecific *Beta vulgaris* × *B. procumbens* hybrids. Can J Genet Cytol 20:177–186

Scandalios JG, Sorensen JC, Ott LA (1975) Genetical control and intracellular localization of glutamate oxaloacetic transaminase in maize. Biochem Genet 13:759–769

Smith GA (1980) Sugar beet. In: Fehr WR, Hadley HH (eds) Hybridization of crop plants. Am Soc Agron, Madison/WI, pp 601–616

Speckmann GJ, Bock ThSM De (1982) The production of alien monosomic additions in *Beta vulgaris* as a source for the introgression of resistance to beet root nematode (*Heterodera schachtii*) from *Beta* species of the section *Patellares*. Euphytica 31:313–323

Speckmann GJ, Bock ThSM De, Jong JH De (1985) Monosomic additions with resistance to beet cyst nematode obtained from hybrids of *Beta vulgaris* and wild *Beta* species of the section *Patellares*. I. Morphology, transmission and level of resistance. Z Pflanzenzücht 95:74–83

Tang KS, Hart GE (1975) Use of isozymes as chromosome markers in wheat-rye addition lines and in triticale. Genet Res 26:187–201

Vallejos CE (1983) Enzyme activity staining. In: Tanksley SD, Orton TJ (eds) Isozymes in plant genetics and breeding. Elsevier, Amsterdam, pp 469–516

Yu MH (1981) Sugar beets homozygous for nematode resistance and transmission of resistance to their progeny. Crop Sci 21:714–717